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February 24, 2012

*via e-mail: [CEQA.Guidelines@ceres.ca.gov](mailto:CEQA.Guidelines@ceres.ca.gov)*

CEQA Guidelines Update  
c/o Christopher Calfee  
Office of Planning and Research  
1400 Tenth Street  
Sacramento, CA 95814

Re: Proposed State CEQA Guidelines Section 15183.3: Streamlining for  
Infill Projects (SB 226)

Dear Mr. Calfee:

The South Coast Air Quality Management District staff ("SCAQMD") appreciates the opportunity to comment on the proposed CEQA Guidelines Section 15183.3 (Streamlining for Infill Projects), which would implement SB 226. (Simitian, 2011.) We have specific comments on three areas in the proposal.

## **VMT Provisions**

The SCAQMD strongly supports the emphasis the Governor's Office of Planning & Research (OPR) has placed upon encouraging development which reduces VMT (vehicle miles traveled) because it is either located in low VMT areas (less than 75% of the regional average) or itself reduces VMT. We also understand the desire to promote environmentally – beneficial design in areas with moderate VMT (75-100% of average regional per capita VMT) by allowing streamlining for such infill projects if they include enhanced efficiency measures in the CALGreen building code.

However, we are concerned that the proposal to allow streamlining for projects associated with high VMT (above average regional per capita VMT), if they use CALGreen tier 2 measures, sends the wrong message to infill developers. As OPR has recognized, "VMT is a metric that advances nearly all of the environmental objectives listed in SB 226." Narrative Explanation, p. 16. Moreover, "green building techniques, which seek to reduce energy use

through building design, are important, but they alone do not have nearly the same greenhouse gas reducing potential as does reducing VMT.” Narrative Explanation, pp. 20-21. The same is true for pollutants for which there is a national ambient air quality standard and which have recognized public health impacts. Therefore, we urge OPR not to provide the benefits of CEQA streamlining to infill projects associated with higher than regional average VMT. Such projects of course may still be approved, but would simply have to comply with current CEQA requirements.

### **Projects Near High-Volume Roadways**

We have several comments on Appendix M: Performance Standards for Infill Projects Eligible for Streamlined Review, specifically regarding “projects near high-volume roadways.” SB 226 offers an important opportunity because it specifically requires projects to further the goal of “protection of public health, including the health of vulnerable populations from air pollution,” (Pub. Res. C. §21094.5.5(b)(7)), in order to obtain streamlined review. OPR should apply this policy requirement to the maximum extent feasible to prevent exposing persons to the risks of air pollution from nearby high volume roadways. The Appendix sets a definition of “high volume roadway” but then allows local agencies to ignore this definition. The Appendix should not allow the term “high-volume roadway” to be “more specifically defined at the local level” unless the local definition is more stringent than the definition in Appendix M. The Appendix applies additional standards to projects within 500 feet of high volume roadways, but then allows the local agency to set a different distance. The Appendix should not allow the local agency to set a different distance than the 500 feet set forth in Appendix M as “appropriate...based on local conditions” unless the local standard is more stringent (i.e., more than 500 feet).

There are over 160 cities within the South Coast AQMD. These cities may easily be tempted to compete with one another in a “race to the bottom” by adopting the least stringent standards for CEQA streamlining in order to attract development to their city as opposed to neighboring cities. Accordingly, Appendix M must set forth minimum standards which may not be relaxed by local jurisdictions. Local jurisdictions should be free, however, to adopt more stringent standards.

Moreover, Appendix M as currently proposed only requires projects to comply with local government plans or policies, or measures, such as enhanced air filtration or project design, that the lead agency determines “will promote the protection of public health.” This provision once again allows local agencies to reduce necessary protections for public health. We urge that the Appendix require such projects to include proven air filtration systems and any other recommendations adopted by the California Air Resources Board at a public meeting after opportunity for public comments.

We note that SB 226 requires the Guidelines prepared under its provisions to “include statewide standards for infill projects” to promote the goal of protection of public health. Pub. Res. C. §21094.5.5(b). In our opinion, the above-cited provisions, which allow the local lead

agency to determine for itself the definition of "high-volume roadway," deviate from the 500-foot extra protection zone, and determine for itself what, if any, health protections to require, clearly violate the statutory requirement for "statewide standards."

Since it may be impractical to fully develop specific statewide standards by your June deadline, we have suggested requiring the use of future standards that may be adopted by CARB.

In view of the public health impacts potentially resulting from exposure to emissions near high volume roadways, we also urge OPR to provide additional streamlining benefits to infill projects that are located more than 500 feet from such roadways.<sup>1</sup>

Finally, the public health protections for projects located within 500 feet of a high-volume roadway is at present limited to residential projects. We are particularly concerned that SB 226 specifically lists "schools" as eligible projects, (Pub. Res. C §21094.5(e)(1)(A)(iv)), but no protections are provided for schools, which are generally considered sensitive receptors. It is well established that children are more vulnerable than adults to the ill effects of air pollution due to their less-developed respiratory systems and greater degree of outdoor activity. Schools must be required to provide the same protections for public health as residential developments in order to qualify for streamlining.

Indeed, there is no reason why any development in which people work should not also be required to provide either a buffer zone or protection from the air pollution impact of high volume roadways in order to qualify for streamlining. Scientific evidence indicates that emissions from roadways are associated with a variety of health impacts.<sup>2</sup> In particular, high volume roadway emissions are associated with higher levels of ultrafine particles<sup>3</sup>, which are

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<sup>1</sup> The appropriate distance for such benefits may be subject to reasonable disagreement and we would welcome the opportunity to further discuss this issue with OPR and other stakeholders. For example, Zhu et al., *infra* note 2, found that ultrafine particle number concentrations at 300m downwind from the freeway were indistinguishable from background, while Hu et al., *infra* note 2, found that particle numbers dropped off much more slowly (background at 2600m) when measured 1 to 2 hours before sunrise (when residents are likely to be home).

<sup>2</sup> Knibbs et al., *A review of commuter exposure to ultrafine particles and its health effects*, ATMOSPHERIC ENVIRONMENT 45 (2011) 2611-2622; *Traffic-Related Air Pollution: A Critical Review of the Literature on Emissions, Exposure, and Health Effects*, HEI SPECIAL REPORT 17, HEI Panel on the Health Effects of Traffic-Related Air Pollution, Health Effects Institute, Boston, MA (2010); Samet, J., *Traffic, Air Pollution, and Health*, INHALATION TOXICOLOGY 19:1021-1027 (2007). [Copies can be provided on request.]

<sup>3</sup> Zhu et al., *Study of ultrafine particles near a major highway with heavy-duty diesel traffic*, ATMOSPHERIC ENVIRONMENT 36 (2002) 4323-4335; Hu et al., *A wide area of air pollutant impact downwind of a freeway during pre-sunrise hours*, ATMOSPHERIC ENVIRONMENT 43 (2009) 2541-2549.

associated with adverse health impacts<sup>4</sup>. There is as yet no state or national ambient air quality standard for ultrafine particles. Nevertheless, it is not prudent for state policy to encourage development which will increase exposure to ultrafine particles given the growing knowledge of their risks. For example, a recent study indicates that the health benefits of living in a “walkable” community may be outweighed by the adverse effects of exposure to pollution from roadways.<sup>5</sup>

Heavily-travelled roadways are also key contributors of diesel particulate matter in the environment. The SCAQMD’s third Multiple Air Toxics Exposure Study (published in 2008) concluded that 84% of the total cancer risk from toxic air contaminants in the region comes from diesel particulate. Importantly, the areas of highest exposure closely track the heavily-travelled roadways (as well as the port and railyard vicinities). We urge OPR to design the criteria for CEQA streamlining to avoid preferentially encouraging development which would expose people, whether workers, students, or residents, to this toxic pollutant.

However, if an infill project is located along a major roadway which has primarily zero-emission or near-zero emission traffic, such as an electrified truck corridor, then these concerns would be lessened. OPR should consider providing the most CEQA incentives to transit stations that service zero-emissions transit, or infill projects along zero-emission corridors.

**Notice of Exemption Where Uniformly Applicable Development  
Policies Will Substantially Mitigate New Specific Effects**

Proposed CEQA Guidelines Section 15183.3(c)(2)(A) provides that a lead agency may determine that no additional environmental review is required and a notice of exemption (NOE) will be prepared if any new significant effects of the project, or effects that are more significant than previously analyzed (in planning-level CEQA documents), would be “substantially mitigated” by “uniformly applicable development policies adopted by cities and counties.” Proposed Guideline §15183.3(c)(1)(E) provides that “substantially mitigate means that the policy or policies will substantially lessen the effect, but not necessarily below the level of significance.” In short, this Guideline would allow the lead agency to rely on a Notice of Exemption even though the infill project has new significant adverse impacts. This

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<sup>4</sup> Araujo et al., *Ambient Particulate Pollutants in the Ultrafine Range Promote Early Atherosclerosis and Systemic Oxidative Stress*, CIRCULATION RESEARCH, March 14, 2008, p. 589; Li et al., *Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage*, ENVIRONMENTAL HEALTH PERSPECTIVES Vol. III No. 4 (April 2003), p. 455; Delfino et al., *Association of Biomarkers of Systemic Inflammation with Organic Components and Source in Quasi-Ultrafine Particles*, ENVIRONMENTAL HEALTH PERSPECTIVES Vol. 118 No. 6 (June 2010), p. 756.

<sup>5</sup> Hankey et al., *Health Impacts of the Built Environment: Within-Urban Variability in Physical Inactivity, Air Pollution, and Ischemic Heart Disease Mortality*, ENVIRONMENTAL HEALTH PERSPECTIVES Vol. 120 No. 2 (February 2012), p.247.

appears to be a violation of CEQA, which requires an EIR wherever a fair argument can be made that the project will cause significant adverse impact. (*No Oil, Inc. v. City of Los Angeles*, 13 C. 3d 68, 75 (1974.)) The language and legislative history of SB 226 does not clearly indicate intent to cause such a dramatic change to CEQA law. We recognize that SB 226 uses the term “substantially mitigate” rather than “mitigate to insignificance.” This does not in our view constitute sufficient evidence of intent to allow notices of exemption for infill projects that have significant adverse impacts. In contrast, Pub. Res. C. §21084(b) explicitly provides that “greenhouse gas emissions in and of themselves shall not cause an exemption...to be inapplicable” if the project complies with applicable GHG plans. We would expect the legislature to be equally clear if it intended that an exemption be available for infill projects despite significant and unmitigated impacts. *See, Fiol v. Doellstedt*, 50 Cal. App. 4<sup>th</sup> 1318, 1329 (1998): “If the Legislature had intended such a major shift in the law of agency, it would have expressly so stated.” Likewise, we would expect some discussion in the legislative history if it were intended that notices of exemption be issued to projects with significant adverse impacts on public health, without even requiring any mitigation.

Most troubling, this Guideline would allow a lead agency to approve an infill project (which may reduce VMT and thus improve regional air quality) even though it causes a significant adverse impact on public health by exposing sensitive receptors to high levels of carcinogenic and otherwise dangerous air pollution. And *unlike existing CEQA requirements*, this could occur without requiring all feasible (or any) mitigation measures, without considering feasible alternatives, and without the public policymakers having to face the issue and make findings of overriding considerations. We strongly urge OPR to remove this provision or at least prevent its use in cases where people would be exposed to significant adverse air pollution impacts. This could be done by specifying in the public health “statewide standards” that any impact on public health must be mitigated to insignificance for a project to qualify, or at least mitigated to the maximum extent feasible, including adopting all measures approved by CARB.

Moreover, OPR needs to define what is meant by “substantially” mitigated, if it is concluded that it does not mean mitigate to insignificance. One lead agency could say that “substantially” mitigated means a 90% reduction, whereas another could say it means 30% reduction, or even less. Since this provision is a requirement to qualify for significant CEQA streamlining, OPR should adopt a uniform benchmark to prevent local governments from competing by providing the least protection to the environment and public health.

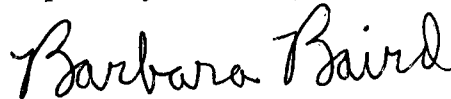
### Conclusion

We look forward to the opportunity to provide further input into this process as it moves forward. In particular, we encourage OPR to adopt specific requirements for a local lead agency to use in determining consistency with an adopted sustainable communities strategy.

We hope to provide more detailed comments on this issue in the future. We recognize OPR is on a tight time schedule since the proposed guidelines must be transmitted to the Natural Resources Agency by July 2012. Therefore, we intend to provide our suggestions quickly.

Should OPR have any questions or wish to discuss the issues raised in this letter, please contact me at 909.396.2302 or bbaird.aqmd.gov.

Respectfully submitted,



Barbara Baird  
District Counsel

BBB:pa

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cc: Barry R. Wallerstein, D.Env. Executive Officer  
Elaine Chang, DrPH, Deputy Executive Officer

Attachments:

1. Knibbs et al., *A review of commuter exposure to ultrafine particles and its health effects*, ATMOSPHERIC ENVIRONMENT 45 (2011) 2611-2622;
2. Zhu et al., *Study of ultrafine particles near a major highway with heavy-duty diesel traffic*, ATMOSPHERIC ENVIRONMENT 36 (2002) 4323-4335
3. Hu et al., *A wide area of air pollutant impact downwind of a freeway during pre-sunrise hours*, ATMOSPHERIC ENVIRONMENT 43 (2009) 2541-2549
4. Araujo et al., *Ambient Particulate Pollutants in the Ultrafine Range Promote Early Atherosclerosis and Systemic Oxidative Stress*, CIRCULATION RESEARCH, March 14, 2008, p. 589
5. Li et al., *Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage*, ENVIRONMENTAL HEALTH PERSPECTIVES Vol. 111 No. 4 (April 2003), p. 455;
6. Delfino et al., *Association of Biomarkers of Systemic Inflammation with Organic Components and Source in Quasi-Ultrafine Particles*, ENVIRONMENTAL HEALTH PERSPECTIVES Vol. 118 No. 6 (June 2010), p. 756.
7. Hankey et al., *Health Impacts of the Built Environment: Within-Urban Variability in Physical Inactivity, Air Pollution, and Ischemic Heart Disease Mortality*, ENVIRONMENTAL HEALTH PERSPECTIVES Vol. 120 No. 2 (February 2012), p.247.



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## Review

## A review of commuter exposure to ultrafine particles and its health effects

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## ABSTRACT

Ultrafine particles (UFPs, <100 nm) are produced in large quantities by vehicular combustion and are implicated in causing several adverse human health effects. Recent work has suggested that a large proportion of daily UFP exposure may occur during commuting. However, the determinants, variability and transport mode-dependence of such exposure are not well-understood. The aim of this review was to address these knowledge gaps by distilling the results of 'in-transit' UFP exposure studies performed to-date, including studies of health effects.

We identified 47 exposure studies performed across 6 transport modes: automobile, bicycle, bus, ferry, rail and walking. These encompassed approximately 3000 individual trips where UFP concentrations were measured. After weighting mean UFP concentrations by the number of trips in which they were collected, we found overall mean UFP concentrations of 3.4, 4.2, 4.5, 4.7, 4.9 and  $5.7 \times 10^4$  particles  $\text{cm}^{-3}$  for the bicycle, bus, automobile, rail, walking and ferry modes, respectively. The mean concentration inside automobiles travelling through tunnels was  $3.0 \times 10^5$  particles  $\text{cm}^{-3}$ .

While the mean concentrations were indicative of general trends, we found that the determinants of exposure (meteorology, traffic parameters, route, fuel type, exhaust treatment technologies, cabin ventilation, filtration, deposition, UFP penetration) exhibited marked variability and mode-dependence, such that it is not necessarily appropriate to rank modes in order of exposure without detailed consideration of these factors. Ten in-transit health effects studies have been conducted and their results indicate that UFP exposure during commuting can elicit acute effects in both healthy and health-compromised individuals. We suggest that future work should focus on further defining the contribution of in-transit UFP exposure to total UFP exposure, exploring its specific health effects and investigating exposures in the developing world.

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## 1. Introduction

The study of commuter exposure to traffic-related air pollutants is not a particularly new field of research. Among the first researchers to recognise its significance was Professor Arie Haagen-Smit, who is best-known for his pioneering and enduring work related to characterising photochemical smog and ozone. In 1966, he performed a series of carbon monoxide measurements on heavily trafficked Los Angeles roads (Flachsbart, 2007; Haagen-Smit, 1966). Given population growth and increased motor vehicle use since that time, coupled with the high degree of proximity to vehicle emissions when commuting, the issue of 'in-transit'

exposure to air pollutants is of equal if not greater relevance 45 years later.

Previous reviews of in-transit pollutant exposure, of which there are few, have focussed on CO inside vehicles (El-Fadel and Abi-Esber, 2009), particle mass concentrations and composition in metro (subway) systems (Nieuwenhuijsen et al., 2007) and various pollutants in multiple transport modes (Weisel, 2001). Only the work of Kaur et al. (2007) included a review of ultrafine (<100 nm) particle (UFP) concentrations in several transport modes.

At present, although gaseous pollutants are still the focus of numerous in-transit exposure studies, UFPs are beginning to attract significant attention. They are produced in large quantities by fuel combustion, and have been identified as a causal component of various negative health effects in humans (Knol et al., 2009; Hoek et al., 2010). UFPs typically constitute ~90% or more of particle number count (PNC) in areas influenced by vehicle emissions (Morawska et al., 2008), and we use UFP to describe PNC throughout this article.

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The primary aim of this review is to distil the results of work performed to-date in order to improve understanding of the measurement, characteristics and determinants of in-transit exposure to UFPs, prior to a discussion of gaps in knowledge and suggestions for future research. Here, we extend the work of Kaur et al. (2007) by confining our focus to UFPs and incorporating the substantial body of relevant work that has appeared in the 4 years since its publication, which now constitutes the large majority of available literature. Like Kaur et al. (2007), we restrict our investigation to UFP exposure concentrations, rather than average or integrated exposure for a given time period. We note that dose assessment, which is a complementary yet distinct concept to that of exposure (Ott, 1985), is not the main focus of this review.

This review begins with an overview of the nature of commuter travel prior to a description of the general characteristics of UFPs. This is followed by a detailed analysis of in-transit UFP exposure studies, a discussion of determinant factors, health effects, and suggestions for future research.

### 1.1. Commuting in modern society

The nature of modern society in many countries both affords and expects a high degree of personal mobility. Time-activity patterns define how people apportion their time across a range of environments, and are a keystone of effective exposure analyses. Time-activity pattern studies of varying magnitude performed in different regions have reported that time spent in-transit typically constitutes between about 5 and 10% of the day (Klepeis et al., 2001), depending on location. The transport microenvironment(s) within which this time is spent varies more substantially between regions than the occupancy time, and has a greater dependence on local factors, such as the availability and desirability of various transport options.

In general, there are scant 24 h time-activity pattern data for developing countries. Saxena et al. (2007) reported that time spent travelling among 4311 Delhi residents ranged from 0.8 to 10% of the day, and varied markedly depending on age, sex and occupation, as did the mode of transport used. It is likely that the time-activity patterns of people in rural areas differ significantly from those of their urban counterparts.

### 1.2. Children's and adult's travel choices

Children are particularly susceptible to negative health effects caused by exposure to air pollutants (Gauderman et al., 2004; Brugge et al., 2007; Ashmore and Dimitroulopoulou, 2009), and many millions are required to commute between home and school each weekday. The choice of which transport mode school children utilise is normally at the discretion of others. Whilst children and young people have been reported to possess informed and responsible opinions regarding transport choices and a clear ideal towards cycling and walking, their parent's choices are guided primarily by safety concerns, and place substantial reliance on private automobiles (Lorenc et al., 2008).

Unlike children, adults generally make their own travel choices. A recent survey of 745 employed adults in Queensland, Australia, found that while about half of respondents felt that exposure to air pollutants in-transit negatively affected their overall health and increased their risk of cardiovascular disease, only 13% indicated that exposure to pollutants was a barrier to their adoption of walking or cycling to work, and that other factors were more responsible for their high level (82%) of dependence on private transport (Badland and Duncan, 2009). Furthermore, Badland and Duncan (2009) found that adults who were better educated and lived in urban areas were most cognisant of the negative health

effects of air pollutant exposure during transit. Marshall et al. (2009) reported that the optimum balance between high walkability and low pollution was identified sporadically and typically in higher income neighbourhoods in urban Vancouver (Marshall et al., 2009). Evidently, there may be a significant socio-economic component involved in air pollution exposure during transit, particularly for active transport modes, and this may reflect wider socio-economic and environmental inequalities reported for several traffic pollutants (Marshall, 2008; Tonne et al., 2008; Su et al., 2009). It should be noted that both children and adults in developing countries are unlikely to be afforded the luxury of a travel choice, *per se*, and a relatively high degree of dependency on walking and public transport may result from this (Saxena et al., 2007).

## 2. Characteristics of ultrafine particles

### 2.1. General

UFP concentrations reflect the contribution of anthropogenic processes to a pre-existing background concentration (Morawska et al., 2008). Background concentrations are ascribed to natural processes, such that in most environments free from the immediate influence of anthropogenic activities, UFPs are present and their concentrations readily measured. Despite the numerous natural sources of UFPs, vehicular fossil fuel combustion has repeatedly been shown to be their dominant source in urban areas, with heavy-duty diesel powered vehicles making a disproportionately large contribution to UFP concentrations (Morawska et al., 2008).

An important distinction is between primary and secondary UFPs. The primary variety are emitted from their source as particles, whilst secondary particles are formed following homogenous nucleation of gases (Koutrakis and Sioutas, 1996; Jacobson, 2002). This occurs when a gas, or gases, nucleate in the absence of a pre-existing surface (Jacobson, 2002).

UFPs from vehicles can be emitted as primary particles or generated as a secondary aerosol, such as following homogenous nucleation of  $\text{SO}_2$ ,  $\text{NH}_3$  and  $\text{NO}_x$  into  $\text{SO}_4^{2-}$ ,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Koutrakis and Sioutas, 1996; Morawska et al., 2008). The ratio of primary to secondary particles varies substantially according to fuel type and operating and environmental conditions, but nucleation mode particles can comprise approximately 90% or more of UFPs in diesel exhaust (Kittelson, 1998). However, more recent research indicates that the number of nucleation mode particles in diesel exhaust can be reduced to 40–50% when ultra-low sulphur diesel fuel is used (Ristovski et al., 2006), which is more representative of modern vehicle fleets in many countries.

### 2.2. Typical concentrations

Morawska et al. (2008) performed a meta-analysis of 71 UFP studies performed across a diverse range of environments. They found mean concentrations of 2.6, 4.8, 7.3, 10.8, 42.1, 48.2, 71.5 and  $167.7 \times 10^3$  particles  $\text{cm}^{-3}$  for clean background, rural, urban background, urban, street canyon, roadside, on-road and tunnel environments, respectively. This indicates that greater proximity to vehicles is associated with increased UFP concentrations, and underscores their importance as a UFP source.

### 2.3. Health significance

Once inhaled, UFPs can reach with the alveolar region of the human lung with greater efficiency than larger particles due to their smaller size, and can deposit in alveoli with greater efficiency as a consequence of their rapid diffusion (Daigle et al., 2003; Phalen



et al., 2006; Frampton, 2007). Due to their content of reactive oxygen species (ROS) and large combined surface area, UFPs from vehicle emissions have the potential to damage pulmonary cells (Delfino et al., 2005). Transition metal components in UFPs are believed to play a role in producing ROS, along with pro-oxidative organic hydrocarbons (Li et al., 2003). Additionally, target cells, such as airway epithelial cells and macrophages, produce ROS during biologically catalysed redox reactions occurring in the mitochondria in response to UFP uptake (Li et al., 2003; Nel, 2005). UFPs can evade alveolar macrophage clearance from the lung and enter lung cells, the interstitium and possibly the vascular bed (Geiser et al., 2005; Frampton, 2007), and can travel from the lung via blood and lymphatic circulation to other organs (Elder et al., 2006; Samet et al., 2009). UFPs are more proatherogenic than larger particles due to their greater bioavailability of reactive compounds, content of redox-active compounds, high number concentration and increased lung retention (Araujo and Nel, 2009).

Epidemiologic investigations of UFPs have been constrained by the scarcity of UFP monitoring sites and the substantial spatial heterogeneity of concentrations (Brook et al., 2010). Studies performed to-date in Erfurt, Germany, have indicated that UFP effects on daily mortality may be of comparable magnitude to, yet independent of, those of fine particles (i.e.  $PM_{2.5}$ ), albeit with greater time lag between UFP concentrations and their effects (Wichmann and Peters, 2000). More recent results from the same long-term study have shown statistically significant associations between UFP concentrations and both total and cardio-respiratory daily mortality with a four day lag period (Stölzel et al., 2007). Interestingly, this study found no association between  $PM_{2.5}$  mass concentration and mortality. Mortality from stroke amongst aged residents of Helsinki during summer was associated with both  $PM_{2.5}$  and UFP concentrations on the previous day, and effects were mostly independent (Kettunen et al., 2007).

The effects of UFP concentration on mortality and morbidity due to various causes are less well understood than those of larger particles. A recent elicitation of European experts found that short-term UFP exposure was rated to variously possess a medium to very high likelihood of causality for all-cause mortality, and a low to high likelihood for cardiovascular and respiratory hospital admissions (Knol et al., 2009). Long-term UFP exposure was generally rated to possess a slightly lower likelihood of causality for all-cause mortality, owing mainly to the lack of long-term studies (Knol et al., 2009). The same group of experts estimated that a permanent decrease in annual average UFP concentration of 1000 particles  $cm^{-3}$  across Europe would lead to median decreases of 0.3%, 0.2% and 0.16% in all-cause mortality, and cardiovascular and respiratory hospital admissions, respectively (Hoek et al., 2010). The relatively small number of epidemiological studies (14) and absence of long-term studies, however, resulted in most experts indicating a substantial degree of uncertainty in their estimates (Hoek et al., 2010).

### 3. Studies of UFP concentration in transport modes

#### 3.1. Methods

We searched combinations of the terms “ultrafine particle”, “transport mode”, “commuter”, “exposure”, “public transport”, “microenvironment”, “vehicle”, “car”, “automobile”, “bus”, “cyclist”, “bicycle”, “train”, “metro”, “subway” on PubMed, ISI Web of Knowledge and Google Scholar until October, 2010. The reference lists of studies identified by this method were reviewed for links to additional literature. Furthermore, the authors’ own literature collections were utilized.

We restricted our investigation to studies that presented numeric values of UFP concentrations, and identified 47 that fulfilled this requirement. Tables S1–S7 in the Supplementary Information file contain detailed information on the various studies. These spanned 6 distinct transport modes: automobile, bus, cycling, ferry, rail and walking. Some studies dealt with multiple transport modes, whilst others focussed on a single mode. Of the studies we identified, only 7/47 (15%) had previously been reviewed by Kaur et al. (2007), which highlights the rapid progression of research related to in-transit UFP exposure since publication of their work.

The mean concentrations extracted from the studies identified were weighted by the corresponding number of trips taken, and overall trip-weighted mean UFP exposure concentrations were calculated for each transport mode (see Tables S1–S7 in the Supplementary Information file). The overwhelming majority of studies (93%) reported the number of trips associated with a given mean; the means reported by those that did not report trip number were weighted by a conservative factor of 1 trip. Most studies reported arithmetic mean UFP concentration, while several reported geometric mean and one gave median values. Where possible, data were disaggregated to permit analyses of the effect of variables such as fuel type, presence of exhaust-treatment devices and route.

Given the range of conditions under which they were collected, we did not assess the statistical significance of differences in measured mean UFP concentrations between modes, and instead sought to identify general trends in the data. This is discussed further in sections 4 and 5.

#### 3.2. Results

Across all modes, we identified approximately 3000 individual trips where UFP measurements were performed. There was an uneven distribution of measurement trips; very few have been performed in ferry (13) and rail (49) modes, while a substantial number have been undertaken in bus (505), walking (524), cycling (599) and automobile (1310) modes. The automobile mode was split into non-tunnel (977) and tunnel (333) trips, as previous results indicate that tunnels are a discrete UFP exposure microenvironment distinct from open air roadways (Kaminsky et al., 2009; Knibbs et al., 2010).

Fig. 1 shows the trip-weighted mean UFP concentrations for each mode, and the number of trips on which they were based. Error bars indicate the trip-weighted standard deviation (Bland and

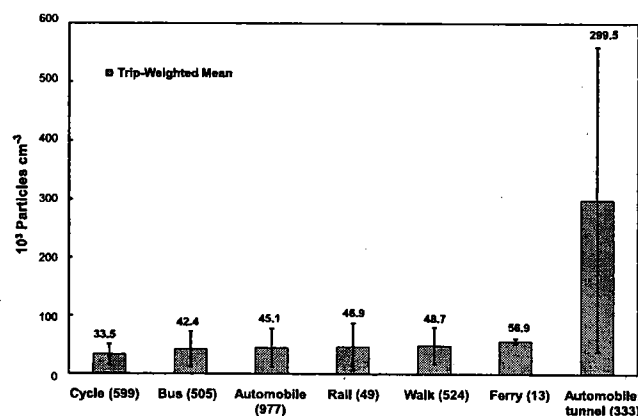


Fig. 1. Trip-weighted mean UFP concentrations in each transport mode, shown as bold numbers. The number of trips taken in each mode is shown in brackets. Error bars denote the trip-weighted standard deviation. The studies from which the data were extracted are listed in the Supplementary Information file.

Kerry, 1998). The range of mean UFP concentrations spanned one order of magnitude, with the lowest measured whilst cycling and the highest in automobiles during tunnel travel;  $3.4 \times 10^4$  (s.d. =  $1.8 \times 10^4$ ) and  $3.0 \times 10^5$  (s.d. =  $2.6 \times 10^5$ ) particles  $\text{cm}^{-3}$ , respectively. Means and standard deviations calculated for the automobile (non-tunnel), bus, ferry, rail and walk modes were 4.5 (3.3), 4.2 (3.1), 5.7 (0.5), 4.7 (4.1) and  $4.9 (3.2) \times 10^4$  particles  $\text{cm}^{-3}$ , respectively.

#### 4. Comparison between modes

Considering the diversity of studies from which they were drawn, the trip-weighted concentrations measured in automobile (non-tunnel), cycle, bus, rail and walking modes exhibited notable coherence, with a maximum to minimum ratio (walk:cycle) of 1.5. A limited number of studies that measured concentrations in different modes simultaneously or near-simultaneously have been reported, and Briggs et al. (2008) found a walk:automobile ratio of 1.4, which is higher than the value of 1.1 presented here. Boogaard et al. (2009) found an automobile:cycle ratio of 1.05, whilst we found a value of 1.3, which was also higher than the value of approximately 1.0 reported by Int Panis et al. (2010).

While the above studies highlighted the relative concentrations encountered in each mode in the absence of bias due to fluctuating UFP concentrations, observed inter-mode contrasts were specific to the conditions of the study (e.g. the ventilation settings in an automobile, or the proximity to traffic on a bike route) and are should therefore not be extrapolated beyond the conditions under which they were collected without appropriate caution.

In studies that measured UFP concentrations in multiple modes non-simultaneously, the mode in which highest concentrations were recorded vacillated between automobiles and buses, whilst those in other modes were markedly lower (Levy et al., 2002; Kaur et al., 2005b; Weichenthal et al., 2008; Cattaneo et al., 2009; Kaur and Nieuwenhuijsen, 2009; Pattinson, 2009; Shrestha, 2009; Knibbs and de Dear, 2010). It is therefore noteworthy that our analysis found that UFP concentrations in buses and automobiles (non-tunnel) were relatively low. We pooled a large number of reported UFP measurements performed under a wide range of conditions, and while the ability to differentiate the observed differences is limited by the level of detail given by the various studies, such an approach is indicative of mean values and general trends. However, the mode in which highest exposures are experienced depends strongly on the determinant factors discussed in the following two sections, and generalisation of results may be of limited value (Int Panis et al., 2010); that is, within mode variability is likely to be substantial.

#### 5. Determinants of UFP concentration in-transit

Despite the convenience it may provide, it is not necessarily appropriate to rank transport modes in order of UFP exposure without certain caveats. For example, Fig. 1 shows the trip-weighted mean UFP concentration in an automobile is higher than the equivalent for cycling. However, an occupant of a relatively airtight automobile in which air is recirculated and filtered will likely experience markedly lower exposure concentrations than a cyclist on a high traffic route. Disentangling the relative roles of determinant factors, their interactions and variability in each mode is a key element required to advance understanding of in-transit UFP exposure. The data reviewed here suggest that while the relationship between UFP concentration and its determinants is often mode-dependent, exposure in all mode types is the result of interplay between multiple factors. These can be viewed as comprising two stages: the first determines the outdoor or on-road

UFP concentration, and the second determines what proportion of this is able to come into contact with a commuter. These factors are addressed, in turn, in the following sections.

##### 5.1. Meteorological variables

Temperature has been variously reported to be positively and negatively correlated with UFP concentrations, although in vehicle-dominated areas the correlation is more likely to be negative due to condensation of volatile compounds in emissions (Morawska et al., 2008). In-transit studies that assessed this relationship uniformly found a negative correlation between temperature and UFP concentration (Krausse and Mardaljevic, 2005; Vinzents et al., 2005; Thai et al., 2008; Weichenthal et al., 2008; Kaur and Nieuwenhuijsen, 2009; Pattinson, 2009; Laumbach et al., 2010). Among studies that reported correlation coefficients, those measured for cycling studies (Vinzents et al., 2005; Thai et al., 2008) were quite high (−0.62 and −0.76, respectively). Multi-mode and automobile studies reported correlations of −0.77 and −0.37, respectively (Kaur and Nieuwenhuijsen, 2009; Laumbach et al., 2010).

Wind speed, which affects dilution and transport of vehicle emissions, was also found to be negatively correlated with UFP concentration in-transit (Krausse and Mardaljevic, 2005; Vinzents et al., 2005; Briggs et al., 2008; Thai et al., 2008; Weichenthal et al., 2008; Kaur and Nieuwenhuijsen, 2009; Pattinson, 2009; Shrestha, 2009; Knibbs and de Dear, 2010), which is in agreement with results reported for various outdoor locations (Morawska et al., 2008). However, the results were not always statistically significant, indicating that temperature may be more consistently and strongly correlated with UFP concentrations. Correlations observed for active transport modes were −0.20 (walk), −0.52 (cycle) and −0.81 (cycle) (Briggs et al., 2008; Vinzents et al., 2005; Thai et al., 2008). Multi-mode and automobile studies reported correlations of −0.14 to −0.49 (Briggs et al., 2008; Kaur and Nieuwenhuijsen, 2009; Knibbs and de Dear, 2010).

For both temperature and wind speed, stronger correlations were generally observed for cycling compared to non-active modes, perhaps reflecting reduced influence of other factors on exposure concentrations encountered when cycling (and walking). The strength of the association between UFP concentration and both temperature and wind speed appears to be mode and location-dependent, and its variability is not well characterised.

While temperature and wind speed are the most frequently reported, other meteorological parameters may affect UFP concentration. The depth of the mixed layer within the atmosphere was found to be negatively correlated with in-transit UFP concentration (Weichenthal et al., 2008), which reflects the tendency of a shallow mixed layer to concentrate pollutants.

##### 5.2. Traffic volume and composition

Very few studies have reported the relationship between traffic volume and in-transit UFP concentrations. Fewer still have examined the effect of traffic composition (i.e. gasoline vehicles, diesel vehicles). Briggs et al. (2008) observed statistically significant correlations between car and truck density and UFP concentrations encountered while walking ( $r = 0.41$ – $0.48$ ) or in an automobile ( $r = 0.43$ – $0.47$ ) in London. In their London-based study, Kaur and Nieuwenhuijsen (2009) similarly found a significant correlation ( $\rho = 0.27$ ) between total traffic count and UFP concentrations in automobile, bus, cycle, taxi and walking modes. Krausse and Mardaljevic (2005) reported road link end description (e.g. signal, left turn, right turn etc.) was a significant determinant of total UFP exposure of car occupants. On-road studies have shown strong

associations ( $R^2 \sim 0.85$ ) between heavy diesel traffic volume and UFP concentrations (Fruin et al., 2008; Knibbs et al., 2009b). Other studies have reported more qualitative assessments of traffic effects; for example, that mean in-transit UFP concentrations increased on high traffic routes and vice-versa (Zhu et al., 2007; Thai et al., 2008; Strak et al., 2010; Zuurbier et al., 2010).

Vehicle emissions are the dominant source of UFPs in urban areas, and heavy diesel vehicles make a contribution that is disproportionate to their volume (Morawska et al., 2008). Coupled with the limited but consistent findings of in-transit studies, this suggests that traffic parameters (volume, density) and composition (gasoline vehicles, heavy diesel vehicles) are an important determinant of in-transit UFP exposure. It should be considered, however, that effects are likely to depend on mode, and that short-term traffic patterns not represented in hourly or daily average data, such as the impact of passing traffic, may be important (Fruin et al., 2008; Boogaard et al., 2009).

### 5.3. Route choice: active transport modes

There are generally fewer mode-specific variables that may affect pedestrians and cyclists compared to other transport modes; that is, traffic and meteorological conditions may be of greater importance as determinants. Most cycling studies were performed on or proximate to major urban roads, however, some studies compared measurements on high and low traffic routes, with the latter typically comprised of a dedicated cycle path. Separating the data into these two categories revealed that 18% of trips were undertaken on low traffic routes, and mean UFP concentrations were  $2.6 \times 10^4$  particles  $\text{cm}^{-3}$ . The mean for high traffic routes was  $3.5 \times 10^4$  particles  $\text{cm}^{-3}$ , suggesting that route selection, within the context of the few studies to address it, can affect cyclist UFP exposure (Pattinson, 2009; Strak et al., 2010; Zuurbier et al., 2010).

Route choice, as a proxy for traffic volume, is likely to be an important determinant of exposure (McCreanor et al., 2007; Hertel et al., 2008), and personal factors (e.g. walking or cycling patterns) may also exert an effect (Kaur et al., 2007). Microscale variations in UFP concentration proximate to roadways may result in higher exposures on the road side of the sidewalk/footpath compared to the building side (Kaur et al., 2005a). Also, the effect of roadway factors on pollutant dispersion (i.e. whether open to the environment or prone to trap pollutants due to geometry of urban canyons) has been shown to be a statistically significant determinant of UFP exposure concentrations encountered when walking (Briggs et al., 2008). Further work focussed on evaluating the effects of these local and microscale route phenomena on UFP exposure is required.

### 5.4. Cabin ventilation

Ventilation rates, whether driven by fans, natural leakage or open windows (Ott et al., 2008; Knibbs et al., 2009a), describe how rapidly outdoor air is capable of entering passenger cabins. Evidence suggests that ventilation is a key determinant of in-cabin UFP concentrations in automobiles, buses (Hammond et al., 2007; Rim et al., 2008; Knibbs and de Dear, 2010; Zhang and Zhu, 2010), ferries (Hill and Gooch, 2007; Knibbs and de Dear, 2010) and rail modes (Hill and Gooch, 2007; Cheng et al., 2009; Knibbs and de Dear, 2010). Quantitative studies support these observations, but are scarce and limited to automobiles (Xu and Zhu, 2009; Knibbs et al., 2010).

Knibbs et al. (2009a) found that air exchange increased linearly with vehicle speed in a group of six test automobiles operating under four distinct ventilation settings, which was in agreement with results obtained by Ott et al. (2008) based on tests performed in four vehicles. Knibbs et al. (2009b, 2010) found that the primary

determinant of on-road UFP concentration in a tunnel bore was hourly heavy diesel vehicle volume ( $R^2 = 0.87$ ), and that cabin ventilation rates explained 81% of the variation in the proportion of on-road UFPs reaching the occupants of 5 automobiles. The proportion reaching the cabin varied from 0.08 (recirculation) to  $\sim 1.0$  (non-recirculation) depending on vehicle and ventilation setting. Thus, ventilation rates controlled the extent to which in-cabin exposure concentrations reflected on-road levels in the tunnel bore, which were largely determined by heavy diesel vehicle volume. Xu and Zhu (2009) reported that cabin ventilation and leakage were predominant factors in their model-based analyses of variables affecting in-cabin/on-road (I/O) UFP ratios, and explained up to  $\sim 60\%$  of on-road UFP ingress. I/O ratios when windows are open can reach 1 due to higher air exchange, and such conditions may also occur when windows are closed but ventilation fan settings are high (Ott et al., 2008; Knibbs et al., 2009a).

Some investigators have successfully performed in-cabin UFP size distribution measurements during transit in automobiles (Zhu et al., 2007) and buses (Zhang and Zhu, 2010). These studies have shown that while in-cabin particle size distributions follow the general shape of those on-roads, the ability of on-road particles to reach the cabin is dependent on particle size and ventilation settings (Zhu et al., 2007). Particle penetration is discussed in section 5.6.

### 5.5. Filtration

Where a vehicle is fitted with a cabin air filter, its efficiency is a key determinant of what proportion of on-road UFPs reach the cabin, and efficiency varies substantially amongst the filters available. Standard automobile cabin filters afford single-pass UFP reductions of between approximately 30 and 60% (Pui et al., 2008; Qi et al., 2008), while this can be increased by employing more advanced filtration technologies (Burtcher et al., 2008). It should be noted that filtration efficiency is affected by the ventilation rate; as filter face velocity increases with mechanical or natural ventilation rates, filtration efficiency decreases due to the reduced time available for particle diffusion inside the filter (Pui et al., 2008; Qi et al., 2008). When air is recirculated in an automobile, Qi et al. (2008) found that UFP concentrations decayed most rapidly in a vehicle capable of filtering recirculated air (single pass efficiency = 46%) than in a vehicle lacking this feature, where UFP removal efficiency without a filter was 27% per recirculation of cabin air. In the former and latter cases, on-road UFP concentrations were reduced to those typical of an office building (4000 particles  $\text{cm}^{-3}$ ) in 3 min and 9–10 min, respectively, indicating the value of recirculation as a simple exposure minimisation mechanism. However, some older, less-airtight vehicles are characterised by outdoor air exchange rates up to  $47 \text{ h}^{-1}$  when air is recirculated (Knibbs et al., 2009a), and the benefit of recirculation in such cases can be substantially diminished (Knibbs et al., 2010).

### 5.6. UFP penetration and deposition

The penetration of UFPs through automobile envelopes is dependent on their size, the number and geometry of cracks, and the pressure difference across these and other ingress pathways (Xu et al., 2010). A recent study reported that penetration efficiency close to 100% was observed for diesel exhaust particles between 100 and 287 nm, and declined to  $\sim 70\%$  for 10 nm particles due to diffusion; although penetration of 10 nm particles increased to  $\sim 90\%$  when pressure differentials reached 200 Pa (Xu et al., 2010). No difference was observed in penetration efficiency amongst different materials.

Given the high surface to volume ratios of many automobile cabins, deposition can be an important UFP removal mechanism, especially under low ventilation conditions (Gong et al., 2009). Gong et al. (2009) found in-cabin deposition rates in automobiles exceed those of indoor environments by a factor of 3–20.

Studies describing UFP filtration, penetration and deposition in bus and rail modes are scarce and the limited data to-date is strongly skewed towards automobiles. Future studies addressing this knowledge gap will be of considerable value.

## 5.7. Fuel type and presence of an emission control device

### 5.7.1. Automobile

The effect of fuel type on UFP concentration in automobiles was assessed by Zuurbier et al. (2010), who found no significant difference in mean levels in diesel and gasoline-powered vehicles (diesel:gasoline concentration ratio = 0.96) based on 14 simultaneous trips under a standard ventilation setting. Their study focussed only on newer vehicles (<6 months) and its relevance to the wider passenger vehicle fleet is unknown. Additionally, it is difficult to separate the effects of fuel type from those due to differences in ventilation under a standard setting between vehicles of different manufacturer (e.g. Knibbs et al., 2009a). Further studies involving test vehicle groups more representative of the heterogeneity present in wider vehicle fleets are required.

### 5.7.2. Bus

Due to their frequency of door opening and the 'stop-start' nature in which they travel, buses have a tendency to self-pollute (Behrentz et al., 2004; Hill et al., 2005; Rim et al., 2008; Liu et al., 2010; Zhang and Zhu, 2010; Zuurbier et al., 2010). Accordingly, the variables most frequently reported by UFP exposure studies were fuel type and the presence of an exhaust or crankcase emission control device. We therefore disaggregated bus trips into 8 categories: diesel, biodiesel, compressed natural gas (CNG), electric, diesel with oxidation catalyst (DOC), diesel with diesel particulate filter (DPF), diesel with crankcase filtration system (CFS), and diesel with combined control (i.e. any combination of two or more of DOC, DPF, CFS and ultra low sulphur diesel). About 70% of trips were performed in diesel buses, with the remainder approximately evenly distributed across the other categories. Five percent of bus trips (26/505) were excluded due to lack of detailed data on fuel type or control device.

Fig. 2 shows the trip-weighted mean UFP concentrations for each category. The lowest mean ( $1.7 \times 10^4$  particles  $\text{cm}^{-3}$ ;  $\text{SD} = 0.8 \times 10^4$ ) was recorded in CNG-powered buses, and the highest ( $4.9 \times 10^4$  particles  $\text{cm}^{-3}$ ;  $\text{SD} = 2.6 \times 10^4$ ) was measured in diesel buses fitted with a CFS, although the latter result was based on a very limited number of trips (13). A similar mean was recorded in diesel buses with no control device ( $4.8 \times 10^4$  particles  $\text{cm}^{-3}$ ;  $\text{SD} = 3.2 \times 10^4$ ). Means and standard deviations calculated for the biodiesel, combined control, DPF, DOC and electric categories were  $1.7$  (–),  $2.0$  (1.8),  $2.4$  (0.9),  $2.8$  (2.0) and  $2.9$  (0.8)  $\times 10^4$  particles  $\text{cm}^{-3}$ , respectively. With the exception of the electric bus category, lowest concentrations were measured in buses powered by alternative fuels. Concentrations inside diesel-powered buses were generally lower when fitted with an emission control device.

Differentiating the effects of self-pollution from those of other factors on in-bus UFP concentrations is challenging. Previous work has shown that self-pollution can be the dominant source of vehicle emissions in the cabin when windows are closed, and constituted 0.01 to 0.3% of air in the cabins of 1975 through 2002 model school buses (Behrentz et al., 2004). Liu et al. (2010) found that self-pollution contributed an overall average of  $1.8 \times 10^4$  particles  $\text{cm}^{-3}$  in two school buses (2000 and 2003 model); the average

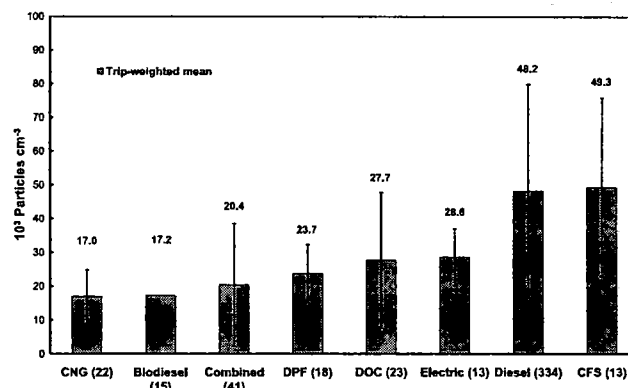


Fig. 2. Trip-weighted mean UFP concentrations measured in buses of different fuel type and emission control device. The number of trips taken in each category is shown in brackets. Error bars denote the trip-weighted standard deviation. See text for abbreviations.

contribution when windows were closed ( $1.0 \times 10^4$  particles  $\text{cm}^{-3}$ ) was less than that when they were open ( $2.6 \times 10^4$  particles  $\text{cm}^{-3}$ ). However, this trend was not in keeping with their results for other measured pollutants, and was attributed to UFP fluctuations due to unidentified non-vehicle sources on the low-traffic routes they studied. Generally, if on-road concentrations are low relative to those in-cabin, open windows will dilute self-pollution (Liu et al., 2010). The reverse can exacerbate its effects.

The relatively small number of trips taken in most categories we analysed and the lack of specific information regarding other possible determinants limits the conclusions that can be drawn, and precluded detailed statistical analyses. However, the results generally suggest that UFP concentrations are greatest in diesel-powered buses, and that reductions may be possible through use of alternative fuels or emission control devices, with best results achieved for diesel buses when two or more of the latter are combined.

### 5.7.3. Rail

In most rail studies we identified, trips were undertaken in vehicles driven by electricity. About 29% of trips were taken in diesel-powered trains, and the weighted mean UFP concentration during these was  $9.0 \times 10^4$  particles  $\text{cm}^{-3}$ . The mean during travel in electric-powered vehicles was  $3.0 \times 10^4$  particles  $\text{cm}^{-3}$ . Based on the limited data available, the power source of the rail vehicle therefore appears to markedly affect UFP exposure concentrations. Moreover, in diesel trains, the position of the locomotive relative to the passenger compartments can affect UFP concentrations; when a locomotive was located in front of passenger cabins, its emission plume can reach the cabin ventilation system intake Hill and Gooch, 2007.

There was insufficient data to investigate the effect of underground and above ground travel on UFP concentrations. Whilst there are numerous mechanical processes that can generate and resuspend particulate matter in electric-powered subway/metro systems, these are more likely to elevate levels of particle mass rather than UFP number count (Nieuwenhuijsen et al., 2007). The limited number of studies reporting UFP measurements on underground platforms tend to support this (Aarnio et al., 2005; Seaton et al., 2005; Raut et al., 2009; Cheng et al., 2009; Nystrom et al., 2010).

## 6. Correlation with other air pollutants

Several in-transit studies measured UFPs and other pollutants simultaneously. A summary of these is provided in Table S8 in the

Supplementary Information file. The correlation between UFP and PM<sub>2.5</sub> concentrations is generally reported to be positive, weak and not statistically significant, although stronger associations have been observed; correlation coefficients range from –0.07 to 0.69 (Aarnio et al., 2005; Kaur et al., 2005a,b; Seaton et al., 2005; McCreanor et al., 2007; Zhu et al., 2008; Berghmans et al., 2009; Boogaard et al., 2009; Knibbs and de Dear, 2010; Laumbach et al., 2010). Although correlation in the rail mode is moderate and relatively consistent across studies, in general there is no clear relationship between the strength of correlation and transport mode. The results are likely to be somewhat location-dependent, in keeping with those for outdoor environments, and the generally poor correlation reflects differences in the sources of particle number and mass and temporal scales involved in their dynamics (Morawska et al., 2008).

Black carbon (BC) and elemental carbon (EC) are often well-correlated with UFP concentrations in urban air, given their shared provenance in vehicle emissions and the large extent to which BC and EC contribute to UFP chemical composition (Morawska et al., 2008). On-road and subway platform studies have shown very good correlation between UFPs and BC; 0.88 and 0.84, respectively (Aarnio et al., 2005; Westerdahl et al., 2005). Correlations were relatively weak in automobile and bus studies (mean = 0.1–0.2), although in-bus relationships were strongly dependent on window position, and mean correlation improved (mean = 0.62) when windows were kept open, which the authors ascribed to self-pollution under the closed window setting (Zhu et al., 2008; Zhang and Zhu, 2010). Very good correlations between UFPs and EC (0.70 and 0.84) have been reported in walking studies (Kaur et al., 2005a; McCreanor et al., 2007).

The correlation between UFP concentrations and those of NO<sub>x</sub> vary extensively from –0.33 to 0.90, and no clear relationship with transport mode is apparent (Westerdahl et al., 2005; McCreanor et al., 2007; Zhu et al., 2008; Laumbach et al., 2010). The relationship with CO concentrations is similarly variable; –0.16 to 0.70 (Kaur et al., 2005a,b; Westerdahl et al., 2005; McCreanor et al., 2007; Zhu et al., 2008; Laumbach et al., 2010). The specifics of the measurement location in terms of local emission sources are likely to explain the observed variation, and it is important to consider that in-transit measurements of particle and gaseous pollutants may exhibit poor temporal correlation due to the varying emission strength of proximate vehicles (Morawska et al., 2008; Zhu et al., 2008).

In summary, the relationship between in-transit UFP concentrations and those of other pollutants is generally inconsistent. Mode, location and environmental factors may all contribute to the observed variability, and the results gathered here from the limited pool of available studies require further validation in order to develop a more complete understanding of the associations. Currently, there is no data to support prediction of UFP concentrations from those of other pollutants, and such an approach is likely to be insufficient.

## 7. Relationship with fixed site monitors

Since the 1970s (Ott and Eliassen, 1973; Cortese and Spengler, 1976), numerous studies have investigated the ability of fixed site pollutant monitoring stations to estimate personal and commuter exposure. Generally, the ability of fixed site monitors to represent the substantial spatial and temporal variability of in-transit exposures has been sub-optimal, and carries with it numerous attendant limitations, the most important of which is underestimation of exposure (Kaur et al., 2007). UFPs are not a regulated pollutant, and are therefore not routinely monitored outside of research studies. Some investigators have assessed the association between fixed

site UFP concentrations and those measured concurrently in-transit.

Aarnio et al. (2005) reported good correlation ( $R^2 = 0.59$ ) between UFP concentrations in subway stations and those measured at an urban background site, while Seaton et al. (2005) found that the ratio of UFP concentrations measured on London Underground platforms to those above ground ranged from 0.38 to 0.68. These results are likely to reflect the absence of strong local UFP sources in subways (Aarnio et al., 2005). For above ground transport microenvironments, however, this is unlikely to be the case. Vinzents et al. (2005) reported a moderate correlation ( $r = 0.49$ ) between UFP measurements performed at a fixed roadside location and those measured while cycling, but found that the only significant variables in a linear mixed effects model to predict cyclist exposure were temperature and concentrations of CO ( $R^2 = 0.60$ ) and NO<sub>2</sub> ( $R^2 = 0.74$ ) measured at urban background and roadside stations, respectively. Asmi et al. (2009) found that the ratio of UFP concentration in the driver's cabin of buses to that measured at an urban background site varied from 1.2 to 6.9 and was dependent on the age of the bus, time of day and route. Zuurbier et al. (2010) systematically evaluated the relationship between bus, car and bicycle UFP exposures and urban background concentrations in Arnhem, the Netherlands. They reported median mode to background ratios of 1.6 (diesel car, electric bus) to 2.5 (diesel bus) and correlations between 0.01 (diesel bus) and 0.87 (bicycle on low-traffic route).

The limited data available to-date indicates that fixed-site monitors may offer some ability to estimate UFP exposure of commuters in areas less affected by vehicle emissions, such as those using subways or low-traffic bike paths. However, depending on location, such persons are likely to constitute only a minor proportion of the commuting population. In the absence of widespread UFP monitoring networks, the utility of routinely monitored particle and gaseous pollutants or individual UFP monitors to represent in-transit UFP exposure appears significantly constrained (Krause and Mardaljevic, 2005; Vinzents et al., 2005).

## 8. Health effects of in-transit UFP exposure

Studies of health effects due to commuter UFP exposure are summarised in Table S9 in the Supplementary Information file.

### 8.1. Healthy individuals

Nystrom et al. (2010) showed that while a cellular response in the airway epithelium was not elicited, minor biological responses such as increased systemic markers of inflammation and signs of lower airway irritation were observed in 20 healthy individuals exposed to subway air (mean UFP concentration  $1.1 \times 10^4$  particles cm<sup>-3</sup>) for 2 h while alternating between exercising on a bicycle ergometer and resting. However, road tunnel air (median UFP concentration  $1.1 \times 10^5$  particles cm<sup>-3</sup>) elicited an inflammatory response in the lower airways and elevated levels of T-lymphocytes and alveolar macrophages in bronchoalveolar lavages from 16 healthy individuals who followed the same protocol (Larsson et al., 2007). The particle mass concentrations that subjects were exposed to in the two above studies were similar, while UFP and NO<sub>x</sub> concentrations were an order of magnitude higher in the road tunnel study than in the subway study due to the presence of proximate vehicle emissions. Although it is not possible to ascribe the disparity in the results of the two studies to differences in UFP concentration alone, the results are suggestive of a causative role for UFP and NO<sub>x</sub> in airway inflammation observed following exposure to vehicle emissions.

Thirty-eight healthy volunteers who cycled parallel to a major traffic corridor for 20 min (mean UFP concentration  $2.9 \times 10^4$  particles  $\text{cm}^{-3}$ ) experienced a minor increase in blood inflammatory cell distribution compared to cycling in a clean air environment, although the role of UFPs as distinct from  $\text{PM}_{2.5}$  was not clear (Jacobs et al., 2010). UFP and EC exposure in 12 healthy non-smoking individuals cycling in traffic (mean UFP concentration  $2.8$  to  $4.1 \times 10^4$  particles  $\text{cm}^{-3}$ ) for 1 h was weakly associated with acute effects; decreased lung function and increased exhaled NO (as a marker of airway inflammation) were observed 6 h post-exposure (Strak et al., 2010). Oxidative DNA damage observed in 15 healthy subjects was positively correlated with cumulative UFP exposure, to which 1.5 h of cycling during rush hours (mean UFP concentration  $3.2 \times 10^4$  particles  $\text{cm}^{-3}$ ) contributed substantially and resulted in greater damage compared to indoor cycling on an ergometer (Vinzents et al., 2005). Concentrations of other pollutants ( $\text{PM}_{10}$ ,  $\text{NO}_x$ , CO) measured at fixed-sites were not associated with oxidative DNA damage.

UFP exposure resulted in modest effects among 34 healthy subjects that commuted by automobile, bus or bicycle for 2 h (median UFP concentration  $2.7$  to  $4.4 \times 10^4$  particles  $\text{cm}^{-3}$ ); peak expiratory flow decreased slightly and airway resistance increased immediately following exposure, and a significant increase in exhaled NO was observed 6 h post-exposure for automobile and bus commuters, but not cyclists (Zuurbier et al., 2011).

As the respiratory minute ventilation of cyclists is 2–4.5 times that of automobile and bus passengers (Zuurbier et al., 2009; Int Panis et al., 2010), the potential dose of inhaled UFPs received during active transport may be significantly higher than that in non-active modes, and recent health effects studies have already begun to adopt a more dose-oriented approach to reflect this (Zuurbier et al., 2011).

## 8.2. Health-compromised individuals

### 8.2.1. Asthmatics

Asthma exacerbations can be triggered due to oxidative stress and inflammation caused by UFPs in the lungs of susceptible individuals (Weichenthal et al., 2007). Reductions in lung function and increased daily symptoms in asthmatics and COPD patients attributable to elevated UFP concentrations have been observed in epidemiologic studies, with more immediate effects seen first in the respiratory system, and a delayed response of cardiovascular effects (Wichmann and Peters, 2000; Ibalid-Mulli et al., 2002).

Consistent asymptomatic reductions in lung function ( $\text{FEV}_1$ , FVC) and increases in both inflammatory biomarkers and airway acidification were observed in 60 persons with mild or moderate asthma who walked for 2 h along a busy London street affected by diesel exhaust (median UFP concentration  $6.4 \times 10^4$  particles  $\text{cm}^{-3}$ ) (McCreanor et al., 2007). The effects were more frequently associated with UFP and EC concentrations than those of  $\text{PM}_{2.5}$  and  $\text{NO}_2$ . Significantly reduced respiratory effects were observed when subjects walked along a route less affected by traffic emissions (median UFP concentration  $1.8 \times 10^4$  particles  $\text{cm}^{-3}$ ).

Fourteen mild asthmatics exposed to road tunnel air (median UFP concentration  $2.3 \times 10^5$  particles  $\text{cm}^{-3}$ ) for 2 h while alternating between exercising on a bicycle ergometer and resting experienced no changes in bronchial responsiveness and most lung function parameters, although peak expiratory flow decreased, and minor indications of inflammation were measured in nasal lavages, but not blood samples (Larsson et al., 2010).

### 8.2.2. Diabetics

Exposure to pollutants (median UFP concentration  $4.3 \times 10^4$  particles  $\text{cm}^{-3}$ ) during 1.5–1.8 h automobile highway

trips made by 21 type 2 diabetics was shown to elicit a decrease in high-frequency heart rate variability the day after exposure, which was more associated with the interquartile range of UFP concentration compared to those of  $\text{PM}_{2.5}$ ,  $\text{NO}_2$  and CO, albeit not significantly (Laumbach et al., 2010). An increased low frequency to high frequency heart-rate variability ratio was observed immediately post-exposure that was not consistent with other observations, although confounding effects not present in the aforementioned finding may have influenced this result.

### 8.2.3. Elderly persons

Nineteen elderly subjects that were exposed to unfiltered and filtered air during 2 h automobile trips on Los Angeles freeways (mean unfiltered UFP concentration  $0.78$  to  $1.1 \times 10^5$  particles  $\text{cm}^{-3}$ ) experienced a 20% decrease in the incidence of atrial ectopic heartbeats and 30% decrease in cardiopulmonary stress biomarkers under the filtered compared to the unfiltered condition (Cascio et al., 2009; Hinds, 2010). Other measured parameters (lung function, indicators of inflammation, blood pressure) did not vary significantly between the two conditions. The observed atrial arrhythmia was ascribed to increased intra-atrial pressure, and was associated with UFP concentrations rather than gases or particle mass (Cascio et al., 2009; Hinds et al., 2010). The significance of such events is related to their role in causing more sustained arrhythmias.

## 8.3. Summary

Commute-time exposure to traffic and attendant pollutant emissions, noise and stress has been associated with increased risk of serious adverse health effects such as myocardial infarction (Peters et al., 2004). The specific role of UFPs as a causative agent of such effects is not clear, and the findings of the limited number of health effects studies addressing commuter exposure to vehicle emissions are mixed. However, some initial trends are emerging. While it is inherently difficult to separate the effects of UFPs from those other pollutants within the real-world exposure scenarios employed by the studies described above, the observed health effects were generally associated most strongly with UFP concentrations. Furthermore, the use of filtered air exposure scenarios in the Los Angeles freeway study (Cascio et al., 2009; Hinds, 2010) reduced particle concentration by >95% compared to the unfiltered condition but did not affect the level of gaseous pollutants, yet there was a marked difference in the cardiac effects observed between the two scenarios. The effects observed by McCreanor et al. (2007) were greater in those with moderate compared to mild asthma, and the degree to which this is true of other susceptible groups (i.e. increasing effects with increasing disease severity) is unclear. The 10 commuter health effects studies performed to-date have yielded valuable information, however, it is clear that further studies are required in order to better elucidate the role of UFPs.

## 9. Modelling exposure

### 9.1. Approaches employed to-date

The ability to accurately model in-transit UFP exposure concentrations has numerous attractive applications in urban planning, transport and policy development. The majority of published studies that developed models employed a multivariate regression approach that incorporated meteorologic, traffic or other pollutants as independent variables (Krause and Mardaljevic, 2005; Vinzents et al., 2005; Weichenthal et al., 2008; Boogaard et al., 2009; Kaur and Nieuwenhuijsen, 2009). Given

the potential for variability in the strength of associations between the independent variables and measured UFP concentrations discussed in sections 5, the external validity of these models is unknown. However, the models were of the explanatory type, and were developed in order to assess the effect of various parameters on UFP concentration measured in a specific location. Their ability to predict exposure concentrations varied from fair ( $R^2 = 0.35$ ) to very good ( $R^2 = 0.74$ ). The influence of mode-dependent parameters like ventilation were either included in a qualitative sense (e.g. ventilation setting or window position) or not included at all. This limitation was raised by both Briggs et al. (2008) and Weichenthal et al. (2008).

Several recent studies (Pui et al., 2008; Xu and Zhu, 2009; Knibbs et al., 2010) have sought to overcome the limitations described above by adopting a more mechanistic, mass-balance modelling approach for automobiles. This has been based on measurements of the effects of cabin ventilation, filtration, particle penetration or deposition on in-cabin concentrations (Qi et al., 2008; Gong et al., 2009; Knibbs et al., 2009a; Xu et al., 2010). These studies have generally shown very good results when validated with experimental data. The main limitation of such approaches is that they require the input of an initial on-road or in-cabin UFP concentration. Therefore, there is a clear need to couple models capable of predicting outdoor or on-road concentrations with those focussed on predicting what proportion of these concentrations reach occupants, and how particle dynamics will affect concentrations through time. Moreover, further refinement of models for predicting exposure in active transport modes will be of significant utility. In summary, there is both substantial need and scope for development of models capable of accurate prediction of UFP exposure concentrations in-transit.

## 9.2. Spatial and temporal aspects of exposure

Efforts to improve understanding of the spatial and temporal nature of UFP exposures during transit have benefited greatly from the use of Global Positioning Systems (GPS) and Geographic Information Systems (GIS), usually at the measurement and analytical stages, respectively. Gulliver and Briggs (2005) described the development and use of a GIS-based model for predicting exposure to  $PM_{10}$  (particles  $< 10 \mu m$ ) during transit, however, the application of spatial technologies to UFPs has to-date been limited to a handful of in-transit studies (Hvidberg, 2006; Thai et al., 2008; Berghmans et al., 2009; Boogaard et al., 2009; Pattinson, 2009; Int Panis et al., 2010). Synchronised video recordings have been included in some studies (Kaur et al., 2006; Berghmans et al., 2009), which affords an additional perspective from which analyses can be performed.

Given the good level of spatial data quality obtainable from even the more basic mobile telephones at present, the integration of such data into exposure studies will assist data interpretation and help to form a more complete and accurate assessment of pollutant exposure and dose for large study populations (Jerrett, 2010). The appropriateness and capability of mobile telephones to record spatial data and photographs during commuting has already been established by Pooley et al. (2010), and Pattinson (2009) collected such data in addition to UFP measurements when commuting by bicycle.

Land use regression (LUR) is an application of GIS that is gaining momentum as a tool with which to predict exposure to a variety of pollutants (see Hoek et al., 2008). The utility of LUR techniques to predict UFP concentrations and spatial variability is not well-established due to absence of extensive UFP monitoring networks; other (mainly gaseous) pollutants have been the focus of most work performed to-date. However, a recent study has reported

reasonable performance of LUR when applied to UFP concentrations in Amsterdam, and comparable predictive utility was observed between the LUR model for UFPs and those for other pollutants (Hoek et al., 2011). LUR is an emerging technology that will increasingly find applications in prediction of personal exposure to a range of pollutants, albeit with an attendant need for validation based on measurements (Hoek et al., 2008). This highlights the need for high-quality databases of concomitant in-transit UFP and spatial measurements.

## 10. Further research needs

### 10.1. In-transit contribution to daily exposure

The significance of in-transit UFP exposure is highly dependent on personal, demographic and occupational context. UFP concentrations encountered on the commute to and from work will exert much greater influence on the total daily exposure of a non-smoking office worker than a smoker or someone who experiences high occupational exposure. Likewise, the health effects of the same exposure on an adult and child are likely to vary. Without better understanding of the characteristics of 24 h UFP exposure for numerous demographic groups, knowledge of in-transit exposure alone is of reduced utility. However, it is useful to be able to determine, for a given location, the transport mode in which highest concentrations occur and the factors that determine this. Such information has numerous valuable planning and policy applications.

A handful of studies have estimated the influence of measured in-automobile UFP concentrations on total exposure. Two were based on Los Angeles residents (Zhu et al., 2007; Fruin et al., 2008), and their estimates ranged from 10 to 50% and 33 to 45%, respectively. Wallace and Ott (2011) measured UFP concentrations in a wide range of microenvironments in two US cities and estimated the in-automobile contribution to total exposure to be 17%, which they attributed to the relatively low density of traffic and diesel trucks on the roadways they measured compared to LA. In all cases, the time spent in automobiles was assumed to be about 90 min per day. The applicability of the estimates reached by these studies to other regions is unknown, but they have established a range within which automobile commutes in urban areas may be expected to contribute to daily UFP exposure. These estimates have flagged this topic as one requiring further investigation, preferably including several transport modes.

It is important to consider the distinction between UFP concentration and exposure (Krausse and Mardaljevic, 2005). A high concentration experienced for a brief duration can result in a lower exposure than a low concentration for a longer period. This underscores the need for both accurate time-activity pattern data across broad demographic groups and representative UFP measurements within the various microenvironments in which time is spent. Until more expansive UFP exposure studies that follow large groups of people of varying time-activity patterns are completed, the ability to discern the range of commute-time's specific contribution to total exposure is constrained.

### 10.2. High exposure professions

The magnitude of UFP exposures incurred by people whose occupation requires them to spend extended period in-transit is poorly understood. Professional drivers, bicycle couriers, police officers and other groups whose work day is constituted by long periods in transport microenvironments may all be at risk of substantially elevated exposure compared to the general population. Riediker et al. (2004) reported the negative health effects of



in-vehicle PM<sub>2.5</sub> exposure on young and healthy police officers during 9 h shifts in patrol cars. Similar studies focussed towards UFPs are required.

### 10.3. Exposure-health effects link

Various acute human health effects caused by UFP exposures have been investigated in controlled exposure studies using a range of subject groups. However, their relevance to in-transit exposures is unclear. There have been precious few studies that measured the effects of in-transit exposures on health end points, and these were described in section 8. There is a significant need for further studies in this area, as they will serve to bolster the link between exposure and health effects, and this will have implications across policy, planning and public health arenas (de Nazelle and Nieuwenhuijsen, 2010). Furthermore, given the substantial variability in minute ventilation between occupants of different modes (Zuurbier et al., 2009; Int Panis et al., 2010), the transition from an exposure to dose-oriented approach is likely to yield data of greater relevance to studies of health effects.

### 10.4. Data from the developing world

A striking feature of the English language literature we searched is the almost complete absence of studies performed in developing regions; with the exception of only the cycling study performed in Bogota, Columbia by Fanara (2003) and cited by Kaur et al. (2007), no other studies from developing countries were identified. This shortcoming is compounded by the generally poor air quality experienced in these regions (Han and Naeher, 2006) and their large populations and urban density. The effect of this combination of factors is that very high UFP exposures are likely to occur for large numbers of people, but the magnitude of such exposures is unknown. Studies of commuter exposure to particulate mass (RSP, PM<sub>10</sub>) performed in Delhi and Hanoi have reported exceptionally high concentrations (Saksena et al., 2007, 2008). Moreover, in addition to walking, the most popular modes of transport, such as bicycles, scooters, motorcycles and 3-wheelers (tuk-tuks, auto-rickshaw etc), are unlikely to afford significant protection from the emissions of proximate traffic, which can include a substantial proportion of high emitting two-stroke vehicles. There is a clear need to redress the scarcity of research in this area.

### 10.5. Other needs

Major needs in future in-transit UFP exposure studies have been outlined above, and numerous other aspects requiring additional research have been suggested throughout this review. Further investigation of the variability inherent in the determinants of exposure discussed in section 5 is required to permit better appreciation of their effects. There is also an obvious need for improved modelling techniques, incorporating GIS, and for further comprehensive assessments of the health risk-benefit balance for active transport modes (de Nazelle et al., 2009; de Hartog et al., 2010).

## 11. Conclusions

In our analysis of 47 studies comprising approximately 3000 trips undertaken in 6 transport modes, we found that highest trip-weighted mean concentration occurred in automobile cabins during tunnel travel ( $3.0 \times 10^5$  particles cm<sup>-3</sup>), and the lowest whilst cycling ( $3.4 \times 10^4$  particles cm<sup>-3</sup>). Mean concentrations in bus, automobile (non-tunnel travel), rail, and walk modes were generally comparable. However, UFP exposure (and dose) during

time spent in-transit is strongly dependent on a range of mode-specific and more general determinants, including, but not limited to, the effects of: meteorology, traffic parameters, cabin ventilation, filtration, deposition, UFP penetration, fuel type, exhaust treatment technologies, respiratory minute ventilation, route and microscale phenomena. Therefore, direct comparison of concentrations measured in different modes highlights general trends, but should not be extrapolated without detailed consideration of the above factors. Characterising the variability in the effects of these determinants will be an important aspect of future work.

There is preliminary evidence to suggest that time spent in-transit can contribute substantially to total daily exposure, and future studies require comprehensive assessment of 24 h UFP exposures across a broad demographic spectrum. Moreover, the range and variability of acute health effect associated with in-transit exposures are not well understood, and further studies are required to supplement the findings of the limited number performed to-date.

Transport is a ubiquitous component of life, and initial evidence suggests that UFP exposures incurred during this time can contribute substantially to daily exposure and be associated with adverse health effects in susceptible and healthy persons. Further research to better define this link is therefore well-justified, and will be of considerable benefit to urban planning, policy development and public health.

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## Appendix. Supplementary information

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.atmosenv.2011.02.065.

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## Study of ultrafine particles near a major highway with heavy-duty diesel traffic

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### Abstract

Motor vehicle emissions usually constitute the most significant source of ultrafine particles (diameter  $<0.1\ \mu\text{m}$ ) in an urban environment. Zhu et al. (J. Air Waste Manage. Assoc., 2002, accepted for publication) conducted systematic measurements of the concentration and size distribution of ultrafine particles in the vicinity of a highway dominated by gasoline vehicle. The present study compares these previous measurements with those made on Interstate 710 freeway in Los Angeles. The 710 freeway was selected because more than 25% of the vehicles are heavy-duty diesel trucks. Particle number concentration and size distribution in the size range from 6 to 220 nm were measured by a condensation particle counter and a scanning mobility particle sizer, respectively. Measurements were taken at 17, 20, 30, 90, 150, and 300 m downwind and 200 m upwind from the center of the freeway. At each sampling location, concentrations of carbon monoxide (CO) and black carbon (BC) were also measured by a Dasibi CO monitor and an Aethalometer, respectively. The range of average concentration of CO, BC and total particle number concentration at 17 m was 1.9–2.6 ppm, 20.3–24.8  $\mu\text{g}/\text{m}^3$ ,  $1.8 \times 10^5$ – $3.5 \times 10^5/\text{cm}^3$ , respectively. Relative concentration of CO, BC and particle number decreased exponentially and tracked each other well as one moves away from the freeway. Both atmospheric dispersion and coagulation appears to contribute to the rapid decrease in particle number concentration and change in particle size distribution with increasing distance from the freeway. Average traffic flow during the sampling periods was 12,180 vehicles/h with more than 25% of vehicles being heavy-duty diesel trucks. Ultrafine particle number concentration measured at 300 m downwind from the freeway was indistinguishable from upwind background concentration. These data may be used to estimate exposure to ultrafine particles in the vicinity of major highways.

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### 1. Introduction

Epidemiological data from air pollution studies have shown a consistent relationship between increases in particulate matter (PM) exposure and contemporary

increases in mortality and morbidity (Schwartz, 1991; Dockery et al., 1993; Pope et al., 1995; Vedal, 1997). However, the underlying biological causes of the health effects of PM exposure and the correct measurement metric are unclear. For example, it is not clear whether the mass concentration (Osunsanya et al., 2001) or the number concentration (Peters et al., 1997; Penttinen et al., 2001) is most important in causing these adverse

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PM health effects. Currently, there are several hypotheses used to explain the association of PM and observed adverse health effect. One argues that particle surface contaminants, such as transition metals, contribute towards ill health (Fubini et al., 1995; Gilmour et al., 1996), wherein the ultrafine particles are thought to act as vehicles for those contaminants, initiating local lung damage when the particles deposit on the epithelial surfaces. Another hypothesis is that the physical characteristics (e.g. number, size, shape, aggregation properties) are important in producing health effects (Bérubé et al., 1999). Particle shape and size are critical factor controlling where the inhaled particles deposit in the various regions of human respiratory system by the complex action of aerosol deposition mechanisms (Hinds, 1999).

Recent toxicological studies have concluded that ultrafine particles (diameter <100 nm) are more toxic than larger particles with the same chemical composition and at the same mass concentration (Ferin et al., 1990; Oberdörster, 1996, 2001; Donaldson et al., 1998, 2001; Churg et al., 1999; Brown et al., 2000). Currently, however, only the mass concentration of PM <10 µm in aerodynamic diameter (PM<sub>10</sub>) and <2.5 µm (PM<sub>2.5</sub>) are regulated. Information about ultrafine particles is usually not available. In fact, even though ultrafine particles represent over 80% of particles in terms of number concentration in an urban environment (Morawska et al., 1998a,b), the less numerous but much heavier particles of the accumulation (0.1–2 µm) and coarse (2.5–10 µm) modes dominate mass concentration measurements. Thus, number concentration, together with the size distribution of ultrafine particles, is needed to better assess ambient air quality and its potential health effects.

Emission inventories suggest that motor vehicles are the primary direct emission sources of fine and ultrafine particles to the atmosphere in urban areas (Schauer et al., 1996; Shi et al., 1999; Hitchins et al., 2000). Although traffic-related air pollution in urban environments has been of increasing concern, most studies have focused on gaseous pollutants, total mass concentration, or chemical composition of particulate pollutants (Kuhler et al., 1994; Clairborn et al., 1995; Williams and McCrae, 1995; Janssen et al., 1997; Roorda-Knappe et al., 1998a,b; Wrobel et al., 2000). Booker (1997) found that particle number concentration was strongly correlated with vehicle traffic while PM<sub>10</sub> was essentially uncorrelated with traffic. Since the majority of particle number from vehicle exhaust are in the size range 20–130 nm for diesel engines (Morawska et al., 1998a,b) and 20–60 nm for gasoline engines (Ristovski et al., 1998), it is important and necessary to quantify ultrafine particle emission levels, and to determine ultrafine particle behavior after emission as they are transported away from the emission source—busy roads and freeways.

Morawska et al. (1999) measured the horizontal and vertical profiles of submicrometer particulates (16–626 nm) near a major arterial route in the urban area of Brisbane, Australia. They found, with the exception of measurements in close proximity to the road (about 15 m), that the horizontal ground-level profile measurements did not show statistically significant differences in fine particle number concentration for up to 200 m distances away from the road. Hitchins et al. (2000) examined the particle size distribution and concentration in the size range from 15 nm to 20 µm at distances from a road ranging from 15 to 375 m at two sites in Australia. They conducted measurements under different wind conditions and found that when the wind is blowing directly from the road, the concentration of the fine and ultrafine particles decayed to about half of their maximum at a distance of 100–150 m from the road. Shi et al. (1999) measured ultrafine particle number concentrations and size distributions at a busy roadside and at nearby urban background sites in Birmingham, United Kingdom. They observed a faster decline of particle number concentration than mass concentration. In a recent study, Shi et al. (2001) reported that the fraction of particles <10 nm represents more than about 40% of the total particle number concentrations at 4 and 25 m from the roadside curb.

While there have been recent studies of ultrafine particles from traffic in other countries, except for Zhu et al. (2002), no comparable work has been done in the Los Angeles basin, a home to more than 15 million individuals and 10 million vehicles contributing to daily traffic. Previous studies have shown that meteorological conditions may affect substantially the characteristics of PM emitted from vehicles. Kittelson et al. (2001) found in their on-road PM measurements that the concentration of particles in the nuclei mode increases by nearly a factor of 10 as the (air) temperature is reduced from 25°C to 15°C. This observation suggests that there could be significant differences in the tendency to form semi-volatile nanoparticles between, for example, northern Europe and Southern California.

Zhu et al. (2002) conducted a systematic ultrafine particle study near one of the busiest freeways in the Los Angeles basin, Interstate 405. Traffic on that freeway was dominated by gasoline-powered cars and light trucks, with <5% of vehicles being heavy-duty diesel trucks. In the US, spark ignition vehicles usually account for most of the vehicles operating on highways. However, since diesel vehicles emit more PM on a fleet averaged, gram-per-vehicle mile mass basis (Kittelson et al., 2001), and that diesel engine exhaust has been proposed as carcinogen in animals and probably carcinogenic for humans (IARC, 1989), it is necessary and timely to conduct a comprehensive study of ultrafine particles in the vicinity of a diesel vehicle dominated freeway. Thus, the aim of the present paper is

to systematically evaluate ultrafine particles in the vicinity of the 710 freeway in the Los Angeles basin, a freeway where more than 25% of vehicles are heavy-duty diesel trucks. Particle number concentration and size distribution in the size range from 6 to 220 nm are measured along with CO and black carbon (BC) as a function of distances upwind and downwind the 710 freeway. The results from the current study are compared to these by Zhu et al. (2002) which were obtained near the 405 freeway.

## 2. Experimental

### 2.1. Description of sampling site

This study was conducted in the City of Downey along Southern Avenue between 30 August and 27 October 2001. The location was chosen for its proximity to the freeway and the lack of other nearby ultrafine particle emission sources. Southern Avenue is located perpendicular to Interstate 710 Freeway and Garfield Avenue near the Los Amigos Country Club. Freeway 710 runs generally north and south near the sampling site and parallels the Los Angeles River.

This location is ideal for this study for several reasons. First, there are no other major roadways near the sampling sites along Southern Avenue. Second, businesses along Southern Avenue generally have large open land areas with little activities during the day. Thus, there is minimal local traffic influence at the sampling locations. Third, the freeway is at the same elevation as Southern Avenue. The only separation between the freeway and Southern Avenue is a metal chain link fence along the freeway. This allowed measurements as close as 3 m from the edge of the freeway. Fourth, a nearby residential area approximately 200 m upwind from the freeway was easily accessible for sampling.

During the sampling period, a fairly consistent eastward wind developed each day starting at approximately 11:00 AM. This wind carried the freeway vehicular emissions directly to the sampling location. The 710 freeway has eight lanes, four north bound and four south bound. It is approximately 26 m wide including a 1-m-wide median strip. Measurement site locations for this study were designated by their distance from the center of the median strip. Thus, the distance from each sampling location to the nearest traffic lane is 13 m less than the indicated distance.

Freeway 710 is a major truck route in Southern California with a large percent of the traffic consisting of heavy-duty diesel trucks. During the sampling period, traffic density ranged from 180 to 230 vehicles/min passing the sampling site, total for both directions, with approximately 25% of the vehicles being heavy diesel trucks.

### 2.2. Sampling and instrumentation

Wind speed and direction were measured at a fixed site 6 m above the ground level 20 m downwind of 710 freeway, which also served as a particle number concentration control site. Wind data were averaged over 1 min intervals and logged into a computerized weather station (Wizard III, Weather Systems Company, San Jose, CA). Throughout each measurement period, the traffic strength on the freeway, defined as number of vehicles passing per minute, was continuously monitored by a video recorder (camcorder), which captures all eight lanes of the freeway. After each sampling session, the videotapes were replayed and traffic density counted manually. Three 1-min samples were randomly selected from each 10-min interval. Cars, light trucks, and heavy-duty trucks were counted separately to estimate the traffic density by type of vehicle.

Particle number concentration and size distribution in the size range from 6 to 220 nm were measured by a condensation particle counter (CPC 3022A; TSI Inc., St. Paul, MN) and a scanning mobility particle sizer (SMPS 3936, TSI Inc., St. Paul, MN). The sampling flow rate of the SMPS was adjusted to 1.5 lpm in order to measure particles as low as 6 nm as well as to minimize the diffusion losses of ultrafine particles during sampling. Flexible, conductive tubing (Part 3001940, TSI Inc., St. Paul, MN) was used for sampling to avoid particle losses due to electrostatic forces. The sizing accuracy of the SMPS was verified in the laboratory by means of monodisperse polystyrene latex spheres (PSL, Polysciences Inc., Warrington, PA). Data reduction and analysis of the SMPS output was done by the Aerosol Instrument Manager software (version 4.0, TSI Inc., St. Paul, MN). Measurements were taken at 17, 20, 30, 90, 150, and 300 m downwind and 200 m upwind from the center of the freeway 710. At each location, three size distribution samples were taken in sequence with the SMPS. Scanning time for each was 180 s.

In addition to size distribution and the total number concentration, the concentrations of BC and carbon monoxide (CO), were monitored simultaneously at each sampling location. Before each measurement session, all instruments were time synchronized. Data were averaged after collection over the time periods corresponding to the scanning intervals of the SMPS. A Dual Beam Aethalometer (Model AE-20, Andersen Model RTAA-900, Andersen Instruments Inc., Smyrna, GA) was used to measure the BC concentrations every 5 min. Concentrations of CO were measured by a near-continuous CO monitor (Dasibi Model 3008, Environmental Corp., Glendale, CA) every minute. The CO monitor was calibrated by means of standard CO gas (RAE systems Inc., Sunnyvale, CA) in the laboratory and automatically zeroed each time the power was turned on.

Electric power for the control site CPC and Weather Station was obtained by an extension cord to a nearby office. Electric power for other sampling instruments at the sampling locations was supplied by a 1.2 kW gasoline-powered portable power generator (Model EU 1000i, Honda Motor Co., LTD., Tokyo, Japan). The generator was placed approximately 50 m downwind of each sampling location. Both total particle number and CO concentrations were measured at the control site with the generator turned on and with it turned off. No detectable difference was observed.

Table 1 gives the sampling dates and times and summarizes the instruments that were used on each date. The weather station and control CPC were placed at the 20 m downwind control site and sampled throughout the sampling period each day. All other applicable instruments were moved together and sampled simultaneously at each sampling location. It takes about 10 min to complete sampling at each location and 120 min to complete a set, all six locations. Three to four sets were performed on each sampling date.

### 3. Results and discussion

The results presented below include measurements of total particle number concentrations by a control CPC, wind velocity by a Weather Wizard III, both positioned at a fixed location 20 m downwind of the freeway; and CO, BC concentration, and ultrafine particles size distributions upwind and at six downwind distances from freeway 710.

#### 3.1. Wind effects

Changes in wind conditions have been reported to modify dramatically the pattern of total particle number concentration versus distances from a major road (Hitchins et al., 2000). Consistency in wind speed and direction allows data from different days to be averaged together (Zhu et al., 2002). Wind speed and direction were measured, averaged and logged over every 1-min interval throughout each sampling period. One hundred

wind data points were randomly selected out of more than 5000 observations from all the sampling dates and plotted in Fig. 1. The orientation of freeway 710 and the sampling road, Southern Avenue, are also shown in the Fig. 1. The Weather Wizard III instrument recorded wind direction at a 22.5° interval (e.g. 11.25° on either side of N, NNE, etc.) and wind speed at 0.4 or 0.5 m/s intervals. In the figure, duplicate observations were spread out slightly in both directions to better illustrate how strong the wind was and how often the wind came from certain directions. Based on all 5000 observations, the percent of sampling time that the wind came from each 22.5° segment is also shown in Fig. 1. As shown in Fig. 1, about 80% of the time, the wind was coming directly from the freeway towards the sampling road with a speed < 3 m/s. The consistency of observed wind direction and speed is a result of a generally low synoptic wind velocities and a consistent sea breeze in the sampling area.

In this study, we found that not only wind direction, but also wind speed, played an important role in determining the characteristics of ultrafine particles near the 710 freeway, similar to the observations made by Zhu et al. (2002) near the 405 freeway. However the pattern of total particle number concentrations as a function of wind speed is somewhat different for the two studies. Fig. 2 shows total particle number concentrations measured by the control CPC, located 20 m downwind of the 710 freeway versus wind speed. Averaged data for the 405 freeway from Zhu et al. (2002) are also plotted for comparison. The CPC was programmed to archive averaged total particle number concentrations at 1-min interval in synchronization with the averaging time of the meteorological data. Only wind data within  $\pm 22.5^\circ$  of normal to the freeway was used in this figure which accounts for more than 60% of the total observations. The difference between the absolute value of total particle number concentration is due in part to the difference in the sampling distance. The control CPC was located 20 m downwind from the 710 freeway but 30 m from the 405 freeway. Assuming the fitted exponential decay characteristics of ultrafine particles holds right to the edge of the freeway, it is thus

Table 1  
Sampling dates, time and instruments used

| Date     | Time        | Weather Wizard III | Control CPC | SMPS | CO monitor | Aethalometer |
|----------|-------------|--------------------|-------------|------|------------|--------------|
| 08/30/01 | 10:00–15:30 | ×                  | ×           | ×    | ×          | ×            |
| 09/05/01 | 10:30–16:00 | ×                  | ×           | ×    |            |              |
| 09/21/01 | 10:00–15:00 | ×                  | ×           | ×    | ×          | ×            |
| 09/25/01 | 10:30–16:00 | ×                  | ×           | ×    | ×          | ×            |
| 10/05/01 | 10:30–16:00 | ×                  | ×           | ×    | ×          | ×            |
| 10/24/01 | 10:00–15:30 | ×                  | ×           |      | ×          | ×            |
| 10/30/01 | 10:00–15:30 | ×                  | ×           | ×    |            |              |

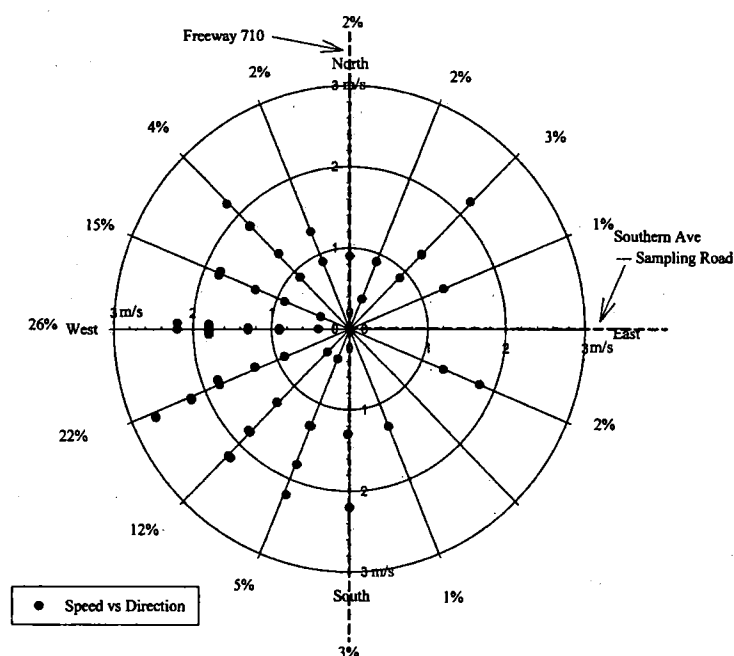


Fig. 1. Wind direction and speed at sampling site.

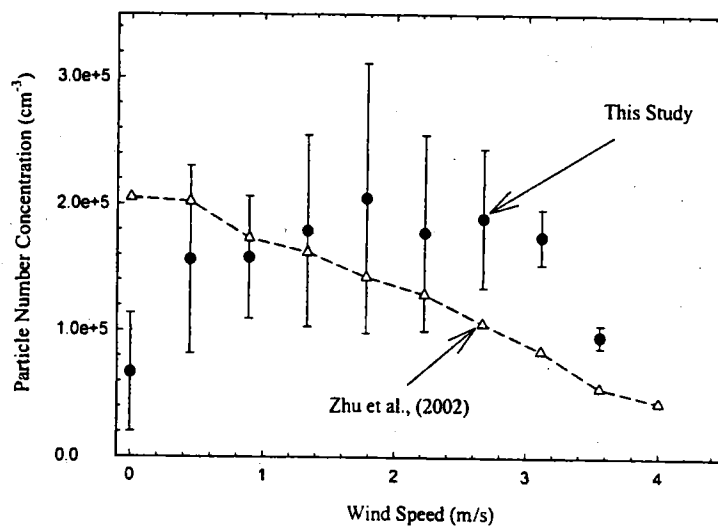


Fig. 2. Total particle number concentration measured by CPC located at 20 m downwind from freeway 710 versus wind speed. Bars indicate one standard deviation.

not surprising, as discussed below, that the CPC will read a greater total particle number concentration at 20 m in the present study than at 30 m in that by Zhu et al. (2002), given similar traffic load on both freeways. However, the relative particle number concentration as function of wind speed are somewhat different in these two studies. The relative particle number concentration

decreased as the wind speed increased near the 405 freeway. In contrast, particle number concentration in the 710 freeway first increases, reaches a maximum around 1.5 m/s, and then decreases. There is no obvious explanation for the observed difference. In both studies, data showed large error bars, and the data of low wind speed ( $< 1$  m/s) were very limited. In addition, the 405

freeway is elevated approximately 4.5 m above the surrounding terrain, while, the 710 freeway is at ground level, the same as the sampling location. Lower speed wind would be expected to cause less atmospheric dilution, and thus lead to greater particle number concentrations, as Zhu et al. (2002) reported. However, at extremely low wind speeds, it would take a considerably longer time for the wind to carry particles to the sampling port of the CPC, which gives ultrafine particles more time to coagulate with either themselves or with larger particles, a phenomenon that would decrease the total particle number concentration. This may partially explain the observed “n” shape curve in the current study.

### 3.2. Traffic effects

The portion of freeway 710 passing through the City of Downey is a major truck shipping route. The average traffic volume per hour during the measurement period was: 8730 cars, 870 light trucks, 2580 heavy trucks, and 12180 total vehicles. It is apparent from these numbers that diesel emission vehicles on the 710 freeway represent about 30% of vehicles while on the 405 freeway they represent <5% (Zhu et al., 2002). Fig. 3 compares the traffic volume on both the 405 and the 710 freeways. Error bars represent one standard deviation. It is seen that the 710 freeway has about 7 times as many diesel vehicles and 70% of gasoline vehicles as the 405 freeway. The total vehicle numbers on both freeways are quite similar 12,180 versus 13,900/h for the 405 freeway.

Zhu et al. (2002) reported that a traffic slowdown on freeway 405 was associated with a drop in total particle

number concentration indicating that fewer ultrafine particles are emitted during such events. In this study, the traffic speed on the 710 freeway stayed constant throughout the sampling period. No traffic slow down was observed. The difference in the variability of traffic volume on both freeways is indicated by the error bars in Fig. 3.

Zhu et al. (2002) reported that both wind speed and traffic density affected the characteristics of ultrafine particles near the 405 freeway, and the control CPC responded to these effects reasonably well. Thus, subsequent data for ultrafine particle analysis at increasing distances from the freeway were all normalized to the control CPC's reading. An average CPC reading,  $\overline{C_N}$ , was obtained based on all the measurements. In Figs. 4–6, number concentration and size distribution data were scaled to  $\overline{C_N}$  by dividing each measurement by the ratio of CPC reading for the period of measurement to  $\overline{C_N}$ .

### 3.3. Change in ultrafine particle size distribution with increasing distance

Fig. 4 depicts ultrafine particle size distributions at 17, 20, 30, 90, 150 and 300 m downwind and 300 m upwind of freeway 710. The size distributions are smoothed and shown together with common scales for both axes. The horizontal axis represents particle size on a logarithmic scale, while the vertical axis represents normalized particle number concentration in the size range of 6–220 nm as measured by the SMPS. Data were averaged for all applicable sampling dates for each distance from the freeway. As shown in Fig. 4, ultrafine particle size

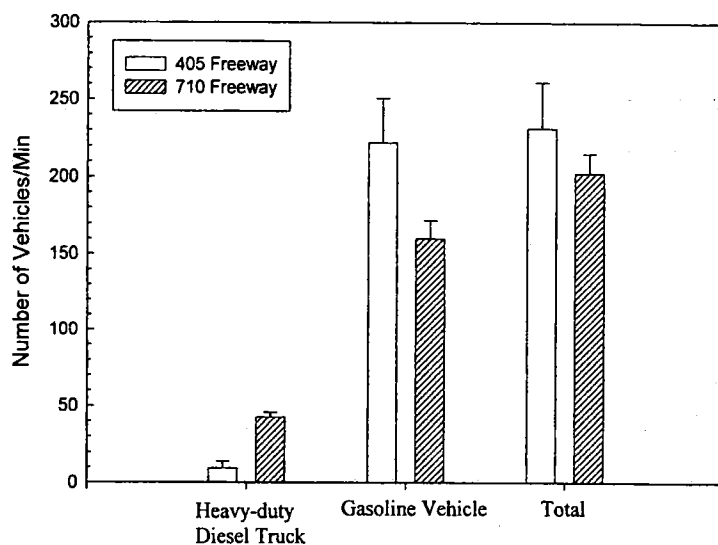


Fig. 3. Traffic volume comparison for the 405 and 710 freeway. Bars indicate one standard deviation.



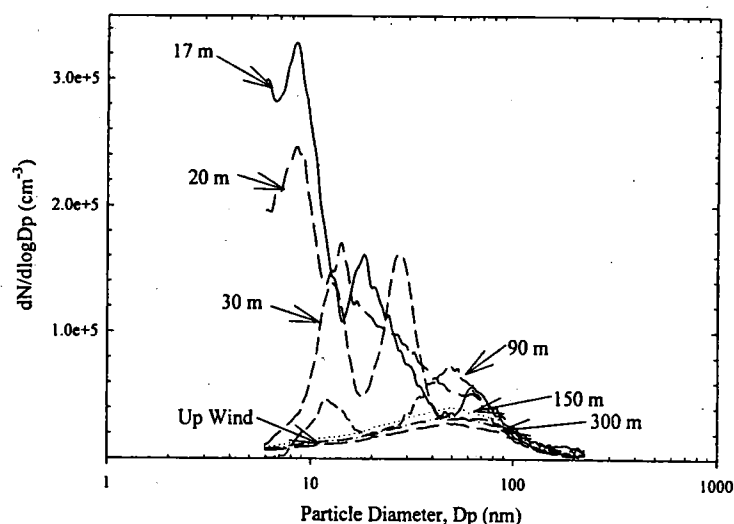


Fig. 4. Ultrafine particle size distribution at different sampling locations near the 710 freeway.

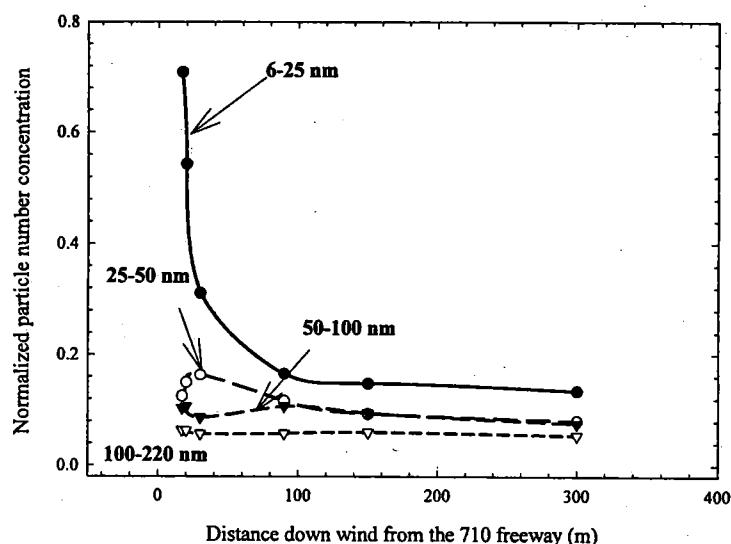


Fig. 5. Normalized particle number concentration for different size ranges as a function of distance from the 710 freeway.

distribution changed markedly and its number concentration dropped dramatically with increasing distance. At the nearest sampling location, 17 m downwind from the center of the freeway, the dominant mode was around 10 nm with a modal concentration of more than  $3.2 \times 10^5/\text{cm}^3$ . This mode remained at 10 nm for the second sampling location, 20 m downwind from the freeway, but its concentration dropped to  $2.4 \times 10^5/\text{cm}^3$ . It shifted to larger size range and its concentration kept decreasing for farther sampling locations. This mode was not observed at distance  $> 150$  m downwind from the freeway. The dramatic decrease of particle number concentration in the size range around 10 nm was likely

due to atmospheric dilution and several atmospheric aerosol particle loss mechanisms that favor small particles, diffusion to surfaces, evaporation, and coagulation. The smaller the particle, the greater its diffusion coefficient and its Brownian motion. Particles of 10 nm diffuse about 80 times faster than particles of 100 nm (Hinds, 1999). As particle size gets smaller, the Kelvin effect becomes more important, making it easier for molecules to leave the particle's surface by evaporation. In addition, when two small particles collide due to their Brownian motion (coagulate), they form a bigger particle. Thus, coagulation reduces number concentrations and shifts the size distribution to larger sizes.

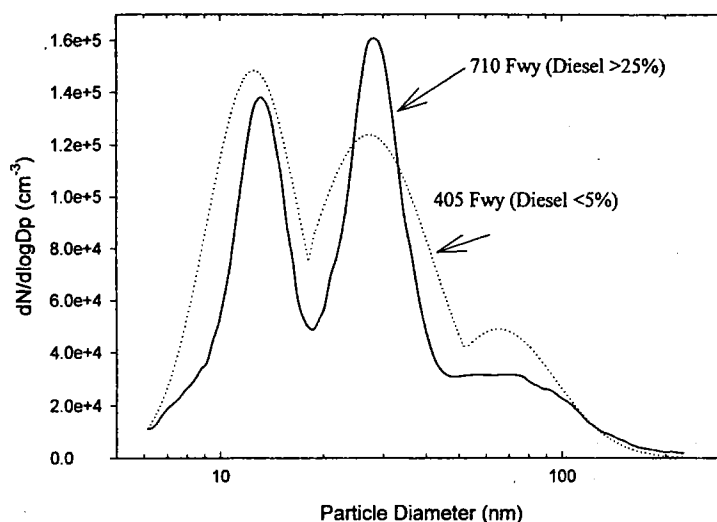


Fig. 6. Comparison of ultrafine particle number concentration at 30 m downwind from 405 and 710 freeway.

In Fig. 4, the second mode at 17 m downwind from the freeway was around 20 nm with a concentration of  $1.5 \times 10^5/\text{cm}^3$ . This mode remained at similar size range and concentration for the next sampling location, 20 m, but shifted to 30 nm at 30 m downwind from the freeway. It is of particular note that, while the concentration for the primary mode, 10 nm mode, decreased about 60% of its maximum value from 17 to 30 m with a slight shift in its mode, the 20 nm mode concentration did not change significantly but the modal size shifted noticeably. This second mode continued to shift to larger sizes with increasing distance from the freeway. In general number concentrations for smaller particles,  $d_p < 50$  nm, dropped significantly with increasing distances from the freeway, but for larger ones,  $d_p > 100$  nm, number concentrations decreased only slightly. These results are in excellent agreement with what Zhu et al. (2002) reported for freeways impacted mostly by gasoline vehicles, which suggests that coagulation is more important than atmospheric dilution for the smallest ultrafine particles and vice versa for large particles. Ultrafine particle concentrations measured at 150 and 300 m downwind of the 710 freeway were statistically within the variation of the 300 m upwind background concentration. The maximum number concentration that was observed next to the freeway was about 30 times greater than that for the background location. This suggests that people who live or work within 100 m downwind of major traffic sources, or spend a substantial amount of time commuting on such highways, will have a much higher ultrafine particle exposure than those who do not. This result can be used in epidemiological studies to estimate exposure to ultrafine particles.

Based on Fig. 4, it is clear that vehicle-emitted ultrafine particles of different size ranges behave quite differently in the atmosphere. Zhu et al. (2002) showed the decay of ultrafine particle number concentrations in four size ranges 6–25, 25–50, 50–100 and 100–220 nm. They found coagulation played a significant role in modifying the particle size distribution of vehicle-emitted ultrafine particle downwind of a freeway. Fig. 5 was prepared in the same ways as Zhu et al. (2002). The measured particle number concentrations in each SMPS size bin were combined in the corresponding size range, and the result was normalized to averaged wind speed. The general trends of sub-grouped ultrafine particle decay curves are quite comparable to those given by Zhu et al. (2002), Figs. 7a and b. Total particle number concentration in the size range of 6 to 25 nm accounted for about 70% of total ultrafine particle number concentration and dropped sharply, by about 80%, at 100 m, and leveled off after 150 m. Overall, it decayed exponentially throughout the whole measured distance. Number concentrations in the next two size ranges 25–50 and 50–100 nm, all experienced a shoulder between 17 and 150 m. These results are in excellent agreement with what Zhu et al. (2002) observed and can be explained by particles, in smaller size ranges, coagulating with these particles to increase their size.

Fig. 6 compares the ultrafine particle size distributions at 30 m downwind from the 710 and the 405 freeways. Three-mode lognormal fitting was used for 405 freeway. Raw data were smoothed by averaging for 710 freeway. Heavy-duty diesel trucks on the 710 freeway represent more than 25% of traffic while on the 405 freeway they represent <5% (Zhu et al., 2002). Average PM emission rate for heavy-duty diesel trucks is about 0.4 g/mi (California ARB, 2000) while for passenger cars is

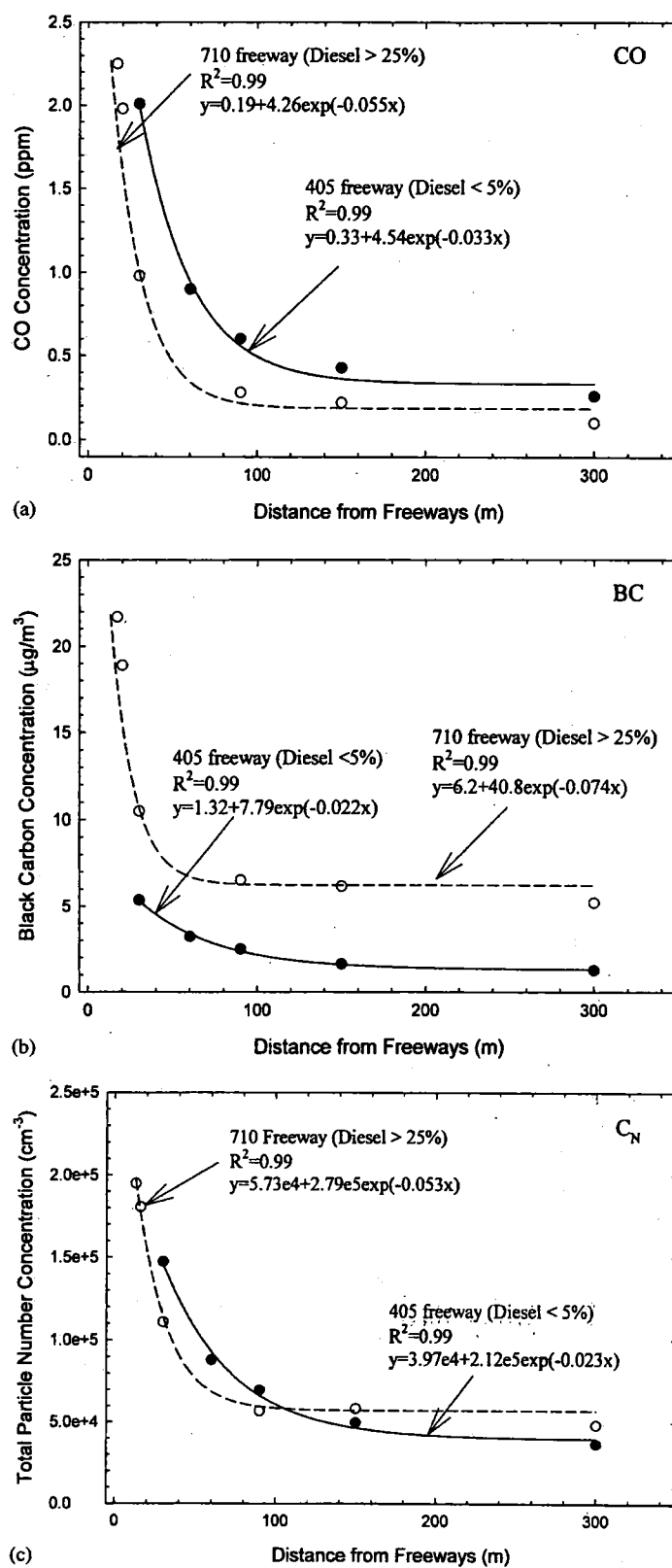


Fig. 7. Decay curves of: (a) CO, (b) BC and (c) particle number concentration near the 405 and 710 freeway.

about 0.08 g/mi (EPA, 2000). Thus, on the 710 freeway, about 60% of PM emission is due to heavy-duty diesel trucks  $((0.25 \times 0.4)/(0.25 \times 0.4 + 0.75 \times 0.08) = 62.5\%)$ . In Fig. 6, both size distributions have three distinct modes. The concentration for the first mode, between 10 to 20 nm, is slightly higher near the 405 freeway. This mode is likely to arise from homogeneous nucleation of semi-volatile materials and is similar to that previously reported for direct laboratory measurement of gasoline vehicle emissions (Ristovski et al., 1998). The concentration for the second mode, around 30 nm, is about 30% higher near the 710 freeway than that near the 405 freeway. This mode probably comprises mainly of BC and is likely due to the much higher diesel emissions on the 710 freeway. The last mode, around 70 nm, represents an insignificant contribution to number concentrations for these two freeways and in both cases are comparable to the background concentrations.

### 3.4. Decay of carbon monoxide, black carbon and particle number concentration

To make this freeway study more comprehensive, the concentrations of CO, BC, and particle number were also measured at increasing distance from the freeway on selected dates, as shown in Table 1. CO and BC were intentionally selected because their ambient concentrations are closely related to vehicular emissions. Averaged concentration and range of values at different distances from the freeway of each measured property are summarized in Table 2. CO and BC concentrations decreased noticeably when moving away from the traffic sources, similar to the findings of the study by Zhu et al. (2002).

Figs. 7a–c were prepared by comparing the decay characteristic of CO, BC and particle number concentrations near the 405, gasoline vehicle dominated, and the 710, diesel vehicle dominated, freeways. Exponential decay was found to be a good estimator for predicting total particle number concentrations at different locations (Zhu et al., 2002). Each data point in the figure

represents an averaged value for all measurements with similar wind directions. The solid line was the best fitting exponential decay curve, determined using SigmaPlot 2000 nonlinear curve fitting procedure. The best fitting exponential decay equations and  $R^2$  values are also given in the figure. It can be seen, in general, all three pollutants decay at a similar rate near both freeways. This implies that atmospheric dilution plays a comparable role in both studies. As discussed previously, the average wind speed for these two studies are all close to 1.5 m/s. The discrepancies of the curves were mainly due to the different traffic fleet compositions on these two freeways. The 710 freeway has more than 25% heavy diesel trucks while the 405 freeway has <5%. It is well known that diesel engines emit less CO and more BC comparing to spark ignition engines (Kittelson et al., 2001). Fig. 7a shows that the concentration of CO near the 710 freeway is generally half of that near the 405 freeway. By comparison, Fig. 7b shows the BC concentration near a diesel vehicle dominated freeway is more than three times greater than that near a gasoline vehicle dominated freeway. As shown in Fig. 7c, the total particle number concentration close to the 405 freeway is somewhat higher than that near the 710 freeway, but drops faster with downwind distance. Since the rate of coagulation increases with decreasing particle size down to 20 nm (Hinds, 1999), the observed result suggests more of the smallest ultrafine particles, mostly in nanosize range, were emitted from the 405 freeway. This may be explained by a total of 20% more vehicles on the 405 freeway. It was previously reported that number emission rates from the spark-ignition vehicles were much lower than from the diesel vehicles under most operating conditions, but were similar under high-speed highway cruise conditions (Riccard et al., 1996; Kittelson, 1998). It should also be noted that the exponential decay characteristic appears to extend to about 3 m downwind from the edge of the freeway for all three pollutants. Based on our results we conclude that atmospheric dilution is so rapid that average concentration decays continuously after leaving the tailpipe.

Table 2  
Measured averaged concentrations at increasing distances from the freeway<sup>a</sup>

| Measurement   | Upwind (m)          | Downwind distance (m) |                     |                     |                    |                     |                     |
|---|---------------------|-----------------------|---------------------|---------------------|--------------------|---------------------|---------------------|
|   | 200                 | 17                    | 20                  | 30                  | 90                 | 150                 | 300                 |
| CO (ppm)  | 0.1<br>(0.0–0.2)    | 2.3<br>(1.9–2.6)      | 2.0<br>(1.5–2.4)    | 1.7<br>(1.1–1.9)    | 0.5<br>(0.2–0.7)   | 0.4<br>(0.1–0.5)    | 0.2<br>(0.1–0.3)    |
| Black carbon ( $\mu\text{g}/\text{m}^3$ )             | 4.6<br>(3.1–5.9)    | 21.7<br>(20.3–24.8)   | 19.4<br>(16.5–21.6) | 17.1<br>(12.6–19.3) | 7.8<br>(4.5–9.3)   | 6.5<br>(3.9–9.2)    | 5.5<br>(3.5–7.7)    |
| Number concentration ( $\times 10^{-5}/\text{cm}^3$ ) | 0.48<br>(0.36–0.57) | 2.0<br>(1.8–2.5)      | 1.8<br>(1.5–2.5)    | 1.6<br>(1.2–1.9)    | 0.72<br>(0.42–1.1) | 0.61<br>(0.35–0.98) | 0.49<br>(0.30–0.59) |

<sup>a</sup> Range given in parenthesis.

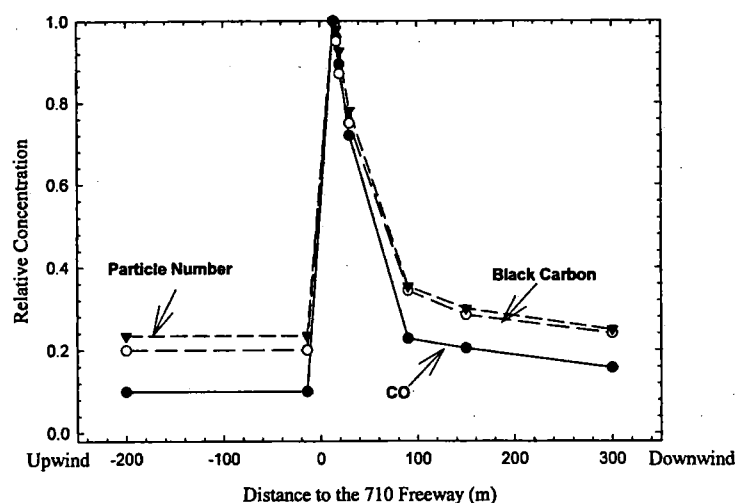


Fig. 8. Relative particle number, BC, CO concentrations versus distance from the 710 freeway.

Fig. 8 shows the decay curves for relative concentrations of CO, BC and total particle number. The curves are normalized and extended to reach 1.0 at the downwind edge of the 710 freeway. Background concentrations are also shown in the figure. It is seen that CO, BC and particle number concentration decreased about 60–80% in the first 100 m and then leveled off somewhat after 150 m, similar to what Zhu et al. (2002) reported. Background CO has a much lower relative concentration while background BC and particle number concentrations are comparable. Thus, CO was diluted more quickly and significantly than BC and particle number concentration. In general, CO, BC and particle number concentrations tracked each other very well. These results confirm the common assumption that vehicular exhaust is the major source for CO, BC and ultrafine particles near a busy freeway. They also support the conclusion made by Zhu et al. (2002) that for the conditions of these measurements the decreasing characteristics of any of these three pollutants could be used interchangeably to estimate the relative concentration of the other two pollutants near freeways.

#### 4. Conclusions and summary

Wind speed and direction are important in determining the characteristic of ultrafine particles near freeways. The average concentrations of CO, BC and particle number concentration at 17 m was 1.9–2.6 ppm, 20.3–24.8  $\mu\text{g}/\text{m}^3$ ,  $1.8 \times 10^5$ – $3.5 \times 10^5/\text{cm}^3$ , respectively. Relative concentration of CO, BC and particle number tracked each other well as one moves away from the freeway. Exponential decay was found to be a good estimator for the decrease of these three pollutants'

concentration with distance along the wind direction starting from the edge of the freeway. Measurements show that both atmospheric dilution and coagulation play important roles in the rapid decrease of particle number concentration and the change in particle size distribution with distance away from a freeway.

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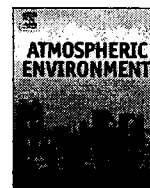
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## A wide area of air pollutant impact downwind of a freeway during pre-sunrise hours

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### ABSTRACT

We have observed a wide area of air pollutant impact downwind of a freeway during pre-sunrise hours in both winter and summer seasons. In contrast, previous studies have shown much sharper air pollutant gradients downwind of freeways, with levels above background concentrations extending only 300 m downwind of roadways during the day and up to 500 m at night. In this study, real-time air pollutant concentrations were measured along a 3600 m transect normal to an elevated freeway 1–2 h before sunrise using an electric vehicle mobile platform equipped with fast-response instruments. In winter pre-sunrise hours, the peak ultrafine particle (UFP) concentration ( $\sim 95\,000\text{ cm}^{-3}$ ) occurred immediately downwind of the freeway. However, downwind UFP concentrations as high as  $\sim 40\,000\text{ cm}^{-3}$  extended at least 1200 m from the freeway, and did not reach background levels ( $\sim 15\,000\text{ cm}^{-3}$ ) until a distance of about 2600 m. UFP concentrations were also elevated over background levels up to 600 m upwind of the freeway. Other pollutants, such as NO and particle-bound polycyclic aromatic hydrocarbons, exhibited similar long-distance downwind concentration gradients. In contrast, air pollutant concentrations measured on the same route after sunrise, in the morning and afternoon, exhibited the typical daytime downwind decrease to background levels within  $\sim 300\text{ m}$  as found in earlier studies. Although pre-sunrise traffic volumes on the freeway were much lower than daytime congestion peaks, downwind UFP concentrations were significantly higher during pre-sunrise hours than during the daytime. UFP and NO concentrations were also strongly correlated with traffic counts on the freeway. We associate these elevated pre-sunrise concentrations over a wide area with a nocturnal surface temperature inversion, low wind speeds, and high relative humidity. Observation of such wide air pollutant impact area downwind of a major roadway prior to sunrise has important exposure assessment implications since it demonstrates extensive roadway impacts on residential areas during pre-sunrise hours, when most people are at home.

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### 1. Introduction

Air quality in the vicinity of roadways can be seriously impacted by emissions from heavy traffic flows. As a result, high concentrations of air pollutants are frequently present in the vicinity of roadways and may result in adverse health effects. These include increased risk of reduced lung function (Brunekreef et al., 1997), cancer (Knox and Gilman, 1997; Pearson et al., 2000), adverse respiratory symptoms (Van Vliet et al., 1997; Venn et al., 2001;

Janssen et al., 2003), asthma (Lin et al., 2002; McConnell et al., 2006), and mortality (Hoek et al., 2002).

Previous studies have shown elevated vehicle-related air pollutant concentrations and gradients downwind of roadways during daytime. Hitchins et al. (2000) measured concentrations of fine and ultrafine particles (UFP) at a distance of 15–375 m from a major roadway during the daytime. They found concentrations decayed to about half of the peak value (at the closest point to the roadway) at approximately 100–150 m from the roadway on the normal downwind side. Particle concentrations were not affected by the roadway at a distance farther than 15 m on the normal upwind side, indicating a sharp gradient of fine and ultrafine particles. Similar studies were conducted by Zhu et al. (2002a,b), who measured ultrafine particles, CO, and black carbon (BC) on the

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upwind (200 m) and downwind (300 m) sides of a freeway in Los Angeles during the daytime. Peak concentrations were observed immediately adjacent to the freeway, with concentrations of air pollutants returning to upwind background levels about 300 m downwind of the freeway.

The few near-roadway studies conducted at night indicated larger areas of impact than during daytime. UFP concentrations at night were reported by Zhu et al. (2006), who conducted measurements upwind (300 m) and downwind (500 m) of a freeway from 22:30 to 04:00. Although traffic volumes were much lower at night (about 25% of peak) particle number concentrations were about 80% of the daytime peak 30 m downwind of the freeway, with UFP concentrations of  $\sim 50\,000\text{ cm}^{-3}$  about 500 m downwind of I-405, a major Los Angeles freeway during the night. Fruin and Isakov (2006) measured UFP concentrations in Sacramento, California, near the I-50 freeway between 23:00 and 01:00 and found 30–80% of maximum centerline concentrations (measured on a freeway overpass) 800 m downwind.

In the present study, the use of a full-size, motorized mobile platform (MP) allowed more pollutants to be measured than previous nighttime studies and with improved spatial and temporal resolution. While traveling at normal vehicle speeds, an instrumented mobile platform allows measurements over greater distances and in shorter times (Bukowiecki et al., 2002a,b, 2003; Canagaratna et al., 2004; Kittelson et al., 2004a,b; Khlystov and Ma, 2006; Kolb et al., 2004; Pirjola et al., 2004, 2006; Unal et al., 2004; Weijers et al., 2004; Westerdahl et al., 2005; Yao et al., 2005; Isakov et al., 2007; Baldauf et al., 2008; Fruin et al., 2008). However, to date, such studies have focused almost entirely on daytime and evening periods.

In the present study, air pollutant concentrations were measured over a wide area on the south and north sides of the I-10 freeway in west Los Angeles, California, 1–2 h before sunrise in the winter and summer seasons of 2008 using an electric vehicle mobile platform equipped with fast-response instruments. We observed a much wider area of impact downwind of the freeway than reported in previous daytime and evening studies, consistent with low wind speed, absence of turbulent mixing, and nocturnal radiation inversions. Our pre-sunrise results were also strikingly different from those we observed for the same route during the daytime. Our observation of a wide area of impact during pre-sunrise hours, up to about 600 m upwind and 2000 m downwind, has significant implications for exposures in residential neighborhoods adjacent to major roadways.

## 2. Methods

### 2.1. Mobile platform and data collection

A Toyota RAV4 sub-SUV electric vehicle served as the mobile platform, with self-pollution eliminated by the non-polluting nature of the vehicle. Table 1 shows a complete list of sampling instruments and equipment installed on the mobile platform. The time resolution for most instruments ranged from 5 to 10 s except the Aethalometer, which had 1 min time resolution. The instrument power supply and sampling manifold were similar to that described by Westerdahl et al. (2005).

Calibration checks and flow checks were conducted on a bi-monthly and daily basis, respectively, as described in Kozawa et al. (2009). For calibrations, a standard gas containing a mix of NO and CO was diluted using an Environics 9100 Multi-Gas Calibrator and Teledyne API Zero Air System (Model 701) to calibrate the CO and NO/NO<sub>x</sub> analyzers. CO<sub>2</sub> was calibrated with zero air and span gas cylinders from Thermo Systems Inc. A DryCal DC-lite flow

**Table 1**  
Monitoring instruments on the mobile platform.

| Instrument                              | Measurement Parameter                              | Time Resolution |
|---|--|-----------------|
| TSI Portable CPC, Model 3007            | UFP Count (10 nm–1 $\mu\text{m}$ )                 | 10 s            |
| TSI FMPS, Model 3091                    | UFP Size (5.6–560 nm)                              | 10 s            |
| TSI DustTrak, Model 8520                | PM <sub>2.5</sub> Mass                             | 5 s             |
| Magee Scientific Aethalometer           | Black Carbon                                       | 1 min           |
| EcoChem PAS 2000                        | Particle Bound PAH                                 | 5 s             |
| Teledyne API Model 300E                 | CO   | 20 s            |
| LI-COR, Model LI-820                    | CO <sub>2</sub>                                    | 10 s            |
| Teledyne-API Model 200E                 | NO <sub>x</sub> , NO, NO <sub>2</sub>              | 20 s            |
| Vaisala Sonic Anemometer                | Local Wind Speed and Direction,                    | 1 s             |
| and Temperature/RH Sensor               | Temperature, Relative Humidity (RH)                |                 |
| Stalker LIDAR and Vision Digital System | Traffic Documentation, Distance and Relative Speed | 1 s             |

meter, with a flow range of  $100\text{ ml min}^{-1}$  to  $7\text{ L min}^{-1}$  and an accuracy of  $\pm 1\%$ , was used to check the flows of each instrument.

### 2.2. Route

For pre-sunrise measurements, the mobile platform was driven on a fixed route over three days in the winter season and two days in the summer season of 2008. The route covered a total length of about 3600 m approximately perpendicular to the I-10 freeway in Santa Monica, California (Fig. 1). The solid line in Fig. 1 shows the section of the route over which the mobile platform traveled about 8–10 times during each monitoring period, reaching about 1200 m south of the freeway. The dashed line shows the extended section of the route, over which the mobile platform traveled 2–4 times during each monitoring period, reaching about 2600 m south of the freeway. The pre-sunrise route crossed a number of local surface streets; these are shown in Fig. 1 together with their normal distances to the freeway as measured from Google Map. The route was selected because it passed under the I-10 freeway, and because there was little traffic flow on the route itself or on the perpendicular surface streets (e.g. Olympic Blvd., Pico Blvd. etc.) during pre-sunrise hours. Hence, the majority of measurements were not significantly affected by local surface street traffic. The route also passed through a dense residential neighborhood where the elevated air pollutant concentrations have significant exposure implications.

During sampling, the mobile platform was intentionally stopped to avoid localized impacts from individual vehicles whenever necessary. During data reduction, pollutant concentration spikes, if verified from videotape to be caused by a nearby vehicle, were excluded from the analysis.

### 2.3. Real-time traffic flow

Traffic flows were collected or measured on the I-10 freeway, the pre-sunrise route itself, and the major surface streets transecting the pre-sunrise route. Real-time traffic flow on the freeway was obtained from the Freeway Performance Measurement System (PeMS) provided by the UC Berkeley Institute of Transportation. Sensors were located at the Dorchester Station, about 300 m from the intersection of the pre-sunrise route and the freeway. Since there were no on-ramps or exits between the Dorchester Station and our route, the PeMS data accurately represented the traffic flow on the I-10 freeway where our route passed under the freeway. Traffic flow on the pre-sunrise route itself was monitored and recorded by a Stalker Vision Digital System on the mobile platform. The recorded videos were replayed and vehicles on the pre-sunrise

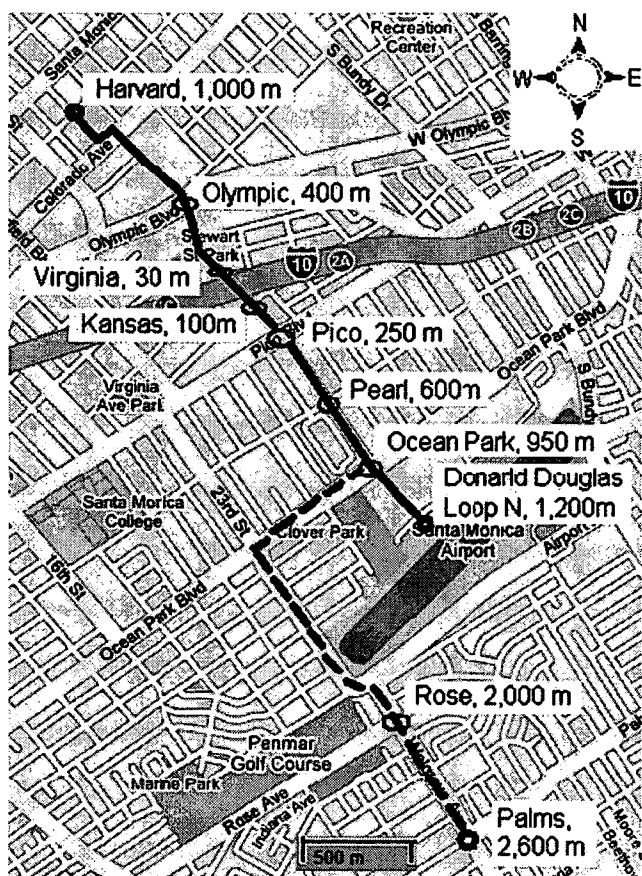


Fig. 1. Pre-sunrise route. The solid line indicates the route 1000 m and 1200 m north and south of the I-10 freeway, respectively. The dashed line indicates the route extended to 2600 m south of the I-10 freeway. Mobile platform measurements on this route were continuous; circles indicate location of cross streets, not fixed site monitoring.

route were manually counted. Traffic flows on the major cross streets (e.g. Olympic Blvd., Pico Blvd., and Ocean Park Blvd.) were manually counted during the winter season on a weekday at times similar to when the pre-sunrise measurements were conducted.

#### 2.4. Data analysis and selection of key pollutants

Data were adjusted for the varying response times of the instruments on the mobile platform to synchronize the measurements.  $\text{NO}_x$ , CO,  $\text{CO}_2$ , and particulate data (UFP, BC, and  $\text{PM}_{2.5}$  mass) were synchronized with particle-bound polycyclic aromatic hydrocarbon (PB-PAH) data measured by the PAS instrument, which had the fastest response time. NO, UFP, and PB-PAH were selected in the present study for detailed spatial analysis because of their rapid and large variation on and near roadways. The overall response time for the PAS instrument was determined by comparing the time of signal peaks in the PB-PAH time-series to the corresponding time of acceleration of a vehicle in front of our mobile platform (as recorded on videotape). This time difference was less than 10–15 s and includes the transport time (typically a few seconds) for the plume from the emitting vehicle to reach the inlet of the sampling duct of the mobile platform. Given the short response times of our instruments and our driving speeds of 5–15 MPH, the spatial resolution of our mobile platform measurements was typically in the range of 25–75 m, with the finer spatial

resolution ( $\sim 25$  m) near the edges of the freeway where we drove more slowly.

Measurements were made continuously over the entire route, not at fixed stationary sites. The measured real-time concentrations of UFP, PB-PAH, and NO along the pre-sunrise route were averaged for each intersection using a few data points measured at and immediately adjacent to the intersection. Although the peak air pollutant concentration always occurred downwind of the I-10 freeway, its value changed with time due to changing traffic volumes on the I-10 freeway and varying meteorological conditions, so peak pollutant concentrations were used to calculate normalized relative pollutant concentrations. For example, in the winter season, the measured averaged peak UFP concentration was about  $95\,000\text{ cm}^{-3}$ , but the instantaneous peak values varied in the range of  $62\,000$ – $135\,000\text{ cm}^{-3}$  (four to nine times the background concentrations).

### 3. Results and discussion

#### 3.1. Meteorological data

Meteorological conditions, including atmospheric stability, temperature, relative humidity, wind speed and wind direction, play an important role in determining air pollutant concentrations and gradients along and downwind of roadways. During each run, the mobile platform was periodically stopped at locations along the pre-sunrise route to obtain wind data from on-board instruments (Table 2). These data were compared with the measurements from the Santa Monica Airport (SMA) located about 1500 m downwind of the I-10 freeway and in the immediate vicinity of the route. Both the averaged wind speeds measured by the mobile platform and by the SMA were quite low during pre-sunrise hours, in a range of  $0$ – $1.0\text{ m s}^{-1}$  and the averaged difference between the two measurements was about  $0.3\text{ m s}^{-1}$ . Temperature and relative humidity were obtained from SMA data.

Fig. 2 shows the wind roses and vector-averaged wind orientation for five days, March 7, 12, 18, June 30, and July 2, from data collected by instruments on the mobile platform. Wind speeds were low during the pre-sunrise hours, with monitoring period averages ranging from  $0.0$  to  $1.0\text{ m s}^{-1}$ . The averaged wind directions measured by the mobile platform indicated a predominant direction of N/NE/NW during the pre-sunrise runs, which agreed reasonably well with airport wind direction data. For this predominant wind direction, the north side of the I-10 freeway was upwind; the south side downwind. Although having a predominant direction from north, the wind was not completely perpendicular to the I-10 freeway. Hence, the distances pollutants traveled from the freeway to various locations along the route, including the major cross-surface streets, were generally longer than indicated by distances shown in Fig. 1. For example, the straight perpendicular distance of Ocean Park Blvd. to the I-10 freeway is  $\sim 950$  m, whereas for the averaged wind direction of  $25^\circ$  for the pre-sunrise run, the distance pollutants traveled was  $\sim 1050$  m. However, due to the variability of meteorological conditions, the perpendicular distances were used to indicate impact distances in the present study.

While detailed thermal structure data for the lowest layers of the atmosphere in the area of our pre-sunrise route were not available, the available data indicate the days sampled had stable (i.e., vertical) temperature profiles or strong nocturnal radiation inversions in the hours before sunrise. Data recorded at the Santa Monica Airport indicated the nights on which sampling took place were clear up to at least 3000 m, and had either offshore flow or a weak land breeze, also consistent with clear skies; clear skies are conducive to the formation of nocturnal surface inversions due to enhanced radiative heat loss in the infrared. Data collected by the

**Table 2**  
Meteorological conditions during pre-sunrise runs (2008).

| Date     | Measurement period     | Sunrise           | Atmospheric Stability from LAX Profiler data | Wind Speed <sup>a</sup> (m s <sup>-1</sup> ) |     | Wind Direction <sup>a</sup> (°) |     | Temperature (°C) | Relative Humidity (%) |
|----------|------------------------|-------------------|--|--|-----|---------------------------------|-----|------------------|-----------------------|
|          |                        |                   |  | MP   | SMA | MP                              | SMA |                  |                       |
| March 7  | 6:20–7:50 <sup>b</sup> | 7:14 <sup>b</sup> | N.D. <sup>c</sup>                            | 0.9  | 1.0 | 13                              | 5   | 11               | 79                    |
| March 12 | 6:00–7:30              | 7:07              | Surface inversion to 250–300 m               | 1.0  | 1.0 | 53                              | 20  | 13               | 66                    |
| March 18 | 6:10–7:20              | 6:59              | Surface inversion to 190 m                   | 0.8  | 1.0 | 6                               | 45  | 9                | 61                    |
| June 30  | 4:00–6:30              | 5:45              | Stable to 190 m, inversion above             | 0.7  | 0.0 | 288                             | 0   | 17               | 87                    |
| July 2   | 4:30–6:45              | 5:45              | Stable to 260 m, inversion above             | 0.7  | 1.0 | 315                             | 340 | 17               | 84                    |

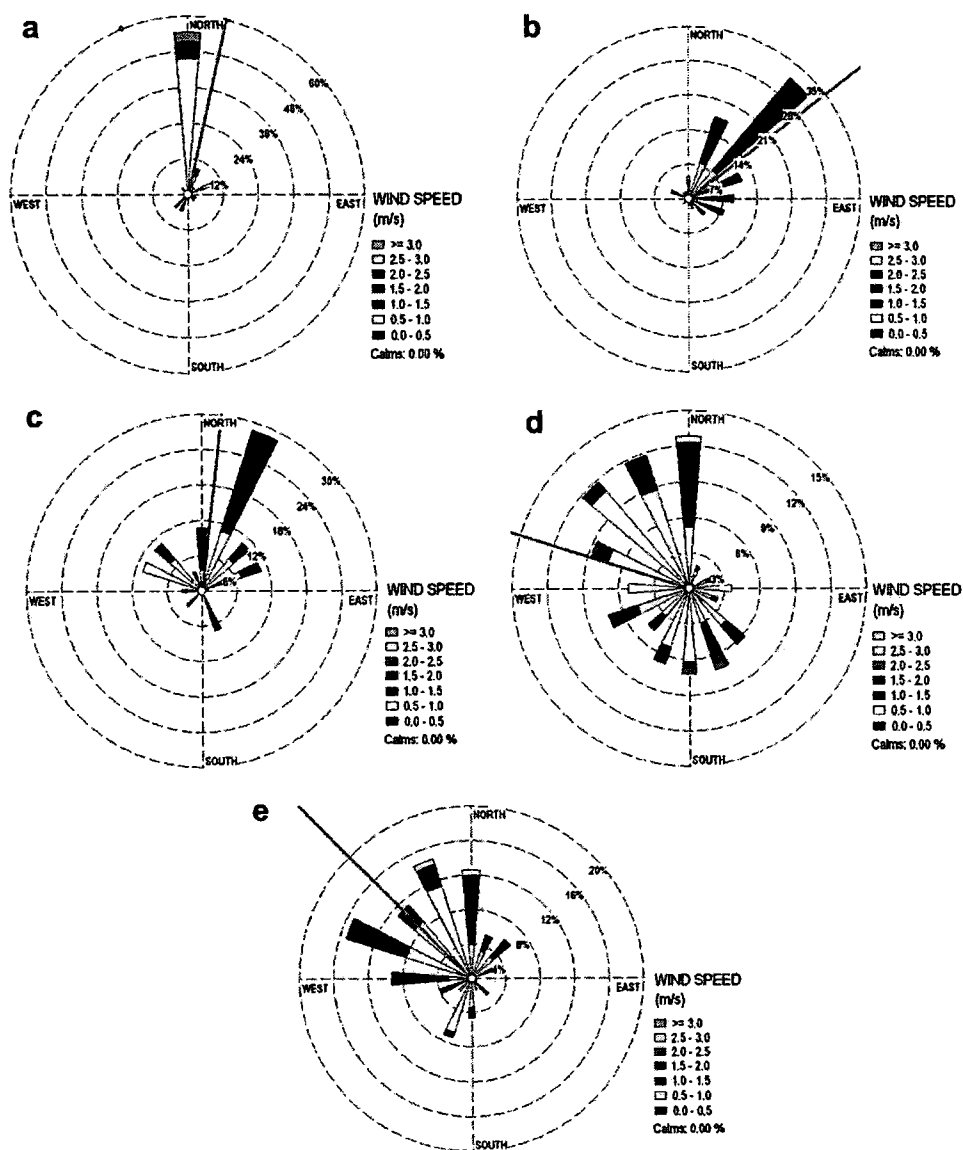
<sup>a</sup> Averaged values for the measured period.

<sup>b</sup> Time corrected to Pacific Day Light Time (PDT); change from PST to PDT occurred on March, 9, 2008.

<sup>c</sup> Profiler came online the following evening. The following night (3/8) experienced a surface inversion for the entire night.

South Coast Air Quality Management District (SCAQMD) at the Los Angeles Airport (LAX), ~8 km south of pre-sunrise route, were also consistent with an inversion or stable conditions at the surface. On 3/10 and 3/18, the data showed temperature inversions from the

lower edge of the measurements at 130 m up to 190 m or more, respectively. On 6/30 and 7/2, the profiles were stable from 130 to 190 or 260 m, respectively, with capping inversion layers above. Wind speeds during the pre-sunrise hours were too low to create



**Fig. 2.** Wind roses for pre-sunrise sampling hours. (a) March 7; (b) March 12; (c) March 18; (d) June 30; (e) July 2. The thin line in each wind rose indicates vector-averaged wind orientation.

appreciable vertical mixing in the presence of these temperature profiles, and the shallow mixed layer was likely thinner in March than in June/July.

### 3.2. Observation of a wide impact area downwind of the freeway during pre-sunrise hours

As shown in Fig. 3, a wide impact area of elevated UFP concentrations, up to 2000 m downwind and 600 m upwind of the I-10 freeway, was observed during the pre-sunrise hours on the monitoring days in the two seasons. In this wide impact area, elevated UFP concentration extended beyond Donald Douglas Loop N located on the south side and 1200 m downwind of the freeway (Fig. 3). Here, 1200 m downwind, the average UFP concentrations during the winter sampling hours, typically 06:00–07:30, were as high as  $\sim 40\,000\text{ cm}^{-3}$ . Only at a downwind distance of about 2600 m (Palms Blvd.), did the UFP concentration drop to  $\sim 15\,000\text{ cm}^{-3}$ , comparable to the upwind background level.

In the winter season, the peak UFP concentration was approximately  $95\,000\text{ cm}^{-3}$  a few tens of meters downwind of the freeway. Upwind, the concentration dropped sharply to around  $40\,000\text{ cm}^{-3}$  30 m upwind (Virginia Avenue) and returned to background levels of  $\sim 15\,000\text{ cm}^{-3}$  at  $\sim 800\text{ m}$  on the upwind side, creating a moderate upwind gradient north of the I-10 freeway (Fig. 3). Interestingly, the upwind impact distance during the pre-sunrise hours,  $\sim 600\text{ m}$ , was far greater than that of  $\sim 15\text{ m}$  observed during the day by Hitchins et al. (2000) and also greater than that measured by Zhu et al. (2002b). This may be caused by the occasionally variable wind direction during the pre-sunrise hours for which the nominal upwind side of the I-10 freeway could temporarily become downwind. These occasional impacts on the nominal upwind side of the freeway appear to have had substantial influence on the averaged upwind UFP concentrations due to their otherwise low levels.

As seen in Fig. 3, the UFP concentration also decreased on the downwind side, but much more slowly than on the upwind side. At a downwind distance of about 600 m from the freeway, UFP concentrations during winter were about twice those on the upwind side ( $50\,000\text{ cm}^{-3}$  vs.  $22\,000\text{ cm}^{-3}$ ). Even 950 m downwind, at the intersection of Ocean Park Blvd., the UFP concentration remained as high as  $45\,000\text{ cm}^{-3}$ , higher than at 30 m upwind. These pronounced differences in gradients of UFP concentrations resulted in strong contrasts between the upwind and downwind sides of the I-10 freeway during pre-sunrise hours (Fig. 3).

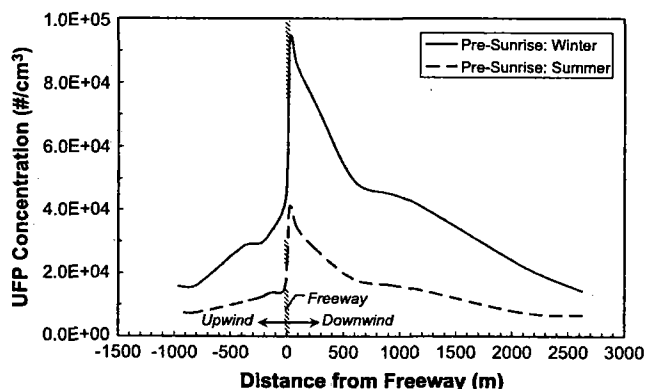


Fig. 3. Ultrafine particle concentrations and gradients along the pre-sunrise route. Positive distances are downwind and negative distances upwind from the I-10 freeway. Data were acquired continuously, up to the edges of the freeway.

As shown in Fig. 4, NO and PB-PAH exhibited concentration gradients similar to UFP along the route during the pre-sunrise hours. Peak concentrations of NO and PB-PAH (on the downwind side) were about 165 ppb and  $55\text{ ng m}^{-3}$ , respectively, in the winter season. Upwind, NO and PB-PAH concentrations dropped rapidly to 70 ppb and  $30\text{ ng m}^{-3}$ , respectively, at a distance of about 150 m. In contrast, on the downwind side, NO and PB-PAH concentrations of 70 ppb and  $30\text{ ng m}^{-3}$ , respectively, extended to a distance of about 1200 m from the freeway (NO and PB-PAH data were unavailable for summer measurement due to instrument problems during the pre-sunrise runs).

Fig. 5 shows normalized UFP concentrations on the two sides of I-10 freeway during the pre-sunrise hours in the winter and summer seasons. UFP concentrations were normalized for each complete run traveled on our route, and then averaged together for all the runs for each season. While there was little or no traffic on our route during the pre-sunrise hours, vehicle counts on the same route during the day were much higher and emissions from these vehicles significantly and frequently affected measurements by the mobile platform. Moreover, the pre-sunrise route was only driven once in the morning after sunrise and once in the afternoon, in contrast to multiple times in the pre-sunrise period. For both of these reasons, comparison between pre-sunrise and morning/afternoon measurements on the pre-sunrise route are not meaningful. Instead, we show normalized data from Zhu et al. (2002b), which were not affected by local traffic, to compare with our pre-sunrise measurements.

As Fig. 5 illustrates, pre-sunrise UFP concentration gradients in the present study exhibited very different behavior than the typical narrow daytime UFP gradients measured by Zhu et al. (2002a,b). In our pre-sunrise measurements, UFP concentrations remained elevated above the background level up to  $\sim 600\text{ m}$  upwind of the freeway versus only  $\sim 17\text{ m}$  upwind for the Zhu et al. (2002b) daytime measurements. On the downwind side in the Zhu et al. (2002b) measurements, UFP concentrations dropped to about 25% of the peak concentration 300 m downwind of the freeway during the day, but in the present study, in strong contrast, the UFP concentrations remained about 40% of the peak as much as 1200 m downwind of the freeway, and was above background levels out to  $\sim 2000\text{ m}$  during the pre-sunrise hours.

To quantify these differences in UFP concentrations an equation of the form  $C = a + e^{-bx}$  was used to fit our observed relative UFP concentrations downwind of the I-10 freeway during pre-sunrise hours, as well as the daytime data reported by Zhu et al. (2002b). As seen in Fig. 6, the decay constant is a factor of five higher for the

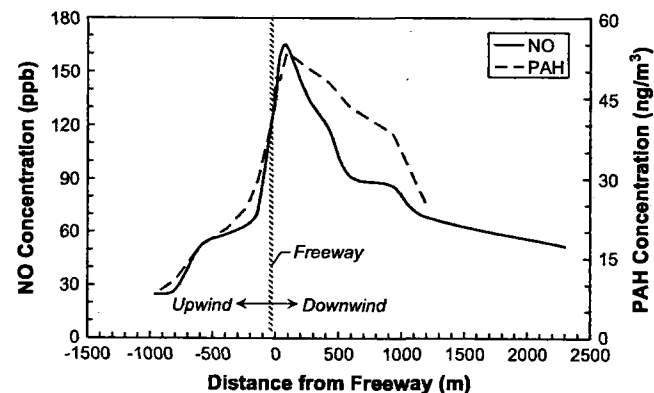


Fig. 4. Average NO and PB-PAH concentrations and gradients, along the pre-sunrise route in the winter season. Positive distances are downwind and negative distances upwind from the I-10 freeway.

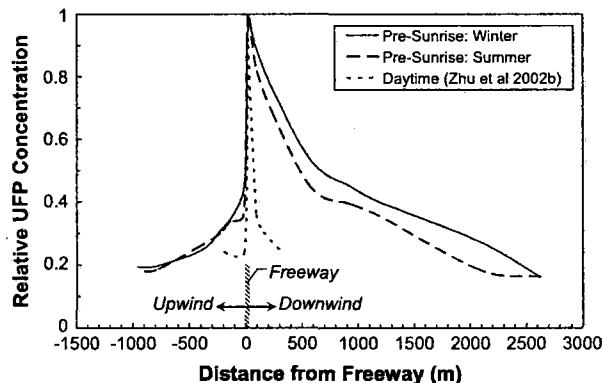


Fig. 5. Relative averaged UFP concentrations and gradients along the pre-sunrise route by season and compared with Zhu et al. (2002b). Positive distances are downwind and negative upwind from the I-10 freeway. Data were acquired continuously for pre-sunrise measurements, up to the edges of the freeway.

daytime vs. the pre-sunrise period, with values of  $b$  of 0.0098 and 0.0018, respectively.

Pre-sunrise relative UFP concentrations exhibited similar trends in both winter and summer (Fig. 5). Although UFP concentrations in the summer were about 40% those in the winter (due to lower traffic flows on the I-10 freeway, as discussed below), the similar trends in relative UFP concentration imply similar UFP propagation during the pre-sunrise hours in the two seasons although meteorological conditions were somewhat different.

### 3.3. Correlation of pollutant concentrations with traffic counts on I-10 freeway

PeMS data showed a similar diurnal traffic pattern on the I-10 freeway on different weekdays during the pre-sunrise hours in both winter and summer (Fig. 7b). Traffic counts on the freeway exhibited an approximately linear increase with the time. However, during 04:00–05:30 (when summer measurements were conducted) traffic counts were lower in summer than in winter. We attribute part of the lower traffic counts in summer to most schools being closed and vacation season in summer, as well as the dramatic increase in gasoline prices between March and July 2008, resulting in a significant overall reduction in vehicle miles traveled. Also, sunrise was about one hour and fifteen minutes earlier in

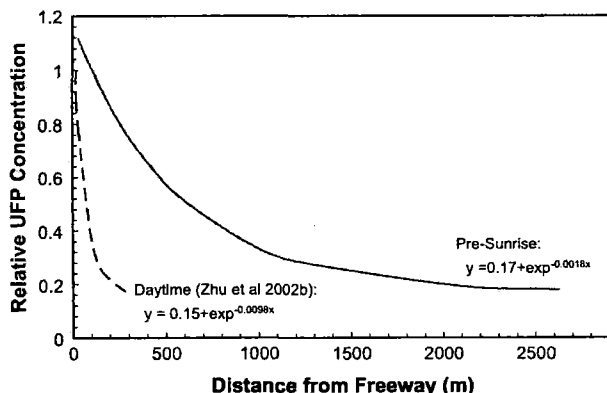


Fig. 6. Exponential fits to the downwind relative UFP concentrations with distance from the I-10 freeway during pre-sunrise hours, compared with fit to daytime data downwind of the I-405 freeway by Zhu et al. (2002b). Data were acquired continuously for pre-sunrise measurements, up to the edges of the freeway.

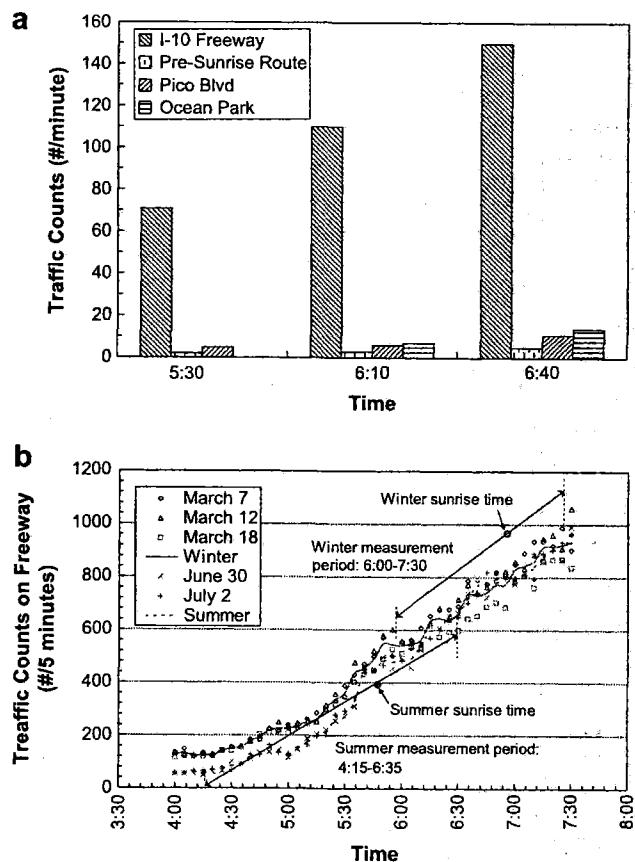


Fig. 7. (a) Comparison of traffic volumes on the I-10 freeway, pre-sunrise route, Pico Blvd., and Ocean Park Blvd. during pre-sunrise hours on a typical weekday; (b) Traffic counts on the I-10 freeway during pre-sunrise measurements; solid line represents averaged count of the three days in the winter season, and dashed line for two days in the summer season. Sunrise times shown here were averaged for each season.

summer (~05:45) than in winter (~07:00), which required an earlier measurement period in summer (~04:15–06:30) compared to winter (~06:00–07:30), and corresponds to much lower overall traffic counts during the pre-sunrise measurement periods in summer.

During the measurement period in winter, traffic counts on the freeway increased from ~530 to ~900 vehicles per 5 min, while in summer counts increased from ~60 to ~620 vehicles per 5 min. Assuming a linear increase of traffic counts with time, the average traffic counts during the pre-sunrise measurements periods, winter versus summer, were ~715 vs. 340 vehicles per 5 min, resulting a ratio of ~2.1. This ratio of seasonal traffic counts compares well with the ratio of the UFP concentrations measured in the winter vs. summer of ~2.2–3.0, depending on distance from the freeway (Fig. 3). It should be noted that the sunrise times during the winter (March) measurements, because they occurred just after the switch to Pacific daylight time (PDT), were close to the latest annual (local) sunrise times, and thus may represent roughly the upper limit for the freeway impact throughout the year.

We attribute the relatively high pollutant concentrations we observed downwind of the I-10 freeway during pre-sunrise hours to emissions of vehicles traveling on the I-10 freeway, combined with strong inhibition of vertical mixing due to stable or inverted temperature profiles near the surface. Fig. 8 shows the UFP and NO concentrations measured at Ocean Park Blvd., ~950 m downwind, vs. the traffic counts on the freeway during the pre-sunrise hours on

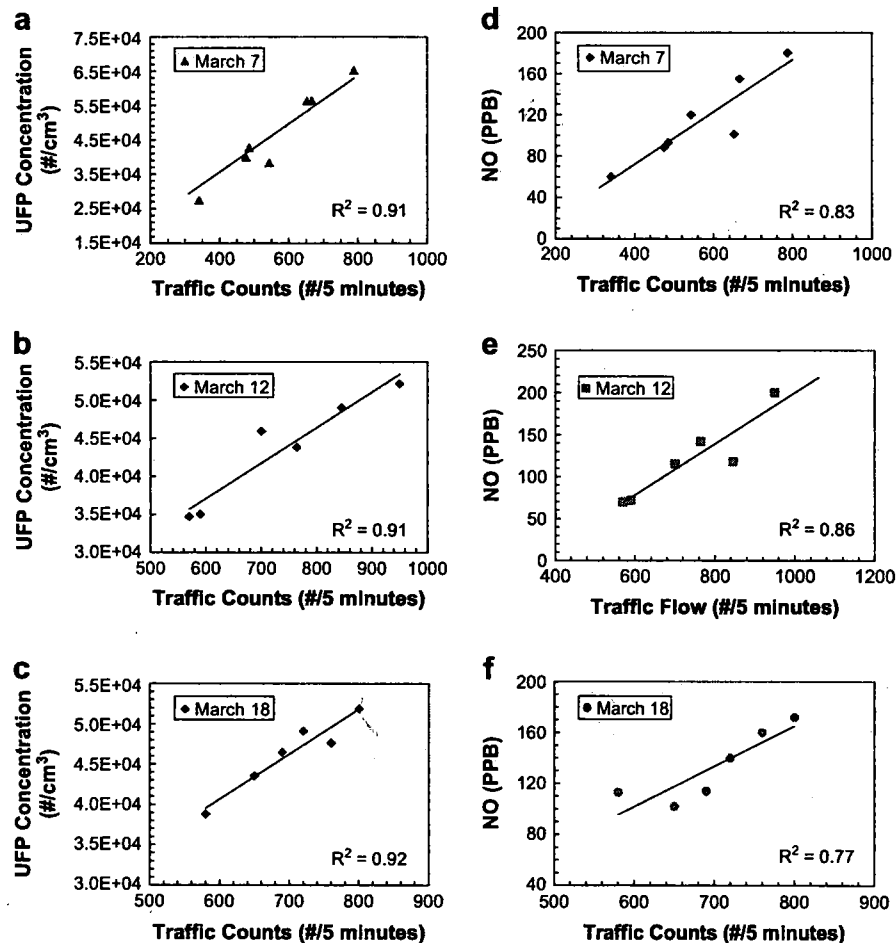


Fig. 8. Linear regressions between UFP and NO concentrations at Ocean Park Blvd. (950 m downwind of I-10 freeway), and traffic counts on the freeway during the pre-sunrise hours in the winter season.

three mornings of the pre-sunrise runs in the winter season. Both the freeway traffic counts (Fig. 7b) and pollutant concentrations increased rapidly during the pre-sunrise hours, and exhibited a strong correlation with each other. For UFP, the values of squared Pearson correlation coefficients ( $r^2$ ) were above 0.90 and for NO, above 0.77 (nitric oxide data were unavailable for summer measurements due to instrument problems during the pre-sunrise runs). Strong correlations at other distances from the freeway were also found between UFP concentrations and traffic counts on the freeway. For example, the correlation coefficients,  $r^2$  for UFP measured at Pearl St. for three winter sampling days, were above 0.85.

Based on our videotape observations and the traffic counts we conducted on surface streets, as well as the strong correlations presented in Fig. 8, we believe the measured concentrations of air pollutants during the pre-sunrise hours were predominantly determined by the traffic counts on the I-10 freeway, and that the impact of local surface street traffic was minor. Traffic volumes on the pre-sunrise route itself were only about 2% of those on the I-10 freeway at corresponding times. Traffic volumes on the three major surface streets crossing the pre-sunrise route, Ocean Park Blvd., Pico Blvd., (downwind of the freeway), and Olympic Blvd (upwind of the freeway) were also low, only about 8%, 6%, and 6%, respectively, of those on the freeway. Most of this early-morning cross traffic for our measurement route encountered green lights. If the emissions of the occasional vehicles on these surface streets were significant, the pollutant concentrations measured downwind of

the streets should have been higher than upwind, but this was not the case; no significant gradients in concentration were observed between the two sides of these streets. Hence, the contribution of emissions from vehicles on the surface streets to our pre-sunrise measurements ranged from minor to insignificant compared to emissions from freeway traffic.

One case in which we find evidence of a minor contribution from non-freeway emissions involves the shallow shoulder in UFP concentrations on Ocean Park Blvd. (~950 m downwind) and shown in Fig. 3. Traffic counts on this major surface street were ~8% of the freeway counts (Fig. 7a), which may have resulted in a small local UFP, NO, and PB-PAH contribution to the measured concentration. A local contribution of ~6% traffic count on Pico Blvd. is not apparent in the measured UFP concentration in Fig. 3, probably due to the closer proximity of Pico Blvd. to the I-10 freeway (~250 m downwind).

Although the mobile platform measurements could be affected by emissions from vehicles occasionally encountered on the pre-sunrise route or cross-surface streets, these encounters typically exhibited only a short, transient spike of elevated concentrations. Furthermore, the overall pre-sunrise concentrations and gradients presented were averaged from 18 to 24 runs in winter and 12–16 runs in summer and for all these reasons were generally not significantly affected by emissions from occasionally encountered nearby vehicles. The Santa Monica Airport (SMA), a small local airport, located south of the pre-sunrise route, had no impact on

any of our pre-sunrise measurements since it has severely restricted hours to minimize noise pollution, and was closed during all of our pre-sunrise experiments.

### 3.4. Size distribution of UFP along pre-sunrise route

The use of a fast mobility particle sizer (FMPS), with its 10 s scans, allowed accurate monitoring of the changing particle size distribution as a function of distance away from the freeway. Fig. 9 shows average UFP size distributions for five downwind and two upwind intersections during the pre-sunrise hours in the winter season, with decreasing particle numbers and increasing sizes as distance downwind increases, until the upwind size distribution was roughly matched at 2600 m. At the downwind intersections up to 1200 m from the freeway, two to four times higher concentrations of ultrafine particles less than 40 nm were observed compared with upwind locations (Fig. 9).

For the intersections nearest the freeway (e.g. Kansas, 100 m downwind, and Pico, 250 m downwind), bi-modal peaks in the size ranges of  $\sim 9$ –12 nm and 16–20 nm were observed. For downwind intersections farther away and for the upwind intersections, UFP peaks observed were typically  $\sim 9$ –12 nm and  $\sim 16$ –20 nm, and 28–35 nm, corresponding to freshly generated UFP and aged particles, respectively. UFP size distributions at a distance of 2600 m downwind (Palms Blvd.) and 1000 m upwind (Harvard St), considered “background” locations, were similar with a dominant mode at 30–60 nm.

In summer, downwind UFP size distributions also had a small mode of 9–12 nm. The persistence of the 9–12 nm peak in UFP concentrations during pre-sunrise hours over a wide area can be attributed to increased condensation of organic vapors and slower rates of conversion to larger particles for the cooler, stable air conditions prior to sunrise during our winter and summer campaigns. These conditions would also promote the more elevated UFP concentrations observed in our pre-sunrise runs compared with daytime runs.

### 3.5. Pre-sunrise vs. daytime concentrations in present study: exposure implications

Although traffic volumes on the freeway during the pre-sunrise hours were markedly lower than during the daytime ( $\sim 30$ –80% of peak congestion traffic volumes), air pollutant concentrations measured prior to sunrise were significantly higher than in the

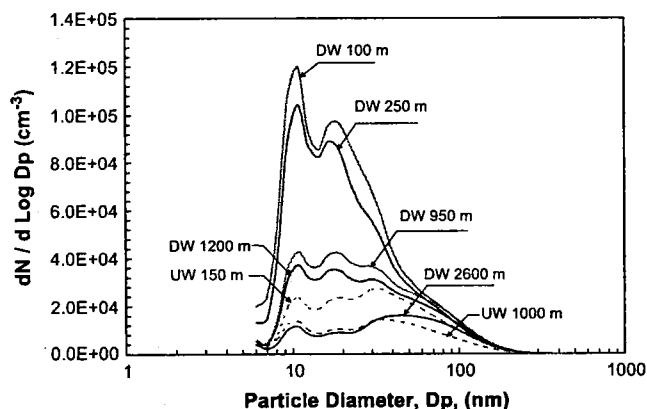


Fig. 9. Size distributions of ultrafine particles measured by a TSI Model 3091 FMPS at upwind (UW) and downwind (DW) intersections during the pre-sunrise hours in the winter season.

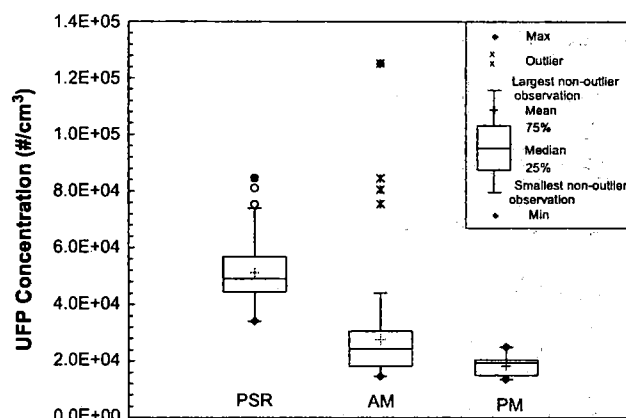


Fig. 10. Comparison of UFP concentrations on Pearl St. (600 m south of I-10 freeway) at different times in winter: pre-sunrise (PSR), morning (AM), and afternoon (PM).

morning or afternoon runs. Fig. 10 shows the UFP concentrations measured at Pearl St.,  $\sim 600$  m south of the freeway, during the pre-sunrise and daytime hours in winter. The median UFP concentrations were  $49\,000\text{ cm}^{-3}$ ,  $24\,000\text{ cm}^{-3}$ , and  $19\,000\text{ cm}^{-3}$  for the pre-sunrise, morning, and afternoon, respectively. Clearly, there was sufficient traffic flow on the I-10 freeway combined with the meteorological conditions during pre-sunrise hours to result in elevated concentrations of UFP, NO, and PB-PAH over a wide area of the downwind (up to  $\sim 2000$  m) and upwind (up to  $\sim 600$  m) residential neighborhoods. Since the pre-sunrise hours are at a time when most people are in their homes, our observations imply the potential for elevated exposures for many more residents in these neighborhoods, adjacent to freeways; far above the numbers of people that live within the  $\sim 300$ –500 m range reported in earlier daytime and evening studies. Additional measurements in the pre-sunrise period downwind of other major roadways should be conducted to confirm our novel findings.

## 4. Conclusions

A wide impact area of elevated pollutant concentrations on the downwind (up to  $\sim 2000$  m) and upwind (up to  $\sim 600$  m) sides of a freeway was measured during the pre-sunrise hours under typical meteorological conditions characterized by weak winds and a strong radiation inversion. To make these measurements, a mobile platform, equipped with fast-response monitoring instruments, drove along a transect crossing under the I-10 freeway and passing through a large residential neighborhood. On the upwind side of the freeway, air pollutant concentrations dropped quickly, but remained elevated up to  $\sim 600$  m. On the downwind side, air pollutant concentrations (UFP, PAH, NO) dropped much more slowly and extended far beyond the typical  $\sim 300$  m distance associated with the return to background pollutant levels observed in previous studies conducted during daytime. For example, elevated ultrafine particle concentration of about  $40\,000\text{ cm}^{-3}$  extended to  $\sim 1200$  m downwind of the freeway in the winter season, which was about 40% of the peak UFP concentration adjacent to the freeway.

Although traffic volumes during the pre-sunrise hours were lower than during the day, the UFP concentrations were significantly higher in the pre-sunrise period. We attribute this pre-sunrise phenomenon to strong atmospheric stability, low wind speeds ( $\sim 0$ –1  $\text{m s}^{-1}$ ), low temperatures ( $\sim 9$ –13  $^{\circ}\text{C}$ ), and high humidities ( $\sim 61$ –79%), facilitating longer lifetimes and slower transport of UFP before dilution and dispersion to background levels. Nocturnal inversions are

a widespread phenomenon particularly on clear nights, and our results suggest broad areas of elevated pollutants around major roadways are expected to be common in the early-morning hours. The implications of these observations for exposures to vehicle-related pollutants should be further explored.

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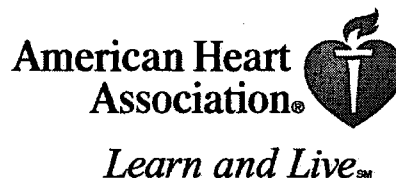
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## **Ambient Particulate Pollutants in the Ultrafine Range Promote Early Atherosclerosis and Systemic Oxidative Stress**

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# Ambient Particulate Pollutants in the Ultrafine Range Promote Early Atherosclerosis and Systemic Oxidative Stress

Jesus A. Araujo, Berenice Barajas, Michael Kleinman, Xuping Wang, Brian J. Bennett, Ke Wei Gong, Mohamad Navab, Jack Harkema, Constantinos Sioutas, Aldons J. Lusis, Andre E. Nel

**Abstract**—Air pollution is associated with significant adverse health effects, including increased cardiovascular morbidity and mortality. Exposure to particulate matter with an aerodynamic diameter of  $<2.5\ \mu\text{m}$  ( $\text{PM}_{2.5}$ ) increases ischemic cardiovascular events and promotes atherosclerosis. Moreover, there is increasing evidence that the smallest pollutant particles pose the greatest danger because of their high content of organic chemicals and prooxidative potential. To test this hypothesis, we compared the proatherogenic effects of ambient particles of  $<0.18\ \mu\text{m}$  (ultrafine particles) with particles of  $<2.5\ \mu\text{m}$  in genetically susceptible (apolipoprotein E-deficient) mice. These animals were exposed to concentrated ultrafine particles, concentrated particles of  $<2.5\ \mu\text{m}$ , or filtered air in a mobile animal facility close to a Los Angeles freeway. Ultrafine particle-exposed mice exhibited significantly larger early atherosclerotic lesions than mice exposed to  $\text{PM}_{2.5}$  or filtered air. Exposure to ultrafine particles also resulted in an inhibition of the antiinflammatory capacity of plasma high-density lipoprotein and greater systemic oxidative stress as evidenced by a significant increase in hepatic malondialdehyde levels and upregulation of Nrf2-regulated antioxidant genes. We conclude that ultrafine particles concentrate the proatherogenic effects of ambient PM and may constitute a significant cardiovascular risk factor. (*Circ Res.* 2008;102:589-596.)

**Key Words:** air pollution ■ ultrafine particles ■ atherosclerosis ■ oxidative stress ■ HDL

It is increasingly being recognized that exposure to ambient particulate matter (PM) contributes to significant adverse health effects and is a risk factor for the development of ischemic cardiovascular events via exacerbation of atherosclerosis, coronary artery disease, and the triggering of myocardial infarctions.<sup>1</sup> Although this association has been documented for PM with a mean aerodynamic diameter of  $<10\ \mu\text{m}$  ( $\text{PM}_{10}$ ), there is increasing evidence that smaller particles may pose an even greater health risk. A growing literature indicates that fine particles (FPs) with an average aerodynamic diameter of  $<2.5\ \mu\text{m}$  ( $\text{PM}_{2.5}$ ) exert adverse health effects of greater magnitude. For example, the "Women's Health Initiative study demonstrated a 24% increase in the incidence of cardiovascular events and a 76% increase in cardiovascular mortality for every  $10\ \mu\text{g}/\text{m}^3$  increase in the annual average  $\text{PM}_{2.5}$  level.<sup>2</sup> It appears that the smallest particles that exist in the urban environment are the most dangerous.<sup>3</sup> Ambient ultrafine particles (UFPs) that have an aerodynamic diameter of  $<0.18\ \mu\text{m}$  are by far the most abundant particles by number in urban environments such as Los Angeles. Because these particles are emitted mainly by

vehicular emissions and other combustion sources, they contain a high content of redox-cycling organic chemicals that could be released deep into the lungs or could even spill over into the systemic circulation. Thus, UFPs may be particularly relevant from the perspective of cardiovascular injury.<sup>3</sup>

In spite of the epidemiological evidence indicating that ambient PM can promote cardiovascular injury and atherosclerosis, the mechanisms of the cardiovascular injury and proatherogenic effects are not clear. However, experimental studies in susceptible animal models have shed some light on disease pathogenesis. For instance, intratracheal administration of ambient  $\text{PM}_{10}$  in Watanabe rabbits<sup>4</sup> or long-term exposure of apolipoprotein (apo)E-null mice to  $\text{PM}_{2.5}$ <sup>5,6</sup> enhanced atherosclerotic plaque growth. Moreover, a cross-sectional exposure study in humans showed a 5.9% increase in carotid intima-medial thickness for every  $10\ \mu\text{g}/\text{m}^3$  rise in  $\text{PM}_{2.5}$  levels,<sup>7</sup> and a prospective cohort study supported an association between long-term residential exposure to high-traffic levels of  $\text{PM}_{2.5}$  and coronary atherosclerosis, as assessed by coronary artery calcification scores,<sup>8</sup> demonstrating

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From the Department of Medicine (J.A.A., B.B., X.W., B.J.B., K.W.G., M.N., A.J.L., A.E.N.), David Geffen School of Medicine, and Department of Civil and Environmental Engineering (C.S.), University of Southern California, Los Angeles; Department of Community and Environmental Medicine (M.K.), University of California, Irvine; and Department of Pathobiology and Diagnostic Investigation (J.H.), Michigan State University, East Lansing.

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that the proatherogenic effects of PM are clinically relevant.<sup>7,8</sup> Air pollution has also been linked to the triggering of acute coronary ischemic events in humans, including myocardial infarction.<sup>9</sup>

We have demonstrated that ambient PM exerts proinflammatory effects in target cells such as endothelial cells,<sup>10</sup> macrophages,<sup>11</sup> and epithelial cells<sup>12</sup> through the generation of reactive oxygen species (ROS) and oxidative stress.<sup>11,12</sup> These prooxidative effects are mediated, in part, by redox-cycling organic chemicals and transition metals that are present on the particle surface.<sup>11</sup> Ambient PM can synergize with oxidized phospholipids in the induction of a wide array of genes involved in vascular inflammatory processes such as atherosclerosis.<sup>10</sup> Moreover, when comparing concentrated ambient particles (CAPs) of various sizes in the Los Angeles basin, UFPs were shown to have the highest content of redox cycling chemicals and therefore displayed the largest prooxidant potential, both abiotically and biotically.<sup>13</sup> We hypothesized, therefore, that UFPs may concentrate some of the PM proatherogenic effects by promoting prooxidant and proinflammatory effects. We used the particle concentrator technology available in the Southern California Particle Center to evaluate the atherogenic potential of concentrated UFPs versus concentrated PM<sub>2.5</sub> in apoE-null mice. In addition, we evaluated the effects of particle exposures on the plasma high-density lipoprotein (HDL) antiinflammatory activity as well as markers of systemic oxidative stress. Our data show that UFPs are more proatherogenic, exert the strongest prooxidative effects, and are associated with the largest decrease in HDL protective activity. These data are of considerable significance from a regulatory perspective.

## Materials and Methods

Detailed methods about histology, immunohistochemistry, blood chemistry, monocyte chemotactic assays, lipid peroxidation assay, RNA extraction, and real-time RT-PCR can be found in the online data supplement at <http://circres.ahajournals.org>.

## Animals and Diet

The Animal Research Committee at The University of California at Los Angeles (UCLA) approved all animal protocols. ApoE<sup>-/-</sup> (C57BL/6J background) male mice were obtained from The Jackson Laboratory (Bar Harbor, Me). Animals were brought to the UCLA animal facility at 4 weeks of age. Mice were fed a regular chow diet (NIH-31 modified 6% diet; Harlan Teklad, Madison, Wis). Both water and food were administered ad libitum. Animals were randomly assigned to 3 groups (n=17/group) that were sent to a mobile inhalation toxicology laboratory located 300 meters from the 110 Freeway. This freeway carries a high volume of gasoline and diesel motor vehicle transit, resulting in high levels of PM<sub>2.5</sub> mass and UFP counts at the exposure site (Table). The mobile research laboratory (AirCARE 1) is owned by Michigan State University.<sup>14</sup> Mice were subjected to CAP exposures starting at 6 weeks of age over a 40-day period. One mouse in the FP group and 2 in the UFP group died during the course of the study. Animals were euthanized 24 to 48 hours after completion of the last CAP exposure, and aortas and various organs were harvested. Between exposures, mice were housed in a Hazelton chamber<sup>15</sup> that was ventilated with air from which 99.9% of the incident particles were removed by a HEPA filter.

## CAP Exposures and Chemical Characterization

Whole-body exposures were performed simultaneously in sessions of 5 hours per day, 3 days per week, for a combined total of 75 hours.

**Table. Characteristics of Experimental Exposure Protocol of ApoE-Null Mice Fed a Normal Chow Diet**

| Experimental Parameter  |                              |
|---|------------------------------|
| Groups  | FA, FP, UFP                  |
| Exposure time (dates)   | 11/03/2005 to 12/12/2005     |
| Exposure time (hours)   | 75                           |
| Total ambient particle no. (particles/cm <sup>3</sup> )                         | 3.42 (±0.96)×10 <sup>4</sup> |
| No. concentration in FA chamber (particles/cm <sup>3</sup> )                    | <5000                        |
| No. concentration in FP chamber (particles/cm <sup>3</sup> )                    | 4.56 (±1.06)×10 <sup>5</sup> |
| Calculated UFP no. concentration in the FP chamber (particles/cm <sup>3</sup> ) | 3.88 (±1.06)×10 <sup>5</sup> |
| No. concentration in UFP chamber (particles/cm <sup>3</sup> )                   | 5.59 (±1.23)×10 <sup>5</sup> |
| Ratio of UFP in the FP vs the UFP chamber*                                      | 1:1.44                       |
| FP chamber particle enrichment factor   | 13.35 (±1.6)                 |
| UFP chamber particle enrichment factor  | 16.4 (±1.8)                  |
| Mass in FP exposure chamber (μg/m <sup>3</sup> )                                | 438.29                       |
| Mass in UFP exposure chamber (μg/m <sup>3</sup> )                               | 112.61                       |
| PM <sub>2.5</sub> mass in ambient air (μg/m <sup>3</sup> )                      | 26.78                        |
| UFP mass in ambient air (μg/m <sup>3</sup> )                                    | 8.43                         |

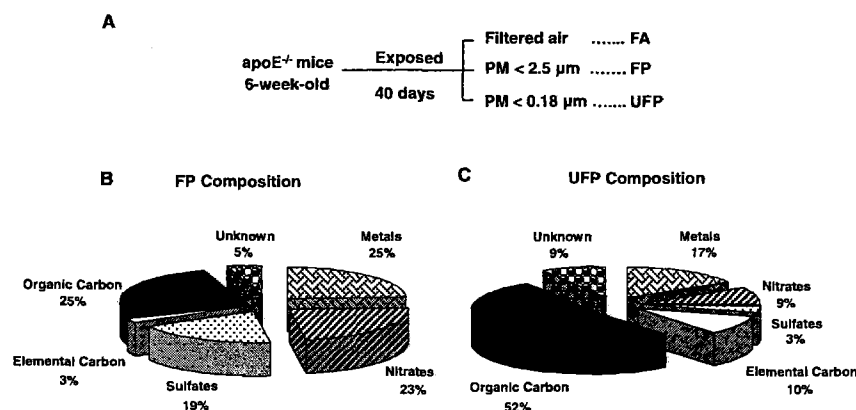
FA, FP, and UFP groups were exposed in a mobile laboratory located in downtown Los Angeles. Values shown are means(±SD). \*This ratio was obtained by reducing the particle no. in the FP chamber by 15%, which represents the contribution of particles in the 0.18–2.5 μm range. This also translates into an ≈2-fold increase in surface area if a spherical particle shape is assumed.

Particle concentrator technology was used to deliver the CAP exposures. Three animal groups were simultaneously exposed to atmospheres containing concentrated particles of <2.5 μm (FPs), particles of <0.18 μm (UFPs), and filtered air (FA). Briefly, ambient air was drawn through an aluminum duct into the VACES (Versatile Aerosol Concentration Enrichment System)<sup>16,17</sup> and delivered to whole-body exposure chambers.<sup>18,19</sup> The FP and UFP aerosol concentrators delivered 0.01- to 2.5-μm and 0.01- to 0.18-μm aerosols, respectively (Table). The FP atmosphere included sub-18 μm particles that were ≈40% fewer particles than in the UFP chamber. Temperature and airflow were controlled to ensure adequate ventilation and minimize buildup of animal-generated dander, ammonia, CO<sub>2</sub>, and thermal stress. Mobilization of mice between the Hazelton chamber and the exposures chambers was performed over the shortest time period possible to limit the exposure to ambient air PM in the trailer. CAP number concentrations were measured with a TSI 3022 Condensation Particle Counter, and particle mass concentration was assessed with a DataRAM Model DK-2000.

Particle mass concentration and elemental CAP composition were measured by particle collection on 37-mm Teflon filters (PTFE 2-μm pore, Gelman Science, Ann Arbor, Mich). Concentrations of inorganic ions (sulfate and nitrate), elemental carbon, organic carbon (OC), polycyclic aromatic hydrocarbon (PAH) content, and particle-bound trace elements and metals was performed as previously described.<sup>16–18</sup>

## Statistical Analysis

All data were expressed as means±SEM unless indicated otherwise. Differences between experimental groups were analyzed by 1-way ANOVA with a 1-tailed Fisher protected least-significance difference (PLSD) post hoc analysis test. Differences were considered statistically significant at *P*<0.05.



**Figure 1.** CAP exposures. A, Experimental protocol. Three groups ( $n=17$ ) of 6-week-old male apoE-null mice were exposed to FA,  $PM_{2.5}$ , and  $PM_{<0.18}$  (UFPs) for 40 days. B and C, Chemical composition of CAPs. UFP air had a greater content of organic and elemental carbon than FP air. Particle chemical composition of the FP (B) and UFP (C) chambers was performed as described in Materials and Methods.

## Results

### UFP Exposures Are Enriched in OC Substances Such As PAHs

Six-week-old male apoE-null mice were exposed in a mobile inhalation toxicology laboratory in downtown Los Angeles to CAPs in the size range of  $<2.5 \mu m$  (FP exposures) or  $<0.18 \mu m$  (UFP exposures). Controls consisted of mice exposed to FA (Figure 1A). Animals were simultaneously exposed to UFPs, FPs, and FA for a total of 75 hours over a 40-day time period while being kept on a chow diet. The atmospheric conditions and particle characteristics in the FP and UFP chambers are summarized in the Table. Because the FP atmosphere included particles of  $<0.18 \mu m$  (UFPs) that accounted for up to 85% of the total particle number, the actual number of these sub- $0.18 \mu m$  particles was  $\approx 44\%$  greater in the UFP chamber (Table), despite a total UFP mass that was approximately one-quarter of the FP mass. Assuming a roughly spherical shape for the particles, this 44% increase in sub- $0.18 \mu m$  particle numbers in the UFP chamber translates into an  $\approx 2$ -fold increase in the particle surface area. This was also accompanied by an  $\approx 2$ -fold increase in fractional OC content (Figure 1B and 1C), which is theoretically more bioavailable than the smaller organic fraction on FPs (Figure 1B). Thus, the increased particle number, greater surface area, and higher fractional carbon composition could combine to deliver a much higher biological effective dose of the injurious components in the UFP compared with the FP chamber. In fact, measurement of a set of signature PAHs in filter samples that were collected concurrently with the CAP exposures, demonstrated that the PAH content of the UFPs was roughly twice as high as the FP content when corrected for a per mass basis (Figure 2). Although there is no definitive evidence that PAHs are those responsible for adverse cardiovascular effects, we have previously demonstrated that their abundance is a good proxy for the prooxidant potential of PM.<sup>13</sup>

### UFP Exposure Promotes Atherosclerosis

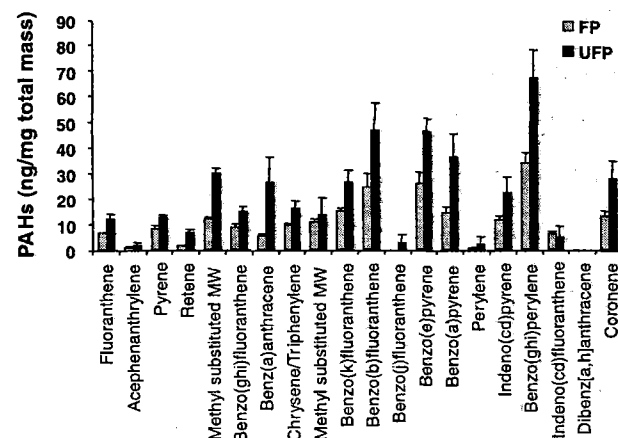
Exposure to the UFP atmosphere for 75 hours over a 40-day interval resulted in 55% greater aortic atherosclerotic lesion development ( $33.011 \pm 3741$ ,  $n=15$ ) as compared with FA controls ( $21.362 \pm 2864$ ,  $n=14$ ;  $P=0.002$ ) (Figure 3). Exposure to the FP atmosphere resulted in a similar trend but of lesser magnitude ( $P=0.1$ ). Interestingly, UFP mice exhibited

a 25% increase in atherosclerotic lesions in comparison with FP mice ( $26.361 \pm 2275$ ,  $n=16$ ,  $P=0.04$ ), which suggests that the smallest particles are indeed more proatherogenic.

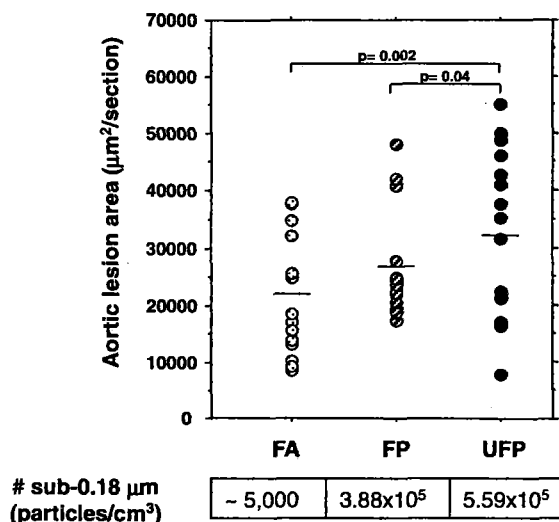
Histological analysis revealed that lesions were predominantly comprised of macrophage infiltration with intracellular lipid accumulation (foam cells) (Figure 4). These cells contributed, on average,  $>85\%$  of the total lesional area in all the groups (supplemental Table I). UFP-exposed animals developed more extensive as well as thicker atherosclerotic plaques that showed the same relative abundance of macrophages and smooth muscle cells, as determined by MOMA-2 and  $\alpha$ -actin immunohistochemical staining (Figure 4 and supplemental Table I).

### Exposure to Ambient CAPs Results in Loss of HDL Antiinflammatory Properties

FP but not UFP exposures resulted in a small but significant increase in plasma total cholesterol in comparison to other groups (supplemental Table II). Although all animals displayed similar levels of plasma HDL cholesterol (supplemental Table II), we did observe a change in HDL antiinflammatory properties. This was demonstrated by comparing the antiinflammatory protective capacity of HDL against LDL-



**Figure 2.** OC composition. Mass concentration fraction of PAHs in the FP (gray) and UFP (black) chambers. Data are shown as nanogram per milligram of PM mass and represent the average of composition analysis performed on filter samples collected for 2 experiments. PAH analysis was performed by means of gas chromatography-mass spectroscopy as described.<sup>16-18</sup>

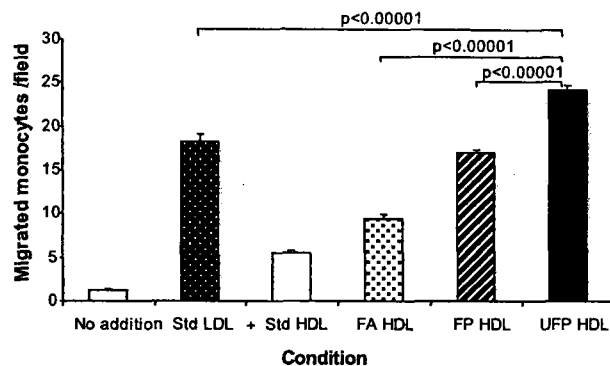


**Figure 3.** UFP is the most proatherogenic PM fraction. Atherosclerotic lesions were quantitatively analyzed in serial aortic root sections and stained with oil red O. Lesional area was scored as square micrometer per section, averaged  $\geq 25$  sections per animal. Group averages are indicated by straight horizontal bars. One FA mouse was an obvious outlier in its group and removed from the atherosclerotic lesion analysis. However, its inclusion did not modify the overall significance. FA mice are represented by dotted circles ( $n=14$ ), FPs by stripped circles ( $n=16$ ), and UFPs by filled circles ( $n=15$ ).

induced chemotaxis (Figure 5). Plasma HDL from both FP and UFP animals exhibited significantly less protective effect than HDL from the FA group (Figure 5). Moreover, the antiinflammatory effect of HDL from the UFP group was significantly decreased compared with the FP group. These results are in good agreement with the extent of vascular lesions in the different animal groups, suggesting that a PM-induced decrease in the HDL antiinflammatory protective capacity could contribute to atherogenesis.

#### UFP Exposure Leads to the Expression of Systemic Biomarkers of Oxidative Stress and Activation of the Unfolded Protein Response

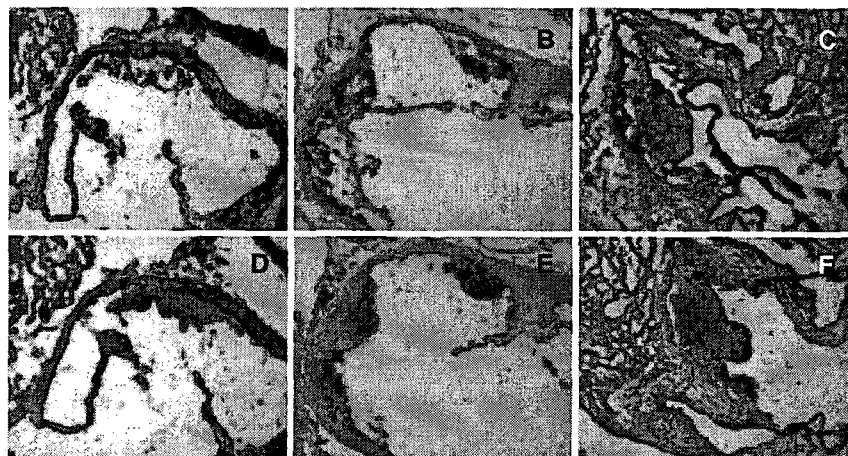
One of the major mechanistic hypotheses regarding PM injury is the ability of the particles to induce ROS production and oxidative stress. To probe for the presence of oxidative



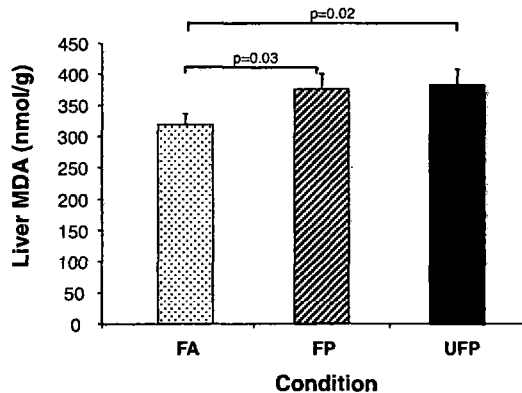
**Figure 5.** PM exposure leads to a loss of HDL antiinflammatory properties. Pooled plasma HDL from FA ( $n=16$ ), FP ( $n=16$ ) and UFP mice ( $n=15$ ) was added to cocultures of human artery wall cells in the presence of standard (Std) human LDL, as described in Materials and Methods. Values are expressed as means  $\pm$  SEM of the number of migrated monocytes in 9 fields. Statistical analysis was performed by 1-way ANOVA (Fisher PLSD).

stress, we explored whether CAP exposure could result in lipid peroxidation in the liver. We observed statistically significant increases in the hepatic malondialdehyde (MDA) levels in the UFP compared with the FA group ( $P=0.02$ ) (Figure 6). FP mice also demonstrated increases in lipid peroxidation compared with the FA group ( $P=0.03$ ). These data suggest that CAP exposure leads to systemic oxidative stress.

We also explored whether differences in lipid peroxidation were accompanied by phase II antioxidant responses that are mediated via the p45-NFE2-related transcription factor 2, Nrf2.<sup>11</sup> This constitutes one of the most sensitive oxidative stress effects that can be traced to prooxidative PM in vitro and in vivo.<sup>11,20</sup> UFP mice exhibited a significant increase in the expression of Nrf2 as well as genes that are secondarily regulated by this transcription factor (Figure 7). Indeed, UFP mice displayed Nrf2 mRNA levels that were 68% greater than FA and FP mice ( $P=0.01$ ). Likewise, as compared with the FA group, UFP mice displayed significantly greater levels of catalase (3.7-fold), glutathione *S*-transferase Ya (5.3-fold), NAD(P)H-quinone oxidoreductase 1 (1.8-fold), and superoxide dismutase 2 (1.4-fold) (Figure 7). Interestingly, increased tissue oxidative stress was also accompanied by the activation



**Figure 4.** Representative histological photomicrographs. A through C, Oil red O staining for neutral lipids in representative aortic root sections of FA (A), FP (B), and UFP (C) mice. D through F, MOMA-2 immunohistochemical staining in adjacent aortic root sections to those shown in the top row, corresponding to the same FA (D), FP (E), and UFP (F) mice. Both oil red O and MOMA-2 staining yielded red-stained areas. UFP mice exhibited more extensive atherosclerotic plaques (C and F) than FP (B and E) or FA animals (A and D), all consisting primarily of foam cells and macrophages (fatty streaks). Original magnification,  $\times 100$ .



**Figure 6.** UFP exposure increases liver lipid peroxidation. MDA was assessed in liver homogenates as described in Materials and Methods. Values are expressed as the means  $\pm$  SEM of MDA (nmol/g) in animals from the FA ( $n=16$ ), FP ( $n=15$ ), and UFP ( $n=14$ ) groups. Statistical analysis was performed by 1-way ANOVA (1-tailed Fisher PLSD).

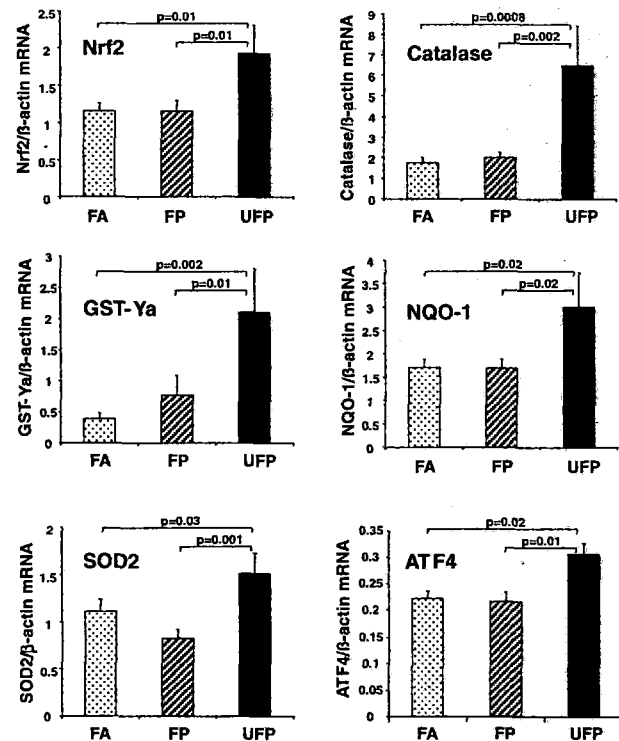
of the unfolded protein response in the liver because the UFP-exposed mice displayed 41% and 37% greater expression of activating transcription factor 4 than FP mice ( $P=0.01$ ) and FA controls ( $P=0.02$ ) (Figure 7).

### Discussion

We demonstrate that atherosclerotic plaque formation in apoE-null male mice is enhanced by exposure to sub- $0.18 \mu\text{m}$  particles. Mice exposed to UFPs alone exhibited greater and more advanced lesions compared with FA- or FP-exposed animals. UFP mice also showed a comparatively greater decline in the antiinflammatory capacity of plasma HDL as well as increased phase II enzyme mRNA expression in the liver. These results support the hypothesis that exposure to UFPs may enhance atherosclerosis via the promotion of systemic prooxidant and proinflammatory effects.

Our study significantly extends previous data showing that PM potentiates atherosclerotic lesion development in animals.<sup>4-6</sup> The fact that FP mice displayed a nonstatistically significant trend to develop more atherosclerotic lesions than FA controls could be attributable to the relatively short duration of our exposure (40 days), which stands in contrast to the 5- to 6-month exposure period that was previously used to demonstrate a 45% to 58% increment in atherosclerotic lesion development during  $\text{PM}_{2.5}$  exposure.<sup>5,6</sup> Of interest, our UFP animals exhibited a similar 55% increment over FA controls despite an exposure duration that was 4 to 5 times shorter, indicating the greater proatherogenic toxicity of sub- $0.18 \mu\text{m}$  particles. This supports the notion that the adverse cardiovascular effects of PM are exaggerated by a small particle size.

A number of injury mechanisms have been proposed to explain the adverse health effects of PM, including its ability to stimulate oxidative stress and inflammation, alter blood clotting, stimulate autonomic nervous system activity, or act as a carrier for endotoxin.<sup>1</sup> A key injury mechanism appears to be the generation of inflammation as a direct consequence of the ability of ambient particles and their adsorbed chemicals to induce ROS and oxidative stress.<sup>21</sup> Oxidative stress



**Figure 7.** UFP exposure leads to upregulation of antioxidant genes in the liver. mRNA levels for antioxidant genes were determined by quantitative PCR in the livers of chow-fed mice exposed to CAPs for 40 days. Values are expressed as the means  $\pm$  SEM of mRNA levels normalized by  $\beta$ -actin mRNA. Ten samples per group were assayed in duplicate. Statistical analysis was performed by 1-way ANOVA (1-tailed Fisher PLSD;  $*P<0.05$ ). ATF4 indicates activating transcription factor 4; GST-Ya, glutathione S-transferase Ya; NQO-1, NAD(P)H-quinone oxidoreductase 1; SOD2, superoxide dismutase 2.

initiates proinflammatory signaling cascades, including the Jun kinase and nuclear factor  $\kappa\text{B}$  cascades<sup>16,22,23</sup> that are relevant to atherogenesis. According to the hierarchical oxidative stress hypothesis, the induction of Nrf2-induced phase II enzyme expression is an integral oxidative stress protective pathway that acts as a sensitive marker for oxidative stress.<sup>24</sup> Indeed, important cytoprotective, antiinflammatory and antioxidant phase II enzymes including catalase, superoxide dismutase 2, glutathione S-transferase Ya, and NAD(P)H-quinone oxidoreductase 1 were all significantly upregulated in the liver of UFP mice (Figure 7) and, together with Nrf2 upregulation, suggest the triggering of a Nrf2-driven antioxidant response.

Our results support the notion that the generation of systemic oxidative stress is responsible for the observed vascular effects. Possible explanations for these systemic effects are: First, inhaled particles may release organic chemicals and transition metals from the lung to the systemic circulation. Second, pulmonary inflammation could lead to the release of ROS, cytokines and chemokines to the systemic circulation. Although we did not observe any major increase in inflammatory cells during the performance of bronchoalveolar lavage in these mice, future studies will need to address whether any subtle proinflammatory effects in the lung could play a role. Third, UFPs could gain access to the

systemic circulation by directly penetrating the alveolar/capillary barrier.<sup>25</sup> However, this possibility is still controversial. Although reports of the systemic translocation of <sup>99m</sup>Tc-labeled ultrafine carbon particles<sup>25</sup> or albumin nanocolloid particles of <80 nm<sup>26</sup> have appeared in the literature, skepticism has been expressed about the stability of the labeling procedures. Moreover, the same has not been demonstrated for ambient air "nanoparticles."

The particles or their chemicals may generate ROS systemically via a number of different pathways, including redox cycling of quinones, metabolism and functionalization of PAHs, activation of leukocyte NADPH oxidase and myeloperoxidase, or interference in 1-electron transfers in the mitochondrial inner membrane.<sup>27</sup> It is also possible that the particles themselves or their chemical components may synergize with oxidized LDL in promoting endothelial cell dysfunction. Indeed, we have shown that ambient PM can synergize with oxidized phospholipids in the induction of a large number of genes in a human microvascular endothelial cell line, many of which belong to antioxidant, proinflammatory, unfolded protein response, or proapoptotic pathways.<sup>10</sup> ROS generation and antioxidant responses constitute a dynamic equilibrium. The greater prooxidant stimulus delivered by the UFPs could be more prone to overwhelm the concomitant generation of a protective antioxidant response. On the other hand, it is interesting that no differences were noted between the FP and UFP exposures in the MDA assay. Although the methodology used is sensitive and specific for the determination of MDA,<sup>28</sup> there are several limitations in this assay in reflecting the degree of lipid peroxidation, as reviewed by Janero et al,<sup>29</sup> such as: (1) MDA yield as a result of lipid peroxidation varies with the nature of the polyunsaturated fatty acids peroxidized (especially its degree of unsaturation) and the peroxidation stimulus; (2) only certain lipid oxidation products decompose to yield MDA; (3) MDA is only one of several (aldehydic) end products of fatty peroxide formation and decomposition; (4) the peroxidation environment influences both the formation of lipid-derived MDA precursors and their decomposition to MDA; (5) MDA itself is a reactive substance that can be oxidatively and metabolically degraded; (6) oxidative injury to nonlipid biomolecules has the potential to generate MDA. Thus, if FP and UFP exposures impacted these factors in a different extent, it may explain a greater degree of lipid peroxidation not reflected by the MDA measurements.

PM-induced systemic inflammation and oxidative stress could also adversely affect lipoprotein function, including interfering in the beneficiary effects of HDL on reverse cholesterol transport<sup>30</sup> and the antiinflammatory<sup>31</sup> effects of this lipoprotein fraction. Indeed, both FP and UFP mice exhibited the development of dysfunctional HDL, which was more severe in the latter group in terms of its proinflammatory potential (Figure 5). Such proinflammatory effects were also supported by the greater expression of activating transcription factor 4 in liver, an unfolded protein response component that we have shown to exert proinflammatory effects in endothelial cells by inducing the expression of interleukin-6, interleukin-8, and monocyte chemotactic protein 1.<sup>32</sup> Likewise, we have also shown that prooxidative

diesel exhaust particle chemicals induce an unfolded protein response in bronchial epithelial cells.<sup>33</sup> Changes in HDL function were observed in the absence of changes on HDL quantitative levels. On the other hand, FP exposures did result in greater total cholesterol levels in the FP versus FA mice, whereas UFP levels were unaffected. These higher cholesterol levels in the FP mice may have resulted in narrowing of the differences in atherosclerosis in between FP and UFP mice that otherwise could have been larger than the 23% observed difference. Consistent with our results, it has been reported that the HDL antiinflammatory profile can be hampered by environmental factors such as the exposure to prooxidative chemicals present in cigarette smoke.<sup>34</sup> For example, mice exposed to second-hand smoke develop dysfunctional HDL.<sup>35</sup> A possible mechanism could be interference with paraoxonase and lecithin cholesterol acyltransferase activities by redox-active chemical compounds. In particular, prooxidative PM chemicals may affect critical thiol groups that are responsible for the catalytic activity of paraoxonase, leading to increased susceptibility to atherosclerosis.<sup>36</sup>

The fact that the FP atmosphere contains both UFPs and particles of >0.18  $\mu$ m makes interpretation of those data complex. However, we have shown that the 25% difference in atherosclerotic lesion scores could be explained by the 44% increase in UFP particle number (Table and Figure 3). Total particle mass was clearly not a determining factor because the FP atmosphere had  $\approx$ 3.9-fold greater mass than the UFP aerosol. What is likely significant is that UFPs have an  $\approx$ 2-fold increase in the OC and PAH content on a per mass basis (Figures 1 and 2). It is possible that these prooxidative components could be delivered from a surface area that is twice as big in particles associated with the UFP atmosphere. Although we cannot claim that the PAHs are actually responsible for the lesion development, these organic chemical compounds are a good proxy for the prooxidative potential of UFPs.<sup>13</sup>

How do our experimental atmospheres relate to real life exposures? The particle numbers in our study were 2- to 6-fold higher than the in-vehicle exposures that commuters may encounter while traveling on Los Angeles freeways.<sup>37</sup> It was not logistically feasible to perform detailed dose- and time-response studies; this type of data will be important to obtain in future studies. Although it would clearly be advantageous to know the minimum exposure that is required for proatherogenic effects, previous epidemiological studies have shown that cardiovascular morbidity and mortality increase linearly without a threshold effect.<sup>38,39</sup> Differences in the physiology of genetically susceptible animals and humans also have to be taken into consideration when extrapolating this work to cardiovascular disease in humans.

In conclusion, we demonstrate that UFP exposures have a higher proatherogenic potential than FP exposures. These effects could be linked to a greater propensity of UFPs to generate systemic oxidative stress and to interfere with the antiinflammatory capacity of plasma HDL. Our findings are important in explaining how ambient PM may contribute to daily total and cardiovascular mortality.<sup>40</sup> Although such an association has been established previously for PM<sub>10</sub> and PM<sub>2.5</sub>,<sup>2,41,42</sup> we demonstrate that UFP exposure could be of

even greater relevance. Further epidemiological and experimental data collection are required to determine the critical physicochemical and toxicological properties of UFPs in humans.

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### Disclosures

None.

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## **ONLINE SUPPLEMENT**

### **MATERIALS AND METHODS**

#### ***Histology and immunohistochemistry***

Atherosclerotic lesions in the aortic root were quantitatively analyzed as previously described<sup>1</sup>. Briefly, the upper portion of the heart and proximal aorta was excised and embedded in OCT compound (Tissue-Tek) and frozen<sup>2</sup>. Serial 10- $\mu$ m-thick cryosections in the aortic root, beginning at the level of the appearance of the aortic valve, were collected for a distance of 500  $\mu$ m. A total of 25 sections, selected as every other section collected over the entire region, were stained with Oil Red O and counterstained with hematoxylin. The lipid-containing area on each section was determined by using a microscope eyepiece grid and expressed in  $\mu$ m<sup>2</sup> lesional area/section. The mean value of lesional areas among the 500  $\mu$ m-spanning sections was referred as the aortic lesion score ( $\mu$ m<sup>2</sup>/section). Cellular composition was assessed by immunohistochemical staining of alternating sections to those stained with Oil Red O, in 3 sections per animal and averaged over four animals per group. Assessment was performed for macrophages (MOMA-2, Beckman Coulter) and smooth muscle cells (smooth muscle  $\alpha$ -actin, Spring Bioscience). Planimetric analysis was performed at 10X using ImagePro Plus software. Relative content of macrophages and/or smooth muscle cells was determined by the percentage of the positively-stained area over the entire lesional area.

#### ***Blood chemistry***

Retro-orbital bleeding was performed under isoflurane anesthesia in 6-hour fasting animals, 1 week prior to the onset (5 weeks of age) as well as at the termination of the exposure protocols (11 weeks of age). Plasma total and HDL cholesterol were determined by enzymatic assays as previously described<sup>3</sup>.

#### ***Monocyte Chemotaxis Assay***

This assay evaluates the protective capacity of HDL against LDL-induced monocyte chemotactic activity. Monocytes were isolated from blood obtained from a large pool of healthy donors at the UCLA Division of Cardiology, Atherosclerosis Research Unit. Human aortic endothelial cells

(HAEC) and human aortic smooth muscle cells (SMC) were isolated from trimmings of fresh surgical aortic specimens from normal donor hearts during transplantation. Endothelial and smooth muscle cells were grown, propagated and used for forming an artery wall model in culture. Cocultures of HAEC and SMC were treated for 18 hours with a standard source of human LDL (100 µg LDL protein/ml), in the absence or presence of a standard source of human or murine HDL (50 µg HDL protein/ml). The LDL and HDL were isolated from normal standard plasma by FPLC<sup>4</sup>. The cells were then washed and incubated in fresh culture medium for 8 hours, following which supernatants were collected to assess monocyte chemotactic activity after 40-fold dilution, which is expressed as the number of monocytes that have transmigrated per high power field, HPF<sup>4</sup>. LDL-induced monocyte chemotactic activity is mostly (70 +/- 4%) a result of the induction of MCP1 secretion, stimulated by oxidized phospholipids that form during the oxidation of LDL by the artery wall cells to generate minimally oxidized LDL<sup>5</sup>. HDL ability to block monocyte chemotaxis correlates with its antioxidant capacity that decreases the generation of minimally oxidized LDL, resulting in inhibition of MCP1 induction and decreased monocyte binding and migration<sup>6-8</sup>.

### ***Lipid Peroxidation Assay***

Malondialdehyde (MDA) content was measured in liver homogenates with a colorimetric assay (OxisResearch, OR) according to the manufacturer's instructions<sup>9</sup>. A standard curve was used to calculate the concentration (nmol/g) of MDA for each sample. The final MDA level represents the average of 14-16 age-matched animals/group.

### ***RNA extraction and real-time RT-PCR***

Total RNA was extracted from liver tissue with the Trizol method (Invitrogen). Reverse transcription was performed using 1 µg of RNA with the iScript cDNA Synthesis kit (Bio-Rad, Hercules, CA). Quantitative real-time polymerase chain reaction (qPCR) was used to measure tissue mRNA expression for heme oxygenase-1 (HO-1), NF-E2-related factor-2 (Nrf2), catalase, superoxide dismutase 2 (SOD2), NAD(P)H-quinone oxidoreductase 1 (NQO1), glutathione S-transferase-Ya (GST-Ya), activating transcription factor (ATF4) and β-actin, utilizing specific PCR primers<sup>10</sup>. The reactions were performed in duplicate on an ABI Prism 7000 (Applied

Biosystems, Foster City, CA, USA) using iQ Sybr Green Supermix (Bio-Rad). Reactions were performed with 0.4  $\mu$ M of primers and 1  $\mu$ g of cDNA template as follows: 95°C for 3 min, 40 cycles of 95°C for 15 sec, 58 - 64°C for 30 sec and 72°C for 30 sec. A standard curve was created from serial dilutions of a pooled sample of cDNA. Gene expression was normalized to  $\beta$ -actin. PCR levels were displayed as arbitrary units.

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**Supplemental Table I.** Cellular composition of atherosclerotic lesions

| Group | MOMA-2 (%) | p (vs. FA) | SMC actin (%) | p (vs. FA) |
|-------|------------|------------|---------------|------------|
| FA    | 88±7       | -          | 14±5          | -          |
| FP    | 86±2       | 0.60       | 10±5          | 0.58       |
| UFP   | 88±3       | 0.91       | 5±7           | 0.42       |

MOMA-2 and SMC  $\alpha$ -actin immunohistochemical staining were performed in 3 sections/animal (n=4 animals/group). Planimetric analysis was performed at 10X using ImagePro Plus software. Data shown represent mean  $\pm$  SE of positive stained area/total lesion area  $\times$  100. Statistical analysis was performed by one-way ANOVA with Fisher's PLSD post hoc analysis. FA: filtered air, FP: fine particles, UFP: ultrafine particles.

**Supplemental Table II. Plasma lipoproteins.**

|                        | Total cholesterol<br>(mg/dl) | HDL cholesterol<br>(mg/dl) |
|------------------------|------------------------------|----------------------------|
| <b>Baseline</b>        |                              |                            |
| FA (n=17)              | 349 +/- 13                   | 11 +/- 1                   |
| FP (n=17)              | 355 +/- 13                   | 11 +/- 1                   |
| UFP (n=17)             | 352 +/- 12                   | 11 +/- 1                   |
| <b>End of protocol</b> |                              |                            |
| FA (n=16)              | 397 +/- 13                   | 9 +/- 1                    |
| FP (n=16)              | 459/- 21 <sup>†‡</sup>       | 8 +/- 1                    |
| UFP (n=15)             | 402 +/- 19                   | 8 +/- 0.5                  |

Mice were bled after 6-hour fasting. Baseline samples were collected one week prior to the beginning of exposure protocols. Samples taken at the end of the protocols were collected 24 hours after the last exposure. Values are given as mean  $\pm$  SE (mg/dl). NM: not measured.  $\dagger$  p (vs. FA group)  $\leq 0.01$ ,  $\ddagger$  p (vs. UFP group)  $< 0.05$ . FA: filtered air, FP: fine particles, UFP: ultrafine particles.

# Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage

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The objectives of this study were to determine whether differences in the size and composition of coarse (2.5–10 µm), fine (< 2.5 µm), and ultrafine (< 0.1 µm) particulate matter (PM) are related to their uptake in macrophages and epithelial cells and their ability to induce oxidative stress. The premise for this study is the increasing awareness that various PM components induce pulmonary inflammation through the generation of oxidative stress. Coarse, fine, and ultrafine particles (UFPs) were collected by ambient particle concentrators in the Los Angeles basin in California and used to study their chemical composition in parallel with assays for generation of reactive oxygen species (ROS) and ability to induce oxidative stress in macrophages and epithelial cells. UFPs were most potent toward inducing cellular heme oxygenase-1 (HO-1) expression and depleting intracellular glutathione. HO-1 expression, a sensitive marker for oxidative stress, is directly correlated with the high organic carbon and polycyclic aromatic hydrocarbon (PAH) content of UFPs. The dithiothreitol (DTT) assay, a quantitative measure of *in vitro* ROS formation, was correlated with PAH content and HO-1 expression. UFPs also had the highest ROS activity in the DTT assay. Because the small size of UFPs allows better tissue penetration, we used electron microscopy to study subcellular localization. UFPs and, to a lesser extent, fine particles, localize in mitochondria, where they induce major structural damage. This may contribute to oxidative stress. Our studies demonstrate that the increased biological potency of UFPs is related to the content of redox cycling organic chemicals and their ability to damage mitochondria. **Key words:** concentrated ambient particles, dithiothreitol assay, heme oxygenase-1, mitochondrial damage, oxidative stress, polycyclic aromatic hydrocarbon, ultrafine particles. *Environ Health Perspect* 111:455–460 (2003). doi:10.1289/ehp.6000 available via <http://dx.doi.org/> [Online 16 December 2002]

Epidemiologic studies have shown associations between ambient air particulate matter (PM) and adverse health outcomes, including increased mortality, emergency room visits, and time lost from work and school [Dockery et al. 1993; Health Effects Institute (HEI) 2002; Samet et al. 2000; Wichmann et al. 2000]. The underlying toxicologic mechanisms by which air pollutant particles induce adverse health effects are of intense scientific interest and have been earmarked as a key scientific priority by the National Academy of Sciences [National Research Council (NRC) 1998]. This includes a call for research on the physicochemical properties that promote particle toxicity (NRC 1998). PM with aerodynamic diameter < 2.5 µm (PM<sub>2.5</sub>) is currently regulated by the U.S. Environmental Protection Agency. Within that spectrum of particle sizes, ultrafine particles (UFPs), defined as having an aerodynamic diameter < 0.1 µm, may have a central role in health effects of PM [Oberdörster and Utell 2002; Samet et al. 2000]. Primary UFPs are formed during gas-to-particle conversion or during incomplete fuel combustion (HEI 2002). Due to their small size, high number concentration, and relatively large surface area per unit mass,

UFPs have unique characteristics, including increased adsorption of organic molecules and enhanced ability to penetrate cellular targets in the lung and systemic circulation [Frampton 2001; HEI 2002; Nemmar et al. 2002; Oberdörster 1996; Utell and Frampton 2000].

Particle composition may also be critical in PM toxicity. We are interested in organic PM compounds because organic extracts made from diesel exhaust particles (DEPs) mimic intact particles in their ability to form reactive oxygen species (ROS) [Hiura et al. 1999, 2000; Kumagai et al. 1997; Nel et al. 1998]. One of the major advances in PM research has been the recognition that the organic and metal PM components can induce proinflammatory effects in the lung due to their ability to cause oxidative stress [Kumagai et al. 1997; Nel et al. 1998, 2001; Saldiva et al. 2002]. Quinones present in PM can act as catalysts to produce ROS directly and may be key compounds in PM-based oxidative stress [Monks et al. 1992; Penning et al. 1999]. PAHs can induce oxidative stress indirectly, through biotransformation by cytochrome P450, epoxide hydrolase, and dihydrodiol dehydrogenase to generate redox active quinones [Penning et al. 1999]. The involvement of quinones and

PAHs was confirmed by demonstrating that compounds present in aromatic and polar fractions of DEP extracts mimic the pro-oxidative effects of intact particles in bronchial epithelial cells and macrophages [Li et al. 2000, 2002b]. DEPs also induce cytochrome P450 1A1 induction in bronchial epithelial cells [Bonvallot et al. 2001]. Animal and human experiments confirm that DEPs and PAHs derived from DEPs promote allergic airway inflammation and cytochrome P450 1A1 induction in the lungs of exposed mice [Miyabara et al. 1998; Nel et al. 1998; Takano et al. 2002; Tsien et al. 1997]. Epidemiologic studies have also shown an association between PM exposure and asthma exacerbation [Nemmar et al. 2002; Penttinen et al. 2001; Utell and Frampton 2000].

The Versatile Aerosol Concentration Enrichment System (VACES), which uses three parallel sampling lines to collect concentrated ambient coarse, fine, and ultrafine particles for biological analysis, is now available for use in toxicologic studies aimed at identifying the relative toxicity of the different particle sizes [Kim et al. 2001a, 2001b]. This technology enables us to probe the relationship between particle size, chemical composition, and toxicity [Li et al. 2002a]. These concentrators are mobile and can be used to test hypotheses about particle toxicity in the Los Angeles basin in California. Concentrated air particulates (CAPs) of different sizes were collected to study their oxidative stress effects and subcellular localization in cultured macrophages and epithelial cells. We demonstrate that UFPs are more potent than fine (< 2.5 µm) or coarse (2.5–10 µm) particles

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toward inducing oxidative stress. This effect may be explained by adsorbed chemicals (organics and metals) capable of generating ROS and the ability of UFPs to localize in mitochondria.

## Materials and Methods

**Ambient particle collection.** Ambient coarse particles (2.5–10  $\mu\text{m}$ ), fine plus ultrafine particles (< 2.5  $\mu\text{m}$ ), and UFPs (< 0.15  $\mu\text{m}$ ) were collected in the Los Angeles basin during November 2001–March 2002 using the VACES (Kim et al. 2001a, 2001b). Coarse particles were concentrated using a single nozzle virtual impactor, while fine and ultrafine particles were concentrated by drawing air samples through two parallel lines, using 2.5  $\mu\text{m}$  and 0.15  $\mu\text{m}$  cut-point preimpactors, respectively, to remove larger-sized particles. These particles are drawn through a saturation–condensation system that grows particles to 2–3  $\mu\text{m}$  droplets, which are subsequently concentrated by virtual impaction. Highly concentrated particle suspensions were obtained by connecting the VACES output to a sterilized liquid impinger (BioSampler; SKC West Inc., Fullerton, CA) (Willeke et al. 1998). Aerosols were collected using ultrapure (Milli-Q; Millipore Corp., Bedford, MA) deionized water (resistivity 18.2 megaohm; total organic compounds < 10 ppb; particle-free; bacteria < 1 colony forming unit/mL) as the collection medium. The concentration enrichment process does not alter the physical, chemical, and morphologic properties of the particles (Kim et al. 2001a, 2001b). We determined the total amount of particulate loading in the collection medium by multiplying the ambient concentration of each PM mode by the total air sample volume collected by each VACES line. The particle concentration in the aqueous medium was then calculated by dividing the particle loading by the total volume collected in that time period. Five sample sets were collected, two at the University of Southern California (USC), and three at Claremont. USC is a typical urban site located 3 km south of downtown Los Angeles. This is a site in which aerosols are mostly generated from fresh vehicular emissions. Claremont is a receptor site approximately 45 km east (i.e., downwind) of downtown Los Angeles. In that location,

ambient PM originates mostly from advection of polluted air parcels originally emitted in urban Los Angeles, after “aging” in the atmosphere for a few hours, as well as from secondary photochemical processes.

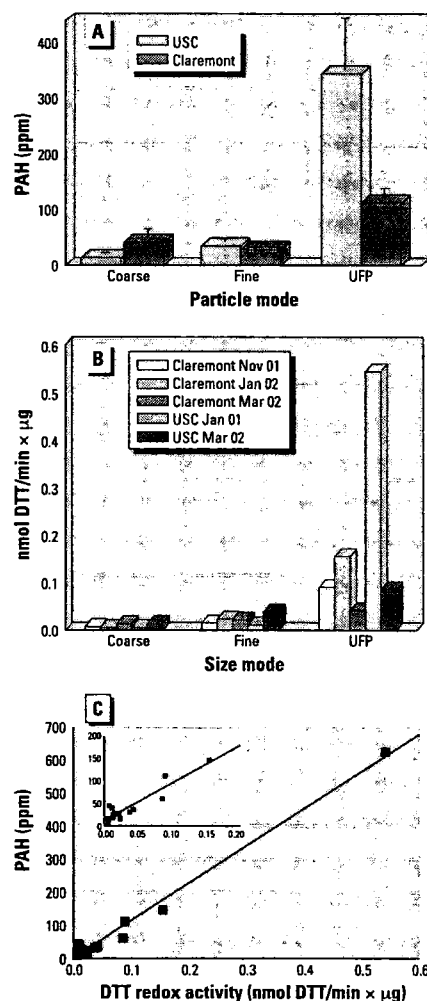
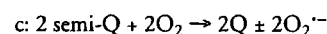
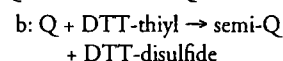
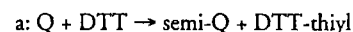
**Particle chemical analysis.** Samples were collected on Teflon and quartz filters with a Micro Orifice Uniform Deposit Impactor (MOUDI; MSP Corporation, Shoreview, MN) for chemical analysis (Li et al. 2002a). We used Teflon filters to determine the metal and trace element content by X-ray fluorescence and quartz filters to determine the organic carbon ( $\text{MnO}_2$ -catalyzed  $\text{CO}_2$  formation), sulfate (ion chromatography), and nitrate (ion chromatography) contents (Li et al. 2002a). PAH content for each CAPs set was determined by an HPLC-fluorescence method that detects a signature group of 16 PAHs (Li et al. 2002a).

**Cellular stimulation and heme oxygenase 1 (HO-1) immunoblotting.** We used two cell lines in the study: RAW 264.7 and BEAS-2B. RAW 264.7 is a murine macrophage cell line that mimics the oxidative stress response of pulmonary alveolar macrophages in response to DEP exposure (Hiura et al. 1999, 2000; Li et al. 2002b). BEAS-2B is a transformed human bronchial epithelial cell line, which mimics the oxidative stress response of primary bronchial epithelial cells (Li et al. 2002b). For RAW 264.7 culture, particle suspensions were reconstituted with Dulbecco's Modified Eagle's Medium powder, a culture medium component that rapidly dissolves in deionized water. This culture medium was further replenished with 10% fetal calf serum and a 1:200 dilution of penicillin/streptomycin/amphotericin B (Li et al. 2002a). For BEAS-2B cells, particle suspensions were made up in hormonally defined F12 medium (Kawasaki et al. 2001). After incubating cells for 16 hr, we used 100  $\mu\text{g}$  of lysate protein for HO-1 immunoblotting (Li et al. 2000, 2002a, 2002b). Densitometric analysis was performed on a laser Personal Densitometer SI using ImageQuant software (both from Amersham Biosciences, Piscataway, NJ).

**GSH/GSSG assay.** Total glutathione and oxidized glutathione (GSSG) were measured in a glutathione reductase recycling assay (Tietze 1969). We calculated the amount of total glutathione and GSSG in the samples

from the standard curves. The amount of reduced glutathione (GSH) was calculated by subtracting the amount of GSSG from that of the total glutathione.

**DTT assay.** The dithiothreitol (DTT) assay quantitatively measures the formation of ROS by quinone catalysis (Kumagai et al. 2002). In the presence of quinones, 1 mol DTT + 2 mol  $\text{O}_2$  generate 1 mol DTT-disulfide +  $2\text{O}_2^{\cdot-}$

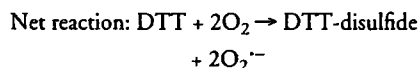


**Figure 1.** Correlation of PAH content with ROS formation. (A) PAH content for each set of CAPs determined by HPLC-fluorescence; values shown are mean  $\pm$  SEM for Claremont ( $n = 3$ ) and USC ( $n = 2$ ). (B) *In vitro* electron transfer capacity of CAPs measured by a colorimetric assay that distinguishes oxidized from reduced DTT (Kumagai et al. 2002). The mean was calculated for three separate measurements; SEM < 0.1. (C) Linear regression analysis demonstrating the correlation between PAH content and 15 DTT data points (5 sites  $\times$  3 samples/site);  $r^2 = 0.98$ . Inset: With the highest point removed,  $r^2$  remains significant at 0.86.

**Table 1.** Mass concentration and fractional composition of CAPs collected in the Los Angeles basin.

| Chemical composition                            | Claremont ( $n = 3$ ) |      |           | USC ( $n = 2$ ) |      |           |
|---|-----------------------|------|-----------|-----------------|------|-----------|
|   | Coarse                | Fine | Ultrafine | Coarse          | Fine | Ultrafine |
| Mass concentration ( $\mu\text{g}/\text{m}^3$ ) | 12.3                  | 17.3 | 1.9       | 21.1            | 20.9 | 3.9       |
| Organic carbon (%)                              | 16                    | 40   | 69        | 20              | 52   | 71        |
| Elemental carbon (%)                            | 1                     | 3    | 13        | 1               | 3    | 11        |
| Nitrate (%)                                     | 27                    | 31   | 4         | 35              | 23   | 3         |
| Sulfate (%)                                     | 5                     | 13   | 5         | 7               | 8    | 6         |
| Metals/total elements (%)                       | 51                    | 13   | 9         | 37              | 14   | 9         |

Values represent the mean fractional composition (%) in which SEM varied < 10%.



The loss of DTT is followed by its reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is converted to 5-mercapto-2-nitrobenzoic (Kumagai et al. 2002). We incubated the PM sample (5–50  $\mu\text{g/mL}$ ) with 10  $\mu\text{M}$  DTT in a Tris buffer at pH 8.9 for 10–90 min. Aliquots of the incubation mixture were transferred to the DNTB solution and the optical density read at 412 nm.

**Electron microscopy.** We performed electron microscopy as previously described (Yang et al. 1987). Thin sections were cut with a Reichert-Jung ultracut and ultramicrotome (Leica, Stuttgart, Germany). Copper grids were stained with lead citrate and uranyl acetate and photographed in a Hitachi electron microscope (Hitachi Instrument Inc., Tokyo, Japan).

## Results

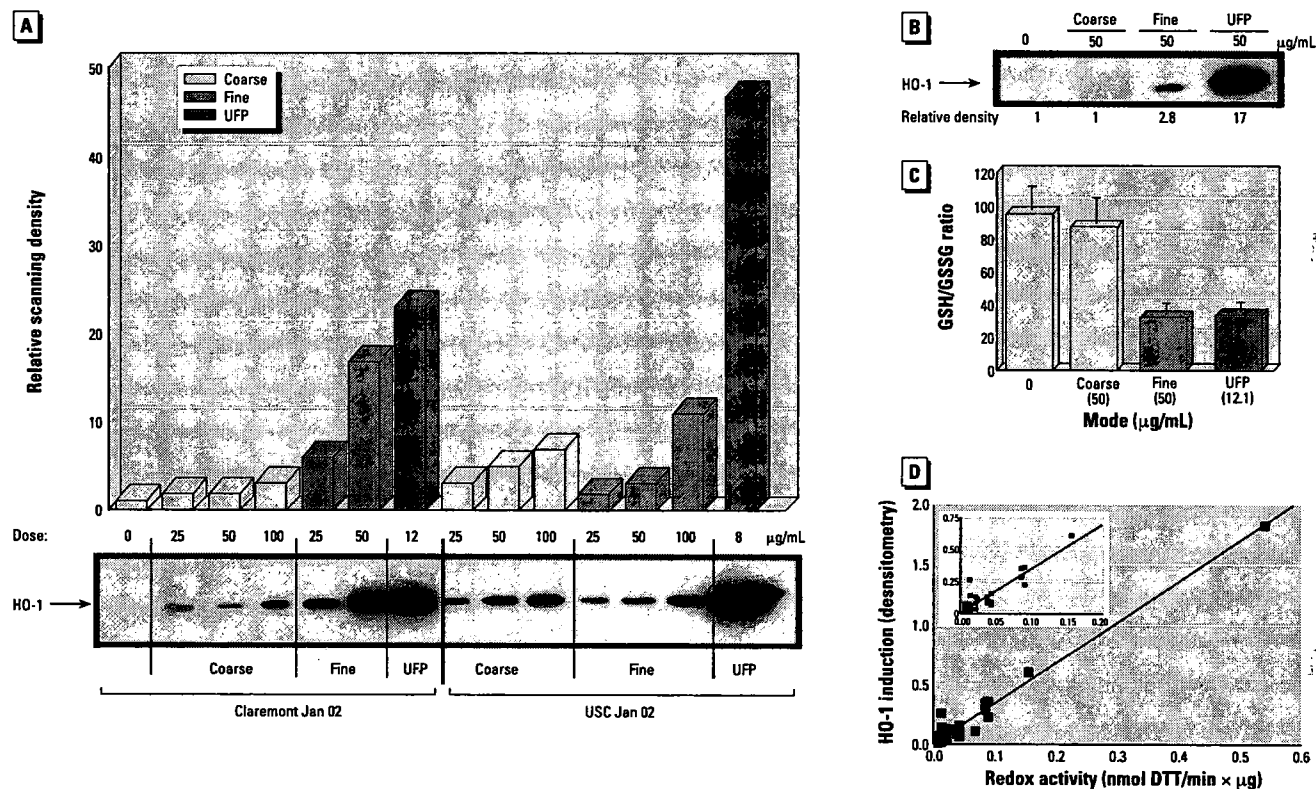
**Particulate organic carbon and PAH content.** To determine whether there is a link between UFP composition and biological effects, CAPs were collected at two outdoor sites in the Los Angeles basin with the VACES. Chemical analysis of the CAPs indicate that

UFPs have a significantly higher organic ( $p < 0.01$ ) and elemental carbon ( $p < 0.001$ ) content than fine plus ultrafine (designated "fine") or coarse particles (Table 1). Coarse and fine particles had a higher metal content than UFPs (Table 1). PAH content for each set of CAPs was determined and averaged for both collection sites; there was a statistically significant difference in total PAH content in UFPs compared to fine ( $p = 0.04$ ) and coarse ( $p = 0.03$ ) PM (Figure 1A). The PAH content of UFPs at USC was significantly higher than the PAH content at Claremont (Figure 1A), which reflects the fact that particles collected at the source site (USC) are derived from primary emissions that are far more abundant in the urban areas of Los Angeles than in receptor areas.

**The DTT assay of ROS formation by particles of varying size.** The ability of PM to generate ROS was assessed with the DTT assay. Quinones with appropriate redox potentials can transfer electrons from DTT to oxygen (Kumagai et al. 2002). We used this reaction to determine the ability of PM to generate ROS *in vitro*. The DTT assay demonstrated that UFPs had significantly higher redox activity than fine and coarse PM (Figure 1B). Averaging of the data revealed that the redox

cycling capacity of UFPs was 21.7- and 8.6-fold greater than coarse and fine PM, respectively (Figure 1B). Regression analysis of the DTT assay and PAH content showed a correlation coefficient ( $r^2$ ) of 0.98, suggesting the electron transfer capacity of CAPs is consistent with their organic chemical content (Figure 1C).

**Particle chemical composition and oxidative stress.** Quinones and other redox-active compounds present in PM generate ROS and oxidative stress (Kumagai et al. 1997; Nel et al. 1998). We have demonstrated that DEP-induced oxidative stress generates hierarchical effects in pulmonary alveolar macrophages and bronchial epithelial cells (Li et al. 2002a, 2002b). Low levels of oxidative stress activate antioxidant defenses, whereas higher levels of oxidative stress lead to proinflammatory and cytotoxic effects (Li et al. 2002a, 2002b). An example of an antioxidant response is HO-1 expression via the antioxidant response element in its promoter (Choi and Alam 1996; Li et al. 2000). Utilizing an immunoblotting technique to assess HO-1 expression in RAW 264.7 cells, UFPs were more potent than fine or coarse particles (Figure 2A). Densitometric analysis demonstrated significantly higher HO-1 expression in ultrafine over fine ( $p = 0.001$ ) and coarse ( $p = 0.001$ ) particles, respectively.



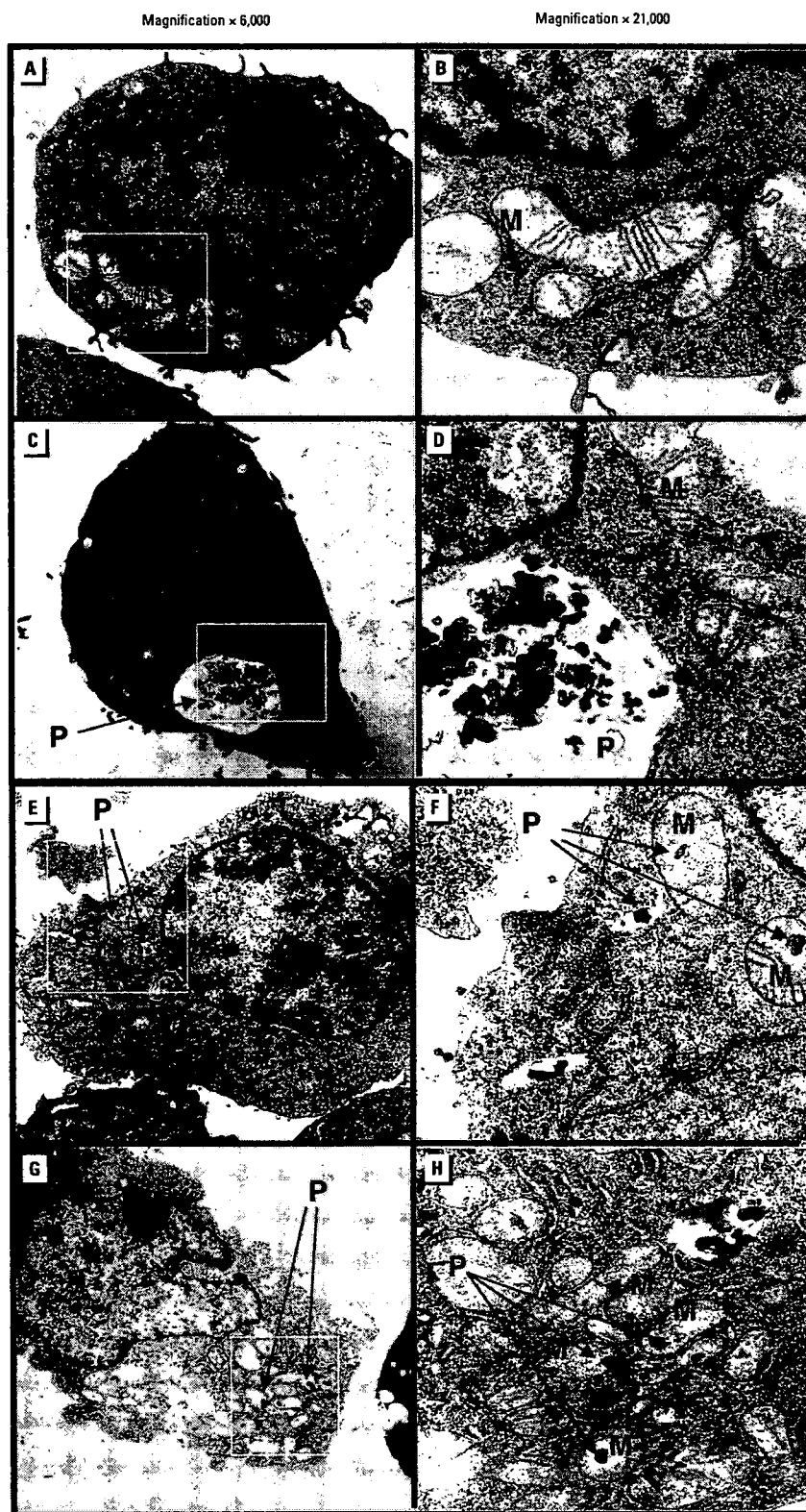
**Figure 2.** Induction of oxidative stress and HO-1 expression. (A) HO-1 expression in RAW 264.7 cells exposed to CAPs (Claremont Mar 02) for 16 hr. (B) HO-1 expression in BEAS-2B cells treated with CAPs (Claremont Jan 02) for 16 hr. (C) Effects of CAPs (Claremont Jan 02) on the intracellular GSH/GSSG ratio in RAW 264.7 cells after 16 hr exposure; GSH/GSSG values shown are mean  $\pm$  SEM from two separate experiments, with duplicate measurements per experiment (Tietze 1969). (D) Regression analysis demonstrating the correlation between *in vitro* redox activity of CAPs and HO-1 induction (15 data points);  $r^2 = 0.97$ . Inset: After removal of the highest data point,  $r^2 = 0.81$ .

The increased potency of UFPs was seen for all CAPs collections (data not shown).

Sufficient CAPs were collected in March 2002 to study HO-1 expression in the bronchial epithelial cell line, BEAS-2B, in parallel with RAW 264.7. The BEAS-2B response mimics the DEP-induced oxidative stress response in human bronchial epithelial cells (Li et al. 2002b). Immunoblot analysis shows that UFPs but not coarse particles induce HO-1 expression in BEAS-2B cells (Figure 2B). To demonstrate that these effects reflect differences in the level of oxidative stress, we compared HO-1 expression to changes in the reduced (GSH) to oxidized (GSSG) glutathione ratio. These data show abundant HO-1 expression by UFPs or 50  $\mu\text{g/mL}$  fine PM (Figure 2A, Claremont Jan 02), which is accompanied by a sizable drop in glutathione ratios (Figure 2C). In contrast, coarse particles had no effect on either biological response (Figure 2C). Regression analysis showed a correlation coefficient ( $r^2$ ) of 0.97 between HO-1 expression and the DTT assay (Figure 2D). Overall, there is a strong correlation between particle size, chemical composition, ROS-generating capacity, and cellular oxidative stress.

**UFP localization and mitochondrial damage.** In defining the mechanistic features of PM toxicity, a key question is the subcellular localization of PM. This may determine ROS generation, as demonstrated by  $\text{O}_2^{\cdot-}$  generation in lung microsomes during incubation with DEP extracts (Kumagai et al. 1997). Subcellular DEP targets include mitochondria, as demonstrated by the ability of organic DEP extracts to induce structural mitochondrial damage (Hiura et al. 1999, 2000; Li et al. 2002b). After exposure to CAPs, there were clear differences in the ultramicroscopic features of RAW 264.7 cells exposed to different particle sizes (Figure 3). Whereas coarse particles collected in large cytoplasmic vacuoles (Figure 3C and 3D), UFPs frequently lodged inside mitochondria (Figure 3G and H). Mitochondrial architecture remained intact in coarse PM incubations, but cells incubated with UFPs showed extensive disruption of mitochondrial cristae, resulting in a vacuolar cellular appearance (Figure 3H). These changes were time dependent, with fewer particles collecting inside mitochondria during shorter incubations (not shown). In cells exposed to fine particulates (which includes some UFPs), some particles lodged inside mitochondria but did not show the same degree of ultrastructural damage (Figure 3E and F).

Electron microscopy showed similar features in BEAS-2B cells—namely, considerable mitochondrial damage by UFPs, resulting in the formation of concentric structures, known as myelin figures (Figure 4). These structures result from the disassociation of lipoproteins, which facilitates water uptake and intercalation



**Figure 3.** Electron micrographs demonstrating effects of different sized particles in RAW 264.7 cells treated with USC-Jan 02 CAPs for 16 hr. (A) and (B) Untreated RAW 264.7 cells. (C) and (D) RAW 264.7 cells exposed to coarse particles. (E) and (F) RAW 264.7 cells exposed to fine particles. (G) and (H) RAW 264.7 cells exposed to UFPs. Notice damage to cristae as well as the presence of particles (P) inside mitochondria (M) in UFP- or fine + UFP-exposed cells.

between lamellar membrane stacks (Figure 4C). Similar to RAW 264.7 cells, UFPs lodged inside damaged mitochondria (Figure 4C). Cells incubated with coarse or fine particles showed lesser mitochondrial damage (not shown). The extent of mitochondrial damage is in accordance with the redox cycling potential of the particles, as well as the HO-1 and glutathione results.

## Discussion

Our data demonstrate that the UFP mode in the Los Angeles basin is more potent than fine and coarse PM toward inducing oxidative stress as measured by the DTT, HO-1, and glutathione assays. Electron microscopy also indicates subcellular penetration and mitochondrial damage by UFPs and, to a lesser extent, fine particles. The findings correlate with PM organic carbon and PAH composition, suggesting a role of organic agents in generating redox activity.

The results from the DTT assay indicate UFPs are capable of producing greater ROS on a microgram basis than fine and coarse particles. This is the first time that a quantitative assay has been used to directly measure ROS generation by CAPs. Kumagai et al. (2002) focused the assay on assessment of quinones, whereas we have applied the assay to UFPs and fine and coarse particles. The DTT assay provides a quantitative measure of the relative redox activity of different PM sizes in the Los Angeles basin.

UFPs contain a higher percentage of organic carbon than fine and coarse particles, and this has relevance to the biologic potency of these particles. The enhanced biologic potency of UFPs is directly correlated with the PAH content. Although PAHs are capable of inducing ROS production in macrophages, it is also possible that these compounds may be a surrogate for other redox cycling chemicals in the DTT assay. We do not exclude a contribution by transition metals, which may interact with organic PM components in ROS generation (Saldiva et al. 2002).

These data are in accordance with the growing awareness that oxidative stress plays a key role in the induction of airway inflammation (HEI 2002; Nel et al. 1998). Recently, we demonstrated that macrophages and epithelial cells exhibit a stratified oxidative stress response to increasing concentrations of DEPs (Li et al. 2002a, 2002b). The stratified response commences with HO-1 expression when the GSH/GSSG ratio is minimally disturbed, proceeds to *Jun* kinase activation at intermediary levels of oxidative stress, and culminates in cellular toxicity at high oxidative stress levels. Ambient CAPs mimic the effects of organic DEP extracts (Li et al. 2002a), with UFPs showing increased potency in depressing the cellular GSH/GSSG ratio (Figure 2C). The significance of *Jun* kinase activation is the transcriptional activation of cytokine, chemokine, and adhesion receptor promoters (Nel et al. 1998). These products play a role in the proinflammatory effects of PM in the lung and possibly also the cardiovascular system (Nel et al. 1998). The finding of a significant correlation between heme oxygenase activity, GSH/GSSG ratio, and redox activity as measured by DTT production provides further evidence for the role of ROS generation in PM toxicity.

The biological significance of HO-1 expression in the lung is the antioxidant effect of its catabolic product, bilirubin (Choi and Alam 1996). In the process of heme catabolism, HO-1 also generates a gaseous substance, CO, which exerts anti-inflammatory effects in the lung and is exhaled in the expired air (Horvath et al. 1998; Maines 1997). It is interesting, therefore, that in a study in which normal human volunteers were exposed to DEPs, CO levels in the expired air was a more sensitive exposure marker than the presence of inflammatory products in the bronchoalveolar fluid (Nightingale et al. 2000). This is in agreement with the exquisite sensitivity of the HO-1 promoter to oxidative stress *in vivo* and *in vitro* (Choi and Alam 1996; Nightingale et al. 2000). HO-1 expression and CO generation are markers for airway inflammation in asthma (Horvath et al. 1998). Monitoring of

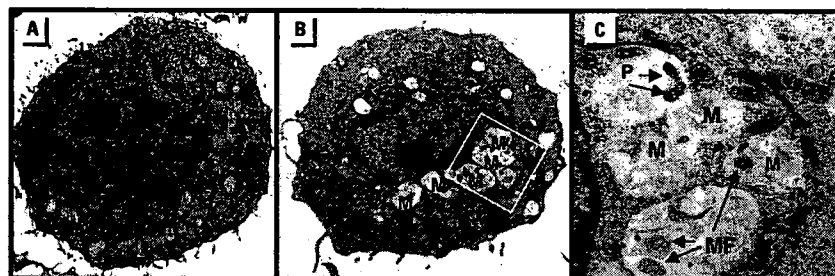
CO levels in the expired air may be a useful marker for evaluating the pro-oxidative and proinflammatory effects of CAPs in the respiratory tract.

How exactly UFPs gain access to and induce mitochondrial damage is unknown. One possibility is that ROS generated outside of the mitochondrion may damage this organelle, allowing access to the particles. This is compatible with the ability of organic DEP extracts to induce ultrastructural mitochondrial damage in the absence of particles (Hiura et al. 2000; Li et al. 2002b). Our previous studies have demonstrated that organic DEP chemicals induced pro-apoptotic effects in macrophages and bronchial epithelial cells (Hiura et al. 1999, 2000; Li et al. 2002b). This effect may be mediated through the perturbation of mitochondrial permeability transition pore, which sets in motion cytochrome *c* release, caspase activation, and superoxide production in the mitochondrial inner membrane (Hiura et al. 2000). Ultramicroscopic visualization of human macrophages and BEAS-2B cells incubated with organic DEP extracts showed that the appearance of apoptotic bodies were accompanied by changes in mitochondrial morphology, including mitochondrial swelling and a loss of cristae (Li et al. 2002b). Another possibility is that UFPs gain access to mitochondria because of their small sizes. These particles might then release redox cycling chemicals that damage the inner membrane. All considered, we propose that enhanced tissue penetrance and ability to generate oxidative stress render UFPs more damaging at cellular level and consequently contribute to the adverse health effects of UFPs in the Los Angeles basin.

These findings may be of importance for PM regulation. Currently, the manufacture of cleaner combustion engines relies on mass output standards but do not consider the output of large numbers of UFPs, which have very low mass. Our data show that UFPs are more potent than PM<sub>2.5</sub> and PM<sub>10</sub> that contribute the majority of mass in the HO-1 and DTT assays. It may be necessary to consider standards based on particle number instead of mass if further studies confirm the differential toxicity of UFPs. Further research to more fully characterize the toxicity of UFPs in relation to particle number, surface area, and chemical composition is needed.

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**Figure 4.** Electron micrographs demonstrating mitochondrial destruction in BEAS-2B cells treated with 8.4 µg/mL of USC-Jan 02 UFPs for 16 hr. (A) Untreated BEAS-2 cells; magnification ×8,500. (B) UFP-treated cells; magnification ×8,500. (C) UFP-treated cells; magnification ×26,300. Notice the disappearance of cristae, formation of myelin figures (MF), and presence of particles (P) inside mitochondria (M).

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## Association of Biomarkers of Systemic Inflammation with Organic Components and Source Tracers in Quasi-Ultrafine Particles

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**BACKGROUND:** Evidence is needed regarding the air pollutant components and their sources responsible for associations between particle mass concentrations and human cardiovascular outcomes. We previously found associations between circulating biomarkers of inflammation and mass concentrations of quasi-ultrafine particles  $\leq 0.25 \mu\text{m}$  in aerodynamic diameter ( $\text{PM}_{0.25}$ ) in a panel cohort study of 60 elderly subjects with coronary artery disease living in the Los Angeles Basin.

**OBJECTIVES:** We reassessed biomarker associations with  $\text{PM}_{0.25}$  using new particle composition data.

**METHODS:** Weekly biomarkers of inflammation were plasma interleukin-6 (IL-6) and soluble tumor necrosis factor- $\alpha$  receptor II (sTNF-RII) ( $n = 578$ ). Exposures included indoor and outdoor community organic  $\text{PM}_{0.25}$  constituents [polycyclic aromatic hydrocarbons (PAHs), hopanes,  $n$ -alkanes, organic acids, water-soluble organic carbon, and transition metals]. We analyzed the relation between biomarkers and exposures with mixed-effects models adjusted for potential confounders.

**RESULTS:** Indoor and outdoor PAHs (low-, medium-, and high-molecular-weight PAHs), followed by hopanes (vehicle emissions tracer), were positively associated with biomarkers, but other organic components and transition metals were not. sTNF-RII increased by 135 pg/mL [95% confidence interval (CI), 45–225 pg/mL], and IL-6 increased by 0.27 pg/mL (95% CI, 0.10–0.44 pg/mL) per interquartile range increase of 0.56 ng/m<sup>3</sup> outdoor total PAHs. Two-pollutant models of  $\text{PM}_{0.25}$  with PAHs showed that nominal associations of IL-6 and sTNF-RII with  $\text{PM}_{0.25}$  mass were completely confounded by PAHs. Vehicular emission sources estimated from chemical mass balance models were strongly correlated with PAHs ( $R = 0.71$ ).

**CONCLUSIONS:** Traffic emission sources of organic chemicals represented by PAHs are associated with increased systemic inflammation and explain associations with quasi-ultrafine particle mass.

**KEY WORDS:** air toxics, biomarkers of effect, cytokines, epidemiology, longitudinal data analysis. *Environ Health Perspect* 118:756–762 (2010). doi:10.1289/ehp.0901407 [Online 2 February 2010]

Cardiovascular hospital admissions and mortality have been associated with ambient mass concentrations of fine particulate matter (PM) air pollution  $\leq 2.5 \mu\text{m}$  in aerodynamic diameter ( $\text{PM}_{2.5}$ ) (Pope and Dockery 2006). Questions remain regarding the underlying causal chemical components and sources responsible for these associations. A recent time-series study of 106 U.S. counties showed stronger associations of cardiovascular hospital admissions with countywide averages of  $\text{PM}_{2.5}$  when there were higher fractions of elemental carbon (EC), nickel (Ni), and vanadium (V), suggesting that important sources included fossil fuel combustion, biomass burning, and oil combustion (Bell et al. 2009).

Unlike  $\text{PM}_{2.5}$ , ultrafine particles (UFPs; generally defined as  $< 0.1 \mu\text{m}$  in diameter) are not regulated by the U.S. Environmental Protection Agency (EPA), yet this is the size fraction that may have the highest toxic potential because it has magnitudes greater number concentrations and surface area than the larger particles that dominate  $\text{PM}_{2.5}$  mass (Oberdörster et al. 2005). On that large surface area, UFPs carry and deliver redox-active organic chemicals, including polycyclic

aromatic hydrocarbons (PAHs), to the respiratory tract in disproportionately higher concentrations than do larger particles (Ntziachristos et al. 2007), possibly leading to a cascade of effects related to oxidative stress and inflammation in the lungs and at extrapulmonary sites (Delfino et al. 2005). These and other effects could underlie associations of morbidity and mortality with air pollutants.

Except for some studies with personal or microenvironmental air pollution data (Chan et al. 2004; Delfino et al. 2008, 2009; Folino et al. 2009; Vinzents et al. 2005), regional ambient air monitoring has been the primary data source used in epidemiologic research on the importance of UFP exposure to cardiovascular outcomes and circulating biomarkers in individual-level studies (de Hartog et al. 2003; Henneberger et al. 2005; Ibaldo-Mulli et al. 2004; Lanki et al. 2008; Pekkanen et al. 2002; Rückerl et al. 2006, 2007; Timonen et al. 2006). These studies of ambient air were all conducted in Europe, and UFPs were measured as particle number concentrations at central regional sites. Exposure error from the use of ambient data is likely, because air monitors may be far from subject locations

and subjects may be exposed to pollutants from local sources, including traffic. UFPs have much higher spatial variability than does  $\text{PM}_{2.5}$  (Sioutas et al. 2005), so exposure error is likely. In addition, UFP mass and particle number do not specifically indicate which particle components or sources are important, although generally in urban areas UFP compositions are dominated by organic chemicals and EC and originate from combustion sources.

We conducted a panel cohort study of elderly subjects with a history of coronary artery disease living in the Los Angeles Basin. This is considered a population that may have among the greatest susceptibility to the adverse effects of air pollution (von Klot et al. 2005). We made repeated measurements of blood biomarkers and air pollutant exposures. To assess the potential importance of UFPs to cardiovascular health, we measured quasi-ultrafine particle mass  $< 0.25 \mu\text{m}$  in diameter ( $\text{PM}_{0.25}$ ). To address the issue of exposure error, we monitored  $\text{PM}_{0.25}$  at the retirement communities of subjects. We previously

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reported positive associations of blood biomarkers of inflammation with  $PM_{0.25}$  but not with larger accumulation-mode particles 0.25–2.5  $\mu m$  in diameter ( $PM_{0.25-2.5}$ ) (Delfino et al. 2009). However, particle mass alone does not provide sufficient information about composition or sources. We also previously found positive associations between the biomarkers and  $PM_{2.5}$  EC (Delfino et al. 2009). Based on these findings, we hypothesized that traffic emission sources of organic chemicals in  $PM_{0.25}$  would be positively associated with systemic inflammation. In the present analysis, we aimed to better delineate which underlying PM components may be responsible for the associations we observed for EC and  $PM_{0.25}$  mass using new data on chemical species in the archived  $PM_{0.25}$  filter samples.

## Materials and Methods

**Population and design.** This was a longitudinal study of repeated measures where each subject acted as his or her own control over time. This limits the impact of confounding by between-subject characteristics. We recruited subjects from four retirement communities. Subjects were eligible for participation if they had a confirmed coronary artery disease history and were  $\geq 65$  years of age, nonsmokers, and unexposed to environmental tobacco smoke. Of 105 volunteers, 21 were not eligible, 19 dropped out, 2 had too few blood draws or valid biomarker data ( $> 5$  of 12 weeks), and 3 had insufficient biomarker data due to exclusions for frequent infections, leaving 60 subjects. We excluded biomarker measurements during weeks with acute infectious illnesses given their well-known impact on measured biomarkers. Table 1 lists subject characteristics.

Two retirement communities were studied in 2005–2006 and two in 2006–2007. Subjects were followed for a total of 12 weeks with weekly blood draws for circulating biomarkers of inflammation in plasma. Each subject contributed 5–12 weekly blood draws ( $n = 578$  total samples).

Each community was studied in two 6-week seasonal phases, a warmer period characterized by higher photochemistry followed by a cooler period characterized by higher air stagnation and lower mixing heights. This seasonal approach was intended to increase the variability in pollutant characteristics, with higher secondary organic aerosols (SOAs) in the warmer phase and higher primary organic aerosols (POAs) in the cooler phase when traffic-related air pollutants increase at ground level. POAs are formed during or shortly after the combustion of fossil fuels. SOAs are largely photochemically produced from gas-to-particle conversion when volatile reactive organic gases from anthropogenic and biogenic sources, and anthropogenic semivolatile organic compounds (SVOCs),

are oxidized to form low-volatility products that condense to produce SOAs. There are few data on the importance of variations in this multipollutant characteristic of PM to human health outcomes. In the present study, POAs are represented by PAHs and hopanes, whereas SOAs are represented by water-soluble organic carbon (WSOC) and organic acids. Most PAHs are considered to be components of POAs. Hopanes are found in the lubricant oils of diesel and gasoline vehicles and are thus tracers of primary vehicular aerosols in the Los Angeles Basin (Schauer et al. 1996, 2000). WSOC (Snyder et al. 2009) and organic acids (Robinson et al. 2006) are tracers of SOAs, although a fraction of WSOC comes from biomass burning (Docherty et al. 2008).

The research protocol was approved by the Institutional Review Board of the University of California–Irvine, and we obtained informed written consent from subjects.

**Biomarkers.** We focused on an informative set of biomarkers of inflammation from the previous analysis of peripheral blood biomarkers and  $PM_{0.25}$  mass (Delfino et al. 2009). We drew blood samples in ethylenediamine-tetraacetic acid tubes on Friday afternoons and processed them and froze the plasma on site within 30 min. Samples were stored at  $-80^{\circ}C$  until assayed. Plasma biomarkers were thawed and assayed using 96-well immunoassay kits for the proinflammatory cytokine interleukin-6 (IL-6) and the cytokine receptor-soluble tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) receptor II (sTNF-RII; Quantikine HS, R&D Systems, Minneapolis, MN). sTNF-RII has a longer half-life than TNF- $\alpha$  (Aderka 1996) and may thus better reflect sustained or lagged effects. Thawed erythrocyte lysates were assayed spectrophotometrically for activities of the antioxidant enzymes copper/zinc-superoxide dismutase (Cu,Zn-SOD) and glutathione peroxidase-1 (GPx-1) (Cayman Chemical, Ann Arbor, MI, USA). Cu,Zn-SOD and GPx-1 values were normalized to units per gram of hemoglobin. These and related biomarkers are predictive of cardiovascular disease risk (Flores-Mateo et al. 2009; Kritchevsky et al. 2005; Pai et al. 2004).

**Exposures.** The methods used to measure components and their relevance to sources of  $PM_{0.25}$  are described in detail in the Supplemental Material [Chemical Measurement Methods (doi:10.1289/ehp.0901407)] and by Arhami et al. (2010). There we also discuss in detail differences by season and community and describe the relation between indoor and outdoor measurements.

Air sampling occurred in the immediate outdoor environment of each retirement community and at an indoor site located in the common areas of the main community buildings. The indoor data are thus representative to some degree of the same indoor environment

of each subject. Our main interest here is in the effects of outdoor-source PM components.

More than 5 days before each blood draw, we collected indoor and outdoor size-segregated particle samples using Sioutas Personal Cascade Impactors (SKC Inc., Eighty Four, PA, USA) with Zeflur filters (3- $\mu m$  pore size; Pall Life Sciences, Ann Arbor, MI, USA). We evaluated components only in the quasi-ultrafine fraction ( $PM_{0.25}$ ). Mass concentrations were determined gravimetrically by weighing the impactor filters and substrates with a microbalance (uncertainty,  $\pm 2 \mu g$ ; Mettler-Toledo, Columbus, OH, USA) in a temperature-controlled and relative humidity-controlled room.

The five weekly  $PM_{0.25}$  filters were composited for chemical analyses. These composites were cut into three sections (one half-section and two quarter-sections). We analyzed the composited half section for 92 different organic compounds using gas chromatography/mass spectrometry (GC/MS) (Stone et al. 2008). For the present analysis, compounds are grouped by their structures, which is the primary control of their chemical interactions. Selected representative organic components were grouped as PAHs, organic (*n*-alkanoic) acids, *n*-alkanes, and hopanes [see Supplemental Material, Table 1 (doi:10.1289/ehp.0901407)]. PAHs were further subdivided into low- (two- to three-ring), medium- (four-ring), and high- (five-ring or larger) molecular-weight PAHs (LMW, MMW, and HMW, respectively), which is loosely connected to volatility and solubility.

**Table 1.** Subjects and biomarker outcomes.

| Variable   | Value             |
|--|-------------------|
| Age (years)  | 84.1 $\pm$ 5.60   |
| Sex  |                   |
| Male   | 34 (56.7)         |
| Female   | 26 (43.3)         |
| Cardiovascular history   |                   |
| Confirmation of coronary artery disease <sup>a</sup>                             |                   |
| Myocardial infarction  | 27 (45.0)         |
| Coronary artery bypass graft or angioplasty                                      | 20 (33.3)         |
| Positive angiogram or stress test  | 10 (16.7)         |
| Clinical diagnosis <sup>b</sup>  | 3 (5.0)           |
| Congestive heart failure   | 13 (21.7)         |
| Hypertension (by history)  | 42 (70.0)         |
| Hypercholesterolemia (by history)  | 43 (71.7)         |
| Medications  |                   |
| Angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists | 24 (40.0)         |
| 3-Hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins)             | 31 (51.7)         |
| IL-6 (pg/mL)   | 2.42 $\pm$ 1.85   |
| sTNF-RII (pg/mL)   | 3,610 $\pm$ 1,489 |

sTNF-RII, soluble tumor necrosis factor- $\alpha$  receptor II. Values are mean  $\pm$  SD or  $n$  (%).

<sup>a</sup>Each category is hierarchical and excludes being in the above diagnostic category. <sup>b</sup>Includes subjects with anginal symptoms relieved with nitrates plus echocardiogram and electrocardiographic evidence of past infarct.

The first composited quarter-section was digested with concentrated acid using microwave digestion followed by analysis to determine 52 trace elements using high-resolution inductively coupled plasma mass spectrometry (Finnigan Element 2; Thermo Fisher Scientific, Waltham, MA, USA) (Herner et al. 2006). We focused our analyses of exposure-response relationships on key transition metals that can generate reactive oxygen species by Fenton-type reactions: vanadium (V), chromium (Cr), iron (Fe), nickel (Ni), copper (Cu), manganese (Mn), lead (Pb), and zinc (Zn).

The second composited quarter was analyzed for WSOC using a General Electric Sievers Total Organic Carbon Analyzer (GE Analytical Instruments, Boulder, CO, USA).

The remaining composited half was analyzed for organic tracer compounds by GC/MS along with field blanks, laboratory blanks, spiked samples, and standard reference material (Urban Dust Standard Resource Material 1649a; National Institute of Standards and Technology, Gaithersburg, MD, USA). Spike recovery after correction for internal standard recoveries was in the range of 96–110% for PAHs, 99–104% for hopanes, and 68–136% for *n*-alkanes. Blank concentrations of MMW PAHs, HMW PAHs, and hopanes were below analytical detection limits ( $\sim 10 \text{ pg/m}^3$  air). The method detection limits for remaining compounds were limited by field and laboratory blanks. Uncertainties for each measurement were estimated based on analytical uncertainties and uncertainties from the blank correction and were used to determine if each measurement was statistically different from zero. The precision of the spike and standard reference material analyses

was used to estimate method precision ( $> 20\%$  for all PAHs, hopanes, and *n*-alkanes).

**Statistical analysis.** We analyzed relations of repeated (within-subject) measures of biomarkers to air pollutant exposures with linear mixed effects models. Random effects were estimated at the subject level, nested within seasonal phase and community, to account for correlated within-individual repeated measures. To focus estimates of associations at the subject level, we adjusted for between-community and between-phase exposure effects as proposed by Janes et al. (2008) by using exposures that were mean-centered across community and phase [see Supplemental

Material, Regression Model, Mean Centering Method (doi:10.1289/ehp.0901407)]. We decided *a priori* to adjust for 5-day average temperature. Magnitudes of association from the mixed models are expressed at pollutant interquartile ranges (IQRs; 25th–75th percentile) to allow strengths of association for different pollutants to be compared by limiting differences due to units of measurement or concentration range.

We evaluated the covariance structure using empirical variograms and found models were best fit as an autoregressive-1 correlation structure. We performed residual analyses to examine deviations from standard linear

**Table 3.** Exposure correlation matrix for outdoor  $\text{PM}_{0.25}$  mass and organic components.

| Pollutant               | WSOC | PAH   |      |      |      | Hopanes | <i>n</i> -Alkanes | Organic acids |
|-------------------------|------|-------|------|------|------|---------|-------------------|---------------|
|                         |      | Total | LMW  | MMW  | HMW  |         |                   |               |
| $\text{PM}_{0.25}$ mass | 0.25 | 0.45  | 0.44 | 0.38 | 0.39 | 0.31    | 0.17              | -0.18         |
| WSOC                    | 1.00 | 0.39  | 0.41 | 0.29 | 0.40 | 0.31    | 0.15              | 0.09          |
| PAHs                    |      |       |      |      |      |         |                   |               |
| Total                   |      | 1.00  | 0.89 | 0.93 | 0.81 | 0.54    | 0.15              | -0.19         |
| LMW                     |      |       | 1.00 | 0.79 | 0.66 | 0.63    | 0.24              | -0.24         |
| MMW                     |      |       |      | 1.00 | 0.67 | 0.51    | 0.12              | -0.33         |
| HMW                     |      |       |      |      | 1.00 | 0.41    | 0.20              | -0.03         |
| Hopanes                 |      |       |      |      |      | 1.00    | 0.08              | -0.26         |
| <i>n</i> -Alkanes       |      |       |      |      |      |         | 1.00              | -0.06         |

All exposures are mean centered by study community and seasonal phase, and results are Spearman rank correlations.

**Table 4.** Exposure correlation matrix for outdoor PAH and source apportioned mass.

| PAH   | Vehicular emissions | Biomass burning | Ship emissions | SOAs | RS dust | NSS sulfate | Sea salt | Unknown |
|-------|---------------------|-----------------|----------------|------|---------|-------------|----------|---------|
| Total | 0.71                | 0.22            | 0.10           | 0.19 | 0.24    | 0.06        | 0.33     | 0.33    |
| LMW   | 0.70                | 0.14            | 0.17           | 0.27 | 0.39    | 0.10        | 0.34     | 0.31    |
| MMW   | 0.66                | 0.36            | -0.01          | 0.04 | 0.19    | -0.06       | 0.27     | 0.30    |
| HMW   | 0.66                | 0.08            | 0.09           | 0.27 | 0.13    | 0.13        | 0.19     | 0.14    |

Abbreviations: RS, resuspended; NSS, non-sea salt. All exposures are mean centered by study community and seasonal phase, and results are Spearman rank correlations. Source apportioned mass data come from Arhami et al. (2010).

**Table 2.** Descriptive statistics of outdoor measurements and indoor/outdoor (I/O) ratios of  $\text{PM}_{0.25}$  organic components and transition metals from 47 weeks of 5-day filter composites.

| Exposure   | Warm season     |      |           |           | Cool season     |       |           |           | IQR overall <sup>a</sup> |
|--|-----------------|------|-----------|-----------|-----------------|-------|-----------|-----------|--------------------------|
|  | Mean $\pm$ SD   | IQR  | Min/max   | I/O ratio | Mean $\pm$ SD   | IQR   | Min/max   | I/O ratio |                          |
| Organic components                                   |                 |      |           |           |                 |       |           |           |                          |
| $\text{PM}_{0.25}$ mass ( $\mu\text{g}/\text{m}^3$ ) | 9.51 $\pm$ 3.46 | 7.24 | 4.67/14.7 | 0.88      | 8.65 $\pm$ 4.51 | 6.07  | 3.31/19.3 | 0.94      | 7.37                     |
| WSOC ( $\mu\text{g}/\text{m}^3$ ) <sup>b</sup>       | 0.52 $\pm$ 0.23 | 0.31 | 0.08/1.01 | 0.95      | 0.38 $\pm$ 0.23 | 0.39  | 0.06/0.94 | 0.94      | 0.37                     |
| PAHs ( $\text{ng}/\text{m}^3$ )                      |                 |      |           |           |                 |       |           |           |                          |
| Total  | 0.88 $\pm$ 0.37 | 0.47 | 0.40/1.75 | 0.84      | 1.04 $\pm$ 0.61 | 0.73  | 0.40/2.70 | 0.99      | 0.56                     |
| LMW  | 0.38 $\pm$ 0.15 | 0.20 | 0.19/0.74 | 0.78      | 0.33 $\pm$ 0.15 | 0.19  | 0.17/0.73 | 1.02      | 0.19                     |
| MMW  | 0.26 $\pm$ 0.12 | 0.18 | 0.09/0.50 | 0.85      | 0.35 $\pm$ 0.24 | 0.33  | 0.09/0.96 | 0.74      | 0.24                     |
| HMW  | 0.24 $\pm$ 0.11 | 0.18 | 0.11/0.50 | 0.97      | 0.37 $\pm$ 0.24 | 0.32  | 0.14/1.01 | 1.04      | 0.21                     |
| Hopanes ( $\text{ng}/\text{m}^3$ )                   | 0.27 $\pm$ 0.34 | 0.36 | 0.06/1.57 | 1.00      | 0.25 $\pm$ 0.25 | 0.35  | 0.06/0.83 | 0.97      | 0.35                     |
| <i>n</i> -Alkanes ( $\text{ng}/\text{m}^3$ )         | 36.3 $\pm$ 23.5 | 43.2 | 9.9/81.2  | 1.39      | 54.8 $\pm$ 111  | 15.9  | 11.7/500  | 1.30      | 29.4                     |
| Organic acids ( $\mu\text{g}/\text{m}^3$ )           | 0.22 $\pm$ 0.17 | 0.30 | 0.06/0.54 | 5.05      | 0.26 $\pm$ 0.22 | 0.26  | 0.07/0.96 | 1.24      | 0.29                     |
| Transition metals ( $\text{ng}/\text{m}^3$ )         |                 |      |           |           |                 |       |           |           |                          |
| V  | 4.83 $\pm$ 2.07 | 2.10 | 1.66/11.3 | 0.75      | 2.10 $\pm$ 1.19 | 2.40  | 0.54/4.25 | 0.77      | 2.95                     |
| Cr   | 10.2 $\pm$ 30.2 | 2.21 | 0.00/139  | 0.89      | 0.26 $\pm$ 0.45 | 0.49  | 0.00/1.24 | 1.00      | 1.18                     |
| Mn   | 3.09 $\pm$ 2.88 | 3.10 | 0.00/13.8 | 0.57      | 2.02 $\pm$ 1.43 | 1.76  | 0.27/6.19 | 0.70      | 2.24                     |
| Fe   | 144 $\pm$ 127   | 167  | 0.00/588  | 0.49      | 92.5 $\pm$ 64.2 | 74.7  | 9.39/287  | 0.74      | 115                      |
| Ni   | 7.21 $\pm$ 18.0 | 3.51 | 0.00/82.8 | 0.83      | 0.20 $\pm$ 0.61 | 0.816 | 0.00/1.44 | 2.27      | 1.64                     |
| Cu   | 6.45 $\pm$ 4.35 | 5.50 | 0.35/16.0 | 0.64      | 4.69 $\pm$ 3.22 | 4.91  | 0.43/11.3 | 0.60      | 4.69                     |
| Zn   | 6.88 $\pm$ 4.16 | 6.39 | 0.00/15.8 | 0.78      | 6.08 $\pm$ 3.51 | 4.81  | 1.75/13.0 | 0.93      | 5.77                     |

Abbreviations: max, maximum; Min, minimum.

<sup>a</sup>Overall IQR used in regression models to estimate expected change in the biomarker from exposure to the air pollutant. <sup>b</sup>WSOC ( $\mu\text{g C}/\text{m}^3$ ) was multiplied by 1.8 to yield mass of organic components ( $\mu\text{g}/\text{m}^3$ ) according to Turpin and Lim (2001).



mixed model assumptions and the presence of influential observations. We found four influential high outliers for IL-6 > 10 pg/mL that were reset to 10 pg/mL (upper limit of its standard curve) to obtain more representative estimates of association. In a model for 5-day average PM<sub>0.25</sub>, including the outliers resulted in an association of 0.41 pg/mL [95% confidence interval (CI), 0.00–0.82] per interquartile change in PM<sub>0.25</sub> of 7.37 µg/m<sup>3</sup>, whereas resetting them to 10 pg/mL resulted in an association of 0.26 pg/mL (95% CI, –0.06 to 0.57). It is important that in the previous analysis of PM<sub>0.25</sub> mass (Delfino et al. 2009), the associations with 1-day and 3-day average PM<sub>0.25</sub> were stronger and had narrower 95% CIs than did the 5-day average for both IL-6 and sTNF-RII.

In exploratory analyses, we retested models for erythrocyte antioxidant enzymes (Cu,Zn-SOD and GPx-1) from our previous publication (Delfino et al. 2009). Random slopes and individual autoregressive models showed small, highly influential subject clusters (seven subjects) with positive associations between air pollutants and antioxidant enzymes, whereas most of the

remaining 53 subjects showed inverse associations. Details of these clusters and their interpretation are presented elsewhere (Delfino et al. 2009). We present these data-driven results with the new air pollutant exposure data primarily in the Supplemental Material, Table 3 (doi:10.1289/ehp.0901407).

## Results

Table 2 provides descriptive statistics for the measured exposures. Seasonal differences were greatest for MMW PAHs, HMW PAHs, and *n*-alkanes, which were higher in the cool season, and for WSOC, which was higher in the warm season, as expected because of photochemistry. Indoor/outdoor ratios were close to 1.0 for PAHs and hopanes, and indoor–outdoor correlations were strong (median *R* was 0.60 for PAH species and 0.74 for hopane species) (Arhami et al. 2010). This suggests high penetration of these outdoor PM<sub>0.25</sub> components into indoor environments and that measured indoor components were largely of outdoor origin. On the other hand, indoor/outdoor ratios were high for *n*-alkanes and *n*-alkanoic acids, with generally low indoor/outdoor correlation coefficients (Arhami et al. 2010). This suggests that indoor sources influenced the indoor levels of *n*-alkanes and *n*-alkanoic acids.

Table 3 shows a correlation matrix for measured outdoor organic components. We found moderate to strong correlations between PM<sub>0.25</sub> mass, PAHs, and hopanes. We also found small negative correlations of these species with organic acids and small positive correlations with WSOC, suggesting that POA and SOA concentrations are relatively independent of each other at the study sites.

To further improve our understanding of the clearly positive associations of biomarkers with summed PAH compounds presented

below, we used the chemical mass balance model (CMB) source apportionment estimates from Arhami et al. (2010) to evaluate the possible sources of PAHs. We briefly summarize methods and source apportionment results in the Supplemental Material, Chemical mass balance (CMB) model (doi:10.1289/ehp.0901407). Table 4 shows a correlation matrix for the relation of PAHs to the CMB-estimated sources. Strong correlations are seen for total PAHs with vehicular emission sources, whereas the apportioned mass from other sources shows weak to null correlations.

In the mixed-model regression analyses, we found positive associations of circulating biomarkers of inflammation (IL-6 and sTNF-RII) with organic components (Table 5, Figure 1). We found the strongest associations with biomarkers for both indoor and outdoor PAHs, including LMW, MMW, and HMW PAHs. The next strongest associations were for hopanes. Indoor but not outdoor hopanes were associated with IL-6, whereas both indoor and outdoor hopanes were associated with sTNF-RII.

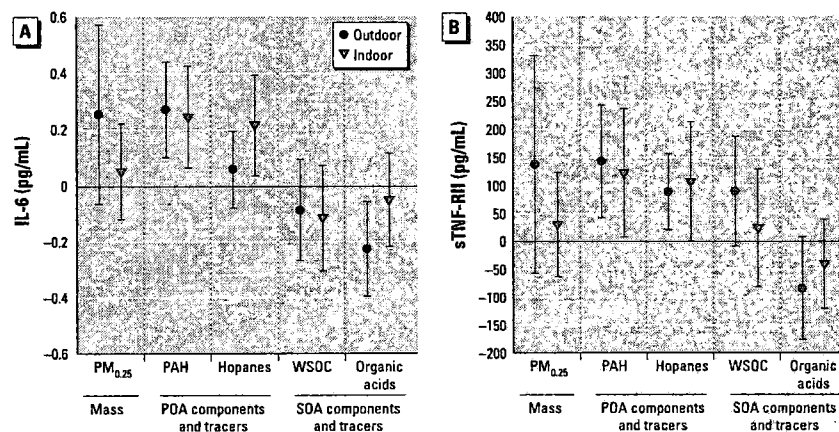
Outdoor WSOC (a marker of SOAs) was positively associated with sTNF-RII, but confidence limits crossed 1.0 (*p* < 0.14), and we found no other associations with SOA markers. The outdoor organic acids (another marker of SOAs) showed a pattern opposite to that of the POA markers, with largely negative regression coefficients in relation to biomarkers of inflammation. To assess whether this was due to inverse correlations with PAHs, we coregressed outdoor total PAHs with outdoor organic acids. We found that associations with PAHs and with organic acids decreased in magnitude to small degrees when coregressed, suggesting that the negative regression coefficients for organic acids with

**Table 5.** Associations of biomarkers of systemic effect with indoor and outdoor 5-day average PM<sub>0.25</sub> mass and organic components [regression coefficient (95% CI)].

| Air pollutant           | IL-6 (pg/mL)             | sTNF-RII (pg/mL)  |
|-------------------------|--------------------------|-------------------|
| PM <sub>0.25</sub> mass |                          |                   |
| Indoor                  | 0.05 (–0.12 to 0.22)     | 18 (–61 to 97)    |
| Outdoor                 | 0.26 (–0.06 to 0.57)     | 125 (–40 to 289)  |
| WSOC                    |                          |                   |
| Indoor                  | –0.11 (–0.30 to 0.08)    | 15 (–77 to 108)   |
| Outdoor                 | –0.08 (–0.27 to 0.10)    | 63 (–19 to 145)   |
| PAHs                    |                          |                   |
| Total                   |                          |                   |
| Indoor                  | 0.25 (0.07 to 0.43)**    | 119 (16 to 223)*  |
| Outdoor                 | 0.27 (0.10 to 0.44)**    | 135 (45 to 225)** |
| LMW                     |                          |                   |
| Indoor                  | 0.30 (0.10 to 0.50)**    | 115 (–2 to 233)   |
| Outdoor                 | 0.22 (0.05 to 0.39)*     | 109 (19 to 200)*  |
| MMW                     |                          |                   |
| Indoor                  | 0.28 (0.07 to 0.48)**    | 138 (22 to 254)*  |
| Outdoor                 | 0.30 (0.12 to 0.48)**    | 143 (47 to 238)** |
| HMW                     |                          |                   |
| Indoor                  | 0.18 (0.02 to 0.35)*     | 91 (1 to 181)*    |
| Outdoor                 | 0.26 (0.07 to 0.44)**    | 137 (39 to 234)** |
| Hopanes                 |                          |                   |
| Indoor                  | 0.22 (0.04 to 0.39)*     | 107 (10 to 204)*  |
| Outdoor                 | 0.06 (–0.08 to 0.20)     | 89 (26 to 151)**  |
| <i>n</i> -Alkanes       |                          |                   |
| Indoor                  | 0.01 (–0.03 to 0.06)     | –6 (–27 to 16)    |
| Outdoor                 | 0.009 (–0.03 to 0.05)    | 14 (–6 to 34)     |
| Organic acids           |                          |                   |
| Indoor                  | –0.05 (–0.22 to 0.12)    | –36 (–109 to 37)  |
| Outdoor                 | –0.22 (–0.39 to –0.06)** | –82 (–164 to 1)   |

Regression coefficients and 95% CIs are for the expected change in the biomarker among 60 subjects associated with an IQR change in the air pollutant (see Table 2), adjusted for temperature.

\**p* < 0.05, \*\**p* < 0.01.



**Figure 1.** Associations of biomarkers with 5-day average outdoor and indoor community PM<sub>0.25</sub> mass, and markers of POAs and SOAs. (A) IL-6. (B) sTNF-RII. Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an IQR increase in the air pollutant concentration (see Table 2), adjusted for temperature.

biomarkers of inflammation may be attributed to other unmeasured factors or chance.

We then tested two-pollutant regression models that included both outdoor  $PM_{0.25}$  mass and total PAHs to assess whether PAHs explained the nominal association with mass. We found that IL-6 and sTNF-RII associations with mass were completely confounded by PAHs in that the regression coefficient for mass decreased to just below zero and the regression coefficient for PAHs was nearly unchanged (Figure 2A,B). We found a similar effect for hopenes, which confounded the nominal association of  $PM_{0.25}$  mass with sTNF-RII (Figure 2C). The variance inflation factor was  $< 3.5$  for exposures, thus showing little evidence of multicollinearity.

Transition metals were not associated with the biomarkers [see Supplemental Material, Table 2 (doi:10.1289/ehp.0901407)].

As previously shown (Delfino et al. 2009), the analysis of the relation of erythrocyte antioxidant enzymes (Cu,Zn-SOD and GPx-1) to air pollutants among all 60 subjects showed regression coefficients were largely negative, suggesting inverse associations, but most upper confidence limits crossed 1.0 (see Supplemental Material, Table 3 (doi:10.1289/ehp.0901407)). The exploratory analysis showed that among seven subjects previously identified as a "positive responder group" (Delfino et al. 2009), we found largely positive associations of Cu,Zn-SOD and GPx-1 with air pollutants, and lower confidence limits were  $> 1.0$  for outdoor  $PM_{0.25}$  mass and several other exposures. In the 53 subjects previously identified as a "negative responder group," we found inverse associations of Cu,Zn-SOD and GPx-1 with indoor and outdoor total, LMW, MMW, and HMW PAHs and with hopenes (all markers of exposures linked to primary combustion). Indoor WSOC was inversely ( $p < 0.07$ ) associated with Cu,Zn-SOD, but we found no other associations with SOA markers in the negative

responder group. Confidence limits were wider for GPx-1 than for Cu,Zn-SOD.

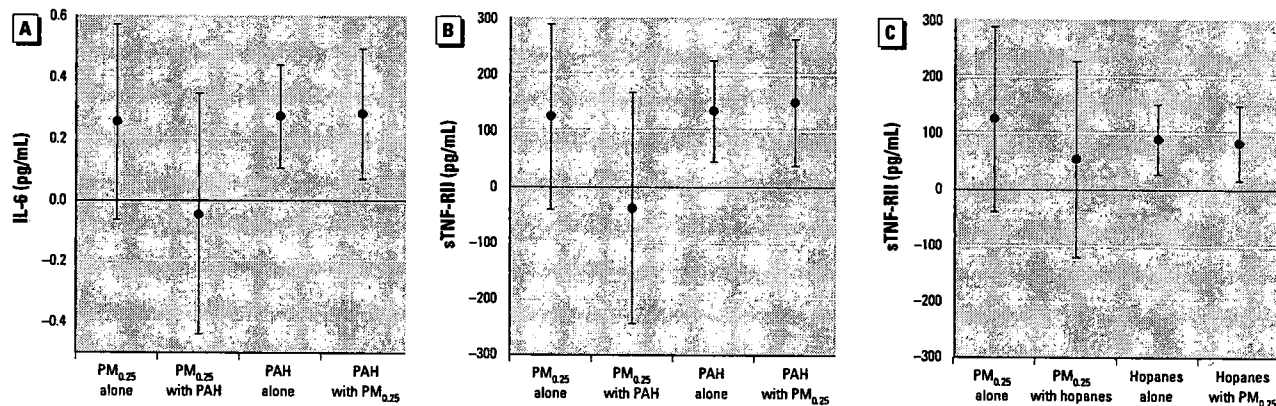
## Discussion

To our knowledge, this is the first report from a panel cohort study to show associations of circulating biomarkers of response in human subjects to specific PM organic compound classes. The measured chemicals serve as indicators and tracers for air pollutant sources and for classes of chemicals with the potential for redox activity in the body. Our prior work has focused on carbonaceous aerosols that provided some differentiation between POAs and SOAs by showing associations of biomarkers of inflammation with primary  $PM_{2.5}$  organic carbon (OC) but not secondary  $PM_{2.5}$  OC (a marker of SOAs) (Delfino et al. 2008, 2009). In the present analysis, we found the strongest biomarker associations with air pollutant variables for all molecular weight classes of PAHs and specific source markers of vehicular emissions (hopenes) measured in  $PM_{0.25}$  with GC/MS. Furthermore, two-pollutant models of the relation between the biomarkers of systemic inflammation and both total PAHs and  $PM_{0.25}$  mass showed that mass associations were completely explained by PAHs. Given the results of the chemical mass balance analysis [see Supplemental Material, Chemical mass balance (CMB) model (doi:10.1289/ehp.0901407) and Arhami et al. 2010], we infer that the confounding of nominal associations between biomarkers and  $PM_{0.25}$  mass by PAHs was through a common set of sources. PAHs likely serve here as a surrogate for redox-active PM chemical components as evidenced in experimental models (Riedl and Diaz-Sanchez 2005). For example, PAHs from diesel exhaust particles and oxidized derivatives of PAHs such as quinones lead to the generation of reactive oxygen species and subsequent oxidant injury and inflammatory responses, including the expression of nuclear

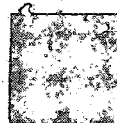
transcription factor- $\kappa B$  (NF $\kappa B$ ) (Riedl and Diaz-Sanchez 2005). NF $\kappa B$  increases the transcription of cytokines and acute-phase proteins that are predictive of coronary artery disease risk (Pai et al. 2004). PAHs can induce oxidative stress responses after biotransformation to quinones by cytochrome P450 1A1 (Bonvallot et al. 2001), perhaps after delivery from the lungs to systemic targets.

In the Los Angeles Basin, most outdoor PAHs in  $PM_{0.25}$  are expected to be from mobile sources (Schauer et al. 1996), and the CMB exposure correlations are consistent with this expectation. PAHs were also correlated with source markers of vehicular emissions (hopenes). Hopenes are the most unambiguous source marker of traffic emissions. However, the moderate but not strong correlation between hopenes and PAHs suggests that the measured PAHs include a different subset of mobile sources than that of hopenes. This may in part be due to the variability in PAHs relative to hopenes by combustion-related problems in the vehicle fleet (Lough et al. 2007).

Overall, the associations of biomarkers with PAHs and hopenes suggest that our previous findings of positive associations of biomarkers with  $PM_{2.5}$ , EC, and primary OC (Delfino et al. 2009) were due to PM of mobile-source origin. PAHs are found in greater concentrations in the quasi-UFP range compared with larger particles (Ntziachristos et al. 2007), and this has been hypothesized to explain enhanced prooxidative and proinflammatory effects of urban UFPs in the lungs and peripheral target organs of rodents (Araujo et al. 2008). The increased biological potency of UFPs may be related to the content of organic chemicals that have the capacity to reduce oxygen, such as quinones and nitro-PAHs, for which PAHs may act, in part, as a surrogate (Ntziachristos et al. 2007) or as a source after biotransformation. From the present results we infer that, although PAHs may have an effect by



**Figure 2.** Associations of circulating biomarkers of inflammation with outdoor  $PM_{0.25}$  mass coregressed with outdoor total PAHs and hopenes in  $PM_{0.25}$ . (A) IL-6, PAHs, and  $PM_{0.25}$ . (B) sTNF-RII, PAHs, and  $PM_{0.25}$ . (C) sTNF-RII, hopenes, and  $PM_{0.25}$ . Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an IQR increase in the air pollutant concentration (see Table 2), adjusted for temperature.



themselves, they are also likely surrogates for other causal species we did not measure that are emitted from the same (traffic) sources.

We found little evidence that tracer variables for SOAs and related components (WSOC and organic acids) were associated with the circulating biomarkers in the expected direction. We have no explanation for the negative regression coefficients for organic acids with biomarkers. Although most of the SOAs are expected to be in larger PM > 0.25  $\mu\text{m}$ , the present results are consistent with our finding of few biomarker associations with PM<sub>2.5</sub> secondary OC or accumulation mode particle mass (PM<sub>0.25-2.5</sub>) in an earlier publication (Delfino et al. 2009). In that study, regression coefficients were also negative for IL-6 in some models with PM<sub>0.25-2.5</sub> and with secondary OC. We speculate that components in outdoor SOAs estimated by our methods (e.g., organic acids), are mostly water soluble and highly oxygenated, and dissolve after deposition on the airway epithelium and then quickly react with extracellular macromolecules and cell membrane constituents. Thus, these PM components may not directly interact with the vasculature, although it has been hypothesized that inhaled particles lead to airway inflammatory responses and subsequent release of activated leukocytes and cytokines into the circulation (Mills et al. 2009).

An important limitation of our characterization of SOAs is that WSOCs and organic acids do not completely characterize the SOA fraction of PM, part of which may come from the photochemical oxidation of low-volatility vapors to form hydrophilic organic components, but whose chemical identity is largely unknown. These precursor vapors include SVOCs that are largely part of POAs. SVOCs evaporate from the particle phase during the process of atmospheric dilution and subsequently react with oxidant gases to form a significant fraction of SOAs (Robinson et al. 2007).

Lipid-soluble components of PM more closely associated with primary emissions, including PAHs, may become bioavailable after deposition followed by distribution of unmetabolized chemicals to the circulation and to extrapulmonary target sites (Gerde et al. 2001). It is also possible that a small fraction of toxic components is carried via various translocation mechanisms into the circulation on UFPs (Mühlfeld et al. 2008). However, translocation may account for a potentially insignificant amount of the impact of UFPs compared with the high retention of UFPs in the lungs (Möller et al. 2008), which may lead to sustained effects through the gradual transfer of redox-active components to the circulation over many days.

Although transition metals are known to be redox active, we found no consistent associations with the biomarkers measured,

possibly because of low concentrations of these trace elements in the study areas.

Finding positive associations of biomarkers with both indoor and outdoor PAHs and hopanes along with the indoor/outdoor ratios of these organic components being close to 1.0 suggests that, even though people spend most of their time indoors, indoor air quality and PM exposures are strongly influenced by PM of outdoor origin. These findings are consistent with our previous analysis for the first half of this panel showing that CMB-estimated indoor PM of outdoor origin (particle number, EC, and primary OC) were associated with the biomarkers to a similar degree as outdoor PM (Delfino et al. 2008).

Briefly, the exploratory (data-driven) findings for GPx-1 and especially Cu,Zn-SOD are consistent with our previous findings for primary OC and EC (Delfino et al. 2009) and suggest antioxidant enzyme inactivation within erythrocytes by traffic-related pollutant components, including PAHs, among a subgroup of people. This inactivation is anticipated to increase oxidative stress and thus inflammation. This is potentially important because these enzymes likely represent important intermediate end points that have been linked to the risk of developing coronary artery disease in prospective cohort and other studies (Flores-Mateo et al. 2009). Given that these findings were far less clear when including the entire 60-subject panel (because a small subgroup of seven subjects had positive associations), these results should be viewed as hypothesis generating and retested in other populations. See Delfino et al. (2009) for further details and discussion concerning potential mechanisms of antioxidant enzyme inactivation versus up-regulation that may explain group differences.

Strengths of the present study lie in exposure measurements in each subject's community microenvironment and in repeated biological marker assessments in a well-characterized patient sample. Limitations include the potential for unmeasured temporal confounding. However, we performed *a priori* adjustment for one of the largest sources of variability in inflammatory mediators that have been documented in the literature (infections), and we also accounted for temperature and for community and seasonal variability in exposures. We also acknowledge that the present study does perform multiple comparisons, although we did narrow the number of hypotheses being tested based on prior evidence of associations from the work of others and ourselves.

The results of the present study suggest that tracer components of mobile source emissions in PM<sub>0.25</sub> are associated with increased systemic inflammation in a potentially susceptible population of elderly individuals. The measured biomarkers likely represent

important intermediate end points (systemic inflammation) that have been linked to the risk of cardiovascular diseases in prospective cohort and other studies (Kritchevsky et al. 2005; Pai et al. 2004). The positive relation between air pollution and cytokine biomarkers may also be indicative of acute risk of adverse cardiovascular outcomes related to vascular dysfunction and atherothrombosis (Mills et al. 2009). We recently reported coherent associations between hourly ambulatory systolic and diastolic blood pressure and hourly air pollutant exposures in the present panel cohort, including stronger associations with primary PM<sub>2.5</sub> OC compared with secondary PM<sub>2.5</sub> OC (Delfino et al. 2010).

We conclude that U.S. EPA-regulated ambient PM<sub>2.5</sub> mass measurements may not adequately represent risk to human health because they are uncharacterized by composition, source, or PM size distribution and are not necessarily representative of personal or local exposure. Confirmatory data are needed in other populations using measurements of organic components across several PM size fractions.

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# Health Impacts of the Built Environment: Within-Urban Variability in Physical Inactivity, Air Pollution, and Ischemic Heart Disease Mortality

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**BACKGROUND:** Physical inactivity and exposure to air pollution are important risk factors for death and disease globally. The built environment may influence exposures to these risk factors in different ways and thus differentially affect the health of urban populations.

**OBJECTIVE:** We investigated the built environment's association with air pollution and physical inactivity, and estimated attributable health risks.

**METHODS:** We used a regional travel survey to estimate within-urban variability in physical inactivity and home-based air pollution exposure [particulate matter with aerodynamic diameter  $\leq 2.5$   $\mu\text{m}$  ( $\text{PM}_{2.5}$ ), nitrogen oxides ( $\text{NO}_x$ ), and ozone ( $\text{O}_3$ )] for 30,007 individuals in southern California. We then estimated the resulting risk for ischemic heart disease (IHD) using literature-derived dose-response values. Using a cross-sectional approach, we compared estimated IHD mortality risks among neighborhoods based on "walkability" scores.

**RESULTS:** The proportion of physically active individuals was higher in high- versus low-walkability neighborhoods (24.9% vs. 12.5%); however, only a small proportion of the population was physically active, and between-neighborhood variability in estimated IHD mortality attributable to physical inactivity was modest (7 fewer IHD deaths/100,000/year in high- vs. low-walkability neighborhoods). Between-neighborhood differences in estimated IHD mortality from air pollution were comparable in magnitude (9 more IHD deaths/100,000/year for  $\text{PM}_{2.5}$  and 3 fewer IHD deaths for  $\text{O}_3$  in high- vs. low-walkability neighborhoods), suggesting that population health benefits from increased physical activity in high-walkability neighborhoods may be offset by adverse effects of air pollution exposure.

**POLICY IMPLICATIONS:** Currently, planning efforts mainly focus on increasing physical activity through neighborhood design. Our results suggest that differences in population health impacts among neighborhoods are similar in magnitude for air pollution and physical activity. Thus, physical activity and exposure to air pollution are critical aspects of planning for cleaner, health-promoting cities.

**KEY WORDS:** active travel, air quality, environmental planning, infill, risk assessment, urban form. *Environ Health Perspect* 120:247–253 (2012). <http://dx.doi.org/10.1289/ehp.1103806> [Online 17 October 2011]

Physical inactivity is associated with increased risk of several adverse health outcomes including heart disease, type 2 diabetes, colon cancer, breast cancer, and mortality (Colditz et al. 1997; Kelley and Goodpaster 2001; Kohl 2001; Verloop et al. 2000). Active commuting, such as walking or biking to work on a daily basis, has been shown to decrease risk of all-cause mortality and cardiovascular disease (Andersen et al. 2000; Hamer and Chida 2008; Zheng et al. 2009). Various attributes of the built environment (e.g., population density, street connectivity, land use mix) have been associated with rates of physical activity at the neighborhood level (Ewing et al. 2003; Frank et al. 2005; Saelens et al. 2003a; Sallis et al. 2009). Furthermore, the type of transportation mode used (public transit vs. car) affects personal energy expenditure (Morabia et al. 2010). Thus, an important research question is whether urban planning can reduce physical inactivity and improve health.

Exposure to outdoor urban air pollution is associated with various adverse health outcomes including heart disease, respiratory disease, lung cancer, asthma, and mortality

(Brunekreef and Holgate 2002; Gent et al. 2003; Pope and Dockery 2006; Pope et al. 2002). Chronic exposures vary at similar magnitudes within-cities as between-cities (Jerrett et al. 2005; Miller et al. 2007), suggesting that neighborhood location, urban design, and proximity to roads can affect exposures (Health Effects Institute 2009; Marshall et al. 2005).

Recently, the World Health Organization (WHO) cited physical inactivity (4th) and exposure to outdoor urban air pollution (14th) among the top 15 risk factors for the Global Burden of Disease (WHO 2009); for high-income countries, these ranks are 4th (physical inactivity) and 8th (outdoor air pollution). Urban planning and the built environment may differentially influence exposures to those two risk factors (Marshall et al. 2009). A small number of studies have investigated the effects of exercise while controlling for air pollution exposure (de Nazelle et al. 2009; Wong et al. 2007) or explored regional- or national-scale theoretical shifts to active travel (de Hartog et al. 2010; Grabow et al. 2011); however, accounting for health

outcomes from exposure to air pollution and physical inactivity among neighborhood types is a little-studied area.

We used risk assessment to explore urban-scale spatial patterns in exposures associated with the built environment. We investigated differences in urban form that have been associated with physical inactivity and air pollution [specifically, particulate matter with aerodynamic diameter  $\leq 2.5$   $\mu\text{m}$  ( $\text{PM}_{2.5}$ ), nitrogen oxides ( $\text{NO}_x$ ), and ozone ( $\text{O}_3$ )] to assess relationships between urban form and public health.

## Methods

Our approach combined four primary sources of information: a geocoded, self-report travel diary to indicate home location and physical activity levels for a specific cohort ( $n = 30,007$ ); modeled and measured estimates of outdoor air pollution concentrations and their variability in space and time; literature-derived estimates relating ischemic heart disease (IHD) rates with physical inactivity and exposure to air pollution; and geographic information system (GIS) land use variables related to walkability. Our method is descriptive (i.e., cross-sectional) and aims to explore long-term health effects of neighborhood characteristics and location. Figure 1 illustrates our risk assessment approach.

**Physical inactivity and air pollution exposures.** We used the year 2001 Post-Census Regional Travel Survey to estimate exposure to physical inactivity and home-based exposure to outdoor air pollution. This survey, which covers southern California communities such as Orange County and Los Angeles, included a geocoded time-activity

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diary that captured self-reported activities and travel during fall 2001 and spring 2002. The survey population consisted of a random sample of residents, recruited by telephone in six southern California counties [Imperial, Los Angeles, Orange, Riverside, San Bernardino, and Ventura; for survey details, see Southern California Association of Governments (SCAG 2003)]. To our knowledge, no other metropolitan-scale travel survey has been used to estimate physical activity and exposure to air pollution (Marshall et al. 2006); in addition, this survey represents one of the largest exposure-relevant surveys available for any urban area in the world.

Of the 40,376 survey respondents, 30,007 (74%) met our inclusion criteria: *a*) geocoded home location [2,346 respondents excluded (5.8%)], *b*) home location within the air pollution modeling domain—the South Coast Air Basin [4,491 respondents excluded (11.1%)], and *c*) complete demographic information [age, sex, and ethnicity; 3,532 respondents excluded (8.7%)]. The survey generally covered 1 weekday per participant. We multiplied each participant's 1-day physical activity record by 7 to obtain an estimate of weekly minutes of physical activity. This approach assumed that physical activity was constant across all days of the week. Population-average levels of physical activity were similar (< 15% difference) between weekdays and weekends (11 vs. 12 min/day, respectively) based on data from a small number of respondents (13%,  $n = 5,104$ ) who participated in an additional weekend survey supplement (see sensitivity analysis 1, below). The survey recorded total physical activity and separately disaggregated that total into active transport (e.g., walking, bicycling) versus recreational activities (e.g., sports, working out at a gym).

Our primary estimates for air pollution exposure were based on monitoring data [U.S. Environmental Protection Agency (EPA) 2010] for  $PM_{2.5}$ ,  $NO_x$ , and  $O_3$  in 2001. We interpolated concentrations [inverse-distance weighted average of the nearest three monitors

(Marshall et al. 2008)] to each survey participant's home location. Each pollutant had several monitoring stations ( $PM_{2.5}$ , 27;  $NO_x$ , 42;  $O_3$ , 52), providing good spatial coverage for the 36,000-km<sup>2</sup> study area. We estimated the annual average of daily 1-hr maximum concentrations for  $O_3$  and annual-average concentrations for  $PM_{2.5}$  and  $NO_x$  at each survey participant's residence to match the metrics used in the epidemiological studies that we used to estimate IHD risks. We used spatial interpolation for the base case because it can be used for all three pollutants and is easily transferable to other urban areas.

**Neighborhood walkability.** We calculated three built environment variables to represent neighborhood type: *a*) population density, *b*) intersection density, and *c*) land use mix. Neighborhoods that were in the upper (lower) tertile of all three built environment variables were defined as high- (low-) walkability neighborhoods. This approach classified 12% of the survey population as living in a high-walkability neighborhood and 18% as living in a low-walkability neighborhood. We used objective measurements of the built environment rather than geographical overlays to match methods commonly used in the urban planning literature. Although no standard measure of walkability exists, most indices include measures of density, connectivity, and land use mix (Ewing and Cervero 2001). As a sensitivity analysis, based on prior research (Marshall et al. 2009) we implemented a second definition that classified 33% of survey participants in high- and 33% in low-walkability neighborhoods [for methods, see Supplemental Material, p. 2 (<http://dx.doi.org/10.1289/ehp.1103806>)]. Results were similar for both definitions; therefore, we report results using the first definition only.

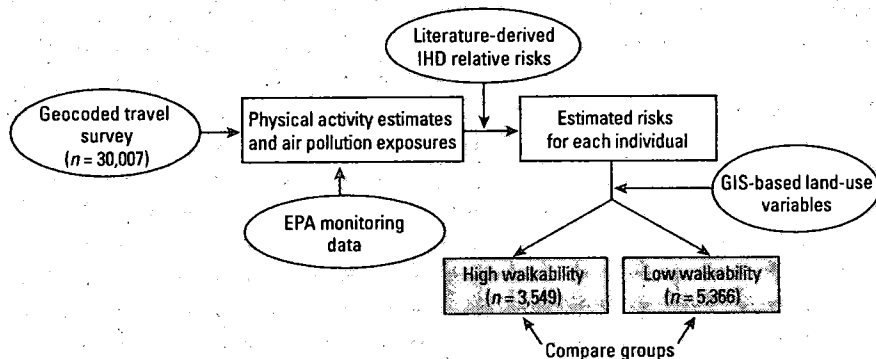
**Population density.** We used U.S. Census data from the year 2000 to calculate population density at the tract level for each household (U.S. Census Bureau 2000). Population density has been shown to be a predictor of per capita

automobile travel (Holtzclaw et al. 2002; Marshall 2008) and trip length (Ewing and Cervero 2001), both of which are predictors of bicycling and walking (Handy et al. 2002).

**Intersection density.** Intersection density was calculated using road TIGER/Line data (U.S. Census Bureau 2000). A 1-km non-freeway network buffer was generated for each household using ArcGIS (version: 9.3.1, ESRI; Redlands, CA, USA). Intersections (more than two road segments) were summed within the buffer, yielding a measure of street connectivity. Previous studies show that street connectivity may reduce vehicle travel and increase walking (Ewing and Cervero 2001; Forsyth et al. 2008).

**Land use mix.** Following Frank et al. (2004), we calculated a land use mix index for each household location. Aerial land use data was obtained from SCAG for the year 2001 (SCAG 2010). The index [see Supplemental Material, pp. 2–3 (<http://dx.doi.org/10.1289/ehp.1103806>)] is a normalized ratio of the mix of four primary land uses (residential, commercial, retail, and institutional) to total land area within the 1-km network buffer. The index ranges from 0 to 1: A value of 1 represents an equal mixture of the four land uses; a value of 0 indicates 100% of land is a single land use. Impacts of land use mix on health include reducing obesity (Frank et al. 2005) and increasing physical activity (Saelens et al. 2003b).

**Dose-response and relative risk estimates.** For each survey participant (i.e., at the individual level), we estimated relative risks (RRs) attributable to outdoor air pollution and physical inactivity for one important health outcome: IHD. IHD is consistently associated with outdoor air pollution and physical inactivity (WHO 2009), is responsible for a large proportion of deaths in the United States (~ 18% of all deaths and 67% of heart disease deaths in 2006) [Centers for Disease Control and Prevention (CDC) 2009], and has been shown to be an important health outcome for both risk factors when considering large-scale shifts to active travel (Woodcock et al. 2009). Because our exposure estimates for air pollution are continuous, we estimated an RR for each survey participant based on a linear dose-response [see Supplemental Material, Figure S2 (<http://dx.doi.org/10.1289/ehp.1103806>)] for the range of observed air pollutant concentrations and the referent exposure levels described below. In contrast, WHO (2004) suggests a three-tier dose-response for physical activity: *a*) active (exercise for > 150 min/week; RR = 1), *b*) insufficiently active (exercise for 1–150 min/week; RR = 1.31), and *c*) inactive (0 min exercise per week; RR = 1.47), allowing for only three possible physical activity RRs for each survey participant. We estimated



**Figure 1.** Conceptual framework for this risk assessment. Ovals are inputs, and boxes are midpoint calculations. Shaded boxes indicate estimated risk separated into two groups for comparison.



attributable fractions for outdoor air pollution and physical inactivity using the mean individual RR in high- or low-walkability neighborhoods.

Air pollution dose-response relationships were identified and selected as follows. We manually searched the tables of contents of four journals (*Journal of the American Medical Association*, *New England Journal of Medicine*, *British Medical Journal*, *Lancet*) for the years 2000–2010 for air pollution risk estimates. We also performed a search of key words in Google Scholar and ISI Knowledge, including (in various combinations) “air pollution,” “O<sub>3</sub>/NO<sub>x</sub>/PM<sub>2.5</sub>,” “ischemic heart disease,” “cardiovascular disease,” “cardiopulmonary disease,” “respiratory disease,” “mortality,” “health effects,” “chronic/acute,” and “dose-response.” We used the “cited by” function in Google Scholar to explore subsequent studies related to each article. Through this process, we identified 62 articles. We then selected studies that focused on within-city variation and included IHD as a health outcome (Table 1).

Each RR for air pollution was estimated from cohort studies of long-term exposures; however, these estimates differed in important ways. For example, Nafstad et al. (2004) studied men 40–49 years of age, meaning our NO<sub>x</sub> results cannot be generalized to other populations [RR = 1.08; 95% confidence interval (CI): 1.06, 1.11]. Jerrett et al. (2005) used a subset of the American Cancer Society (ACS) cohort (Los Angeles, CA, USA) to estimate a within-city RR of 1.25 per 10 µg/m<sup>3</sup> increase in PM<sub>2.5</sub> (95% CI: 0.99, 1.59). Jerrett et al. (2005) did not report a significant RR for PM<sub>2.5</sub> in Los Angeles, but the RR estimate is roughly consistent with two between-city studies that did report statistically significant RRs: Pope et al. (2004; RR = 1.18 per 10 µg/m<sup>3</sup> increase in PM<sub>2.5</sub>; 95% CI: 1.14, 1.23) and Jerrett et al. (2009; RR = 1.21; 95% CI: 1.16, 1.27). The Jerrett et al. (2009) RR for a 10 µg/m<sup>3</sup> increase in O<sub>3</sub> (1.008; 95% CI: 1.002, 1.013) was based on between-city variation (ACS cohort) in 96 U.S. metropolitan statistical areas generated from a one-pollutant model. However, it is important to note that Jerrett et al. (2009) reported a protective effect for O<sub>3</sub> based on a two-pollutant model adjusted for PM<sub>2.5</sub> (RR = 0.97; 95% CI: 0.96, 0.99), and overall there is less evidence in the literature for O<sub>3</sub> associations with IHD compared with those for PM<sub>2.5</sub>. A within-city study of O<sub>3</sub> and IHD was not available.

The referent exposure levels used to estimate individuals' RRs were “active” for physical inactivity (> 150 min of moderate-vigorous activity per week), and the 10th percentile of exposure (survey population based; values: 13.6 µg/m<sup>3</sup> for PM<sub>2.5</sub>, 39.8 µg/m<sup>3</sup> for NO<sub>x</sub>, 80.3 µg/80.3 µg/m<sup>3</sup> for O<sub>3</sub>) for air pollution, consistent with exposures in a relatively clean

neighborhood in the study area. Each survey participant's air pollution RR was estimated based on the difference between their home-location air pollution exposure and the referent exposure level. For example, for PM<sub>2.5</sub>, an individual whose home-location exposure estimate was 23.6 µg/m<sup>3</sup> (10 µg/m<sup>3</sup> above the referent level) would be assigned an RR of 1.25.

**Population-attributable fraction.** We calculated population-attributable fraction (PAF) and estimated attributable IHD mortality rates for each risk factor in high- and low-walkability neighborhoods. PAF for a neighborhood was calculated based on the proportion of individuals exposed to each risk factor and average RR among all individuals in a neighborhood (Baker and Nieuwenhuijsen 2008):

$$\text{PAF} = \frac{p \times (\text{RR} - 1)}{[p \times (\text{RR} - 1)] + 1} \quad [1]$$

Here, RR is the mean individual RR in each group (high- and low-walkability neighborhoods) and risk factor, and  $p$  is the proportion of individuals exposed in each group (defined by our referent exposure levels). We used the 2000–2001 age-adjusted IHD mortality rate in California (191.2 IHD deaths/100,000/year; CDC 2011) to estimate deaths within each group and subsequent attributable IHD mortality rates (except for NO<sub>x</sub> where we used the IHD mortality rate for men in California 45–54 years of age: 81.9 IHD deaths/100,000/year). Attributable mortality due to physical inactivity, PM<sub>2.5</sub>, NO<sub>x</sub>, and O<sub>3</sub> cannot be summed because of confounding among the risk factors and overlap of at-risk populations. Therefore, we report attributable mortality due to the different factors separately.

We separately calculated PAF using a method with multiple exposure levels instead of the dichotomous exposure levels implicit in Equation 1, as described in the Supplemental Material [pp. 5–6 (<http://dx.doi.org/10.1289/ehp.1103806>)] Results based on this alternative method were similar to those reported below.

**Sensitivity analyses.** To explore the robustness of our estimates, we used three sensitivity analyses to assess *a*) different methods of scaling minutes of physical activity, *b*) alternate

modeling approaches for air pollution, and *c*) stepwise versus linear dose-response for physical activity.

**Sensitivity analysis 1: scaling method for minutes of physical activity.** Our approach requires extrapolating weekly exercise rates based on the 1-day travel diary because most physical activity epidemiological literature employs the metric “minutes of physical activity per week.” To test the limitations of this extrapolation for our analysis, we developed a Monte Carlo simulation that relaxes our base-case assumption (i.e., that individuals' physical activity rates are constant by day), by employing two alternative assumptions: that people who are nonsedentary are physically active *a*) every other day or *b*) every third day. The Monte Carlo simulation distributes total minutes of physical activity accordingly, stratifying by age, sex, and ethnicity. The resulting distributions of physical activity better approximate national estimates on the prevalence of physical inactivity (WHO 2004).

**Sensitivity analysis 2: air pollution model.** Our base-case analysis used spatial interpolation of U.S. EPA monitoring data, which are readily available for all three pollutants for many urban areas. We compared results using a Eulerian dispersion model [Comprehensive Air Quality Model with Extensions (CAMx); <http://www.camx.com>; nitrous oxide (NO), nitrogen dioxide (NO<sub>2</sub>), O<sub>3</sub>] and land-use regression (LUR; NO<sub>2</sub>; Novotny et al. 2011). CAMx and LUR provide greater spatial precision than inverse-distance weighting but may or may not be available in other urban areas.

**Sensitivity analysis 3: physical activity dose-response.** We tested the sensitivity of our results to the dose-response curve for physical inactivity. Our base case used the stepwise dose-response from WHO (2004) (Table 1). For this sensitivity analysis, we generated three linear dose-response curves (low, medium, and high slopes) based on the same WHO values.

## Results

Annual-average air pollution exposure for the survey population averaged 49 µg/m<sup>3</sup> for NO<sub>2</sub> [interquartile range (IQR), 41–60 µg/m<sup>3</sup>], 99 µg/m<sup>3</sup> for O<sub>3</sub>

**Table 1.** Summary of RR estimates used for IHD.

| Study               | Risk factor         | Study details  | RR (95% CI)   |
|---------------------|---------------------|--|---|
| Nafstad et al. 2004 | NO <sub>x</sub>     | Within-city; men 40–49 years of age in Oslo, Norway ( $n = 16,209$ )   | 1.08 <sup>a</sup> (1.06, 1.11) per 10 µg/m <sup>3</sup>   |
| Jerrett et al. 2005 | PM <sub>2.5</sub>   | Within-city; subset (Los Angeles, CA) of the ACS cohort ( $n = 22,905$ )                                     | 1.25 <sup>a</sup> (0.99, 1.59) per 10 µg/m <sup>3</sup>   |
| Jerrett et al. 2009 | O <sub>3</sub>      | Between-cities; ACS cohort ( $n = 448,850$ )   | 1.008 <sup>a</sup> (1.002, 1.013) per 10 µg/m <sup>3</sup>  |
| WHO 2004            | Physical inactivity | Meta-analysis of 20 studies from two continents (Western Europe, 8; North America, 12; total $n = 327,004$ ) | Insufficiently active: <sup>b</sup> 1.31 (1.21, 1.41)<br>Inactive: <sup>b</sup> 1.47 (1.39, 1.56) |

<sup>a</sup>Air pollution risk estimates used here were based on long-term cohort studies and chronic health effects. <sup>b</sup>Referent, > 150 min/week; insufficiently active, 1–150 min/week; inactive, 0 min/week.

(86–112  $\mu\text{g}/\text{m}^3$ ; annual average of 1-hr daily maximums), and 22  $\mu\text{g}/\text{m}^3$  for  $\text{PM}_{2.5}$  (20–24  $\mu\text{g}/\text{m}^3$ ; Table 2). Mean  $\text{NO}_2$  exposures were below current ambient-air standards [U.S. EPA and California Environmental

Protection Agency (CalEPA) standards, respectively: 100 and 57  $\mu\text{g}/\text{m}^3$ ].  $\text{PM}_{2.5}$  exposures were approximately 1.5 and 2 times higher than U.S. EPA (15  $\mu\text{g}/\text{m}^3$ ) and CalEPA (12  $\mu\text{g}/\text{m}^3$ ) long-term standards (annual

arithmetic mean), respectively (California Air Resources Board 2010).

Self-reported physical activity levels averaged 77 min/week (IQR, 0–0 min/week; i.e., the 25th and 75th values are 0 min/week; Table 2). Most (83.5%) of the survey participants reported being inactive (0 min/week), 5.6% reported being insufficiently active (1–150 min/week), and 10.9% reported being active (> 150 min/week; physical activity recommendations; U.S. Department of Health and Human Services 1996). Activity levels were notably lower than national averages (U.S. averages: inactive, 29%; insufficiently active, 45%; active, 26%; WHO 2004). Sensitivity analysis 1 addresses this difference in activity levels.

$\text{NO}_x$  and  $\text{PM}_{2.5}$  concentrations were highest near the city center and major roadways, whereas  $\text{O}_3$  concentrations were higher in the outer-lying areas (Figure 2). Because of this spatial pattern, few locations experienced low exposure to all three pollutants. Spatial patterns for physical activity were dependent on the purpose of the activity; there was no discernable spatial pattern for recreational activities, but active transport was clustered near high-walkability neighborhoods (Figure 2).

Average per capita physical activity was 50% higher in high- than in low-walkability neighborhoods (102 vs. 68 min/week; Figure 3). The number of nonsedentary individuals (people with > 0 min/week physical activity) was two times higher in high- versus low-walkability neighborhoods (24.9% and 12.5%, respectively;  $p < 0.001$ ). However, considering nonsedentary individuals only, average physical activity was 24% lower in high- than in low-walkability neighborhoods (410 vs. 543 min/week). This finding suggests that neighborhood type may have differing impacts on the number of people participating in physical activities, average physical activity among all individuals, and average physical activity among nonsedentary individuals.

The self-reported purpose of physical activity differs by neighborhood (Figure 3). For example, active transport accounts for about half of physical activity in the high-walkability neighborhoods but only 20% in low-walkability neighborhoods. Active transport is 3.6 times higher in high- versus low-walkability neighborhoods (a finding that partially corroborates our GIS estimates of walkability), whereas nontravel activity is similar (< 10% difference) in low- versus high-walkability neighborhoods. Activity level and purpose exhibited greater weekend/weekday differences in low-walkability areas than in high-walkability areas [see Supplemental Material, Table S2, Figure S4 (<http://dx.doi.org/10.1289/ehp.1103806>)].

Figure 4 shows estimated attributable IHD mortality rates for each neighborhood type

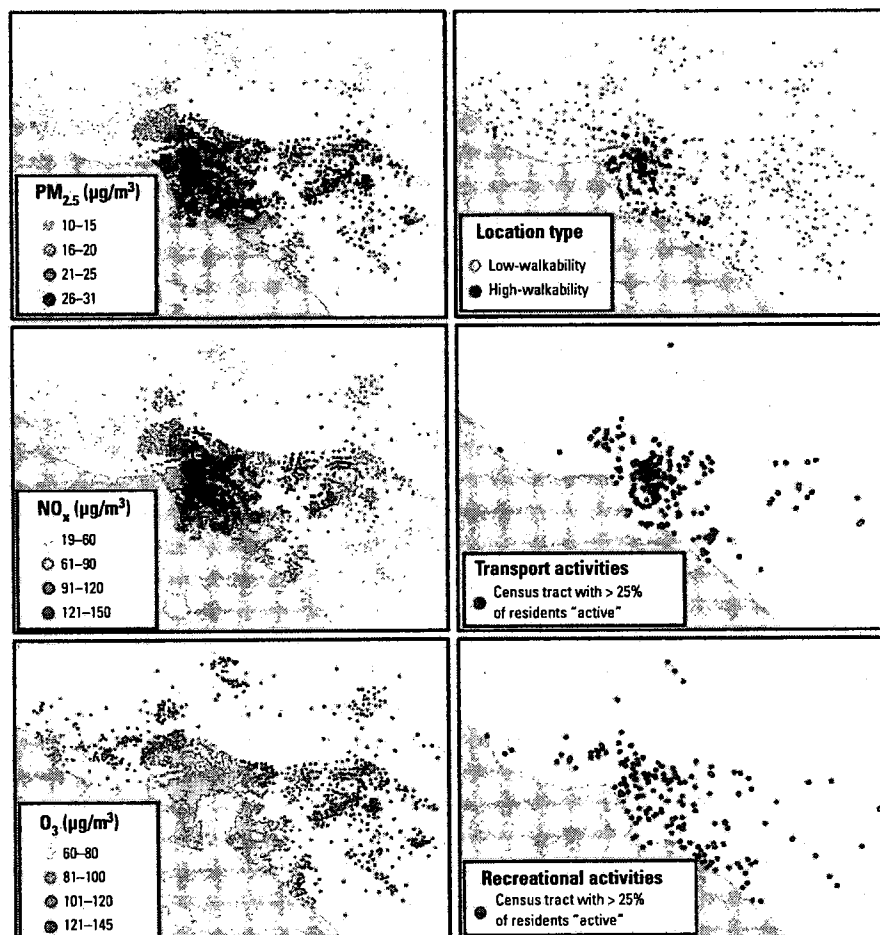
**Table 2.** Descriptive statistics by neighborhood type [mean (IQR)].

| Variable  | All<br>( <i>n</i> = 30,007) | Low walkability<br>( <i>n</i> = 5,366) | High walkability<br>( <i>n</i> = 3,549) |
|---|-----------------------------|--|---|
| Age (years)   | 38 (21–54)                  | 41 (23–58)                             | 34 (20–47)                              |
| Nonwhite (%)  | 40                          | 23                                     | 65                                      |
| Male (%)  | 50                          | 49                                     | 50                                      |
| Income > \$50,000 per year (%)  | 48                          | 57                                     | 31                                      |
| College or more (%)   | 46                          | 52                                     | 40                                      |
| $\text{NO}_x$ ( $\mu\text{g}/\text{m}^3$ ) <sup>a</sup>                     | 85 (68–103)                 | 67 (50–88)                             | 106 (89–130)                            |
| $\text{O}_3$ ( $\mu\text{g}/\text{m}^3$ ) <sup>b</sup>                      | 99 (86–112)                 | 111 (97–124)                           | 86 (82–92)                              |
| $\text{PM}_{2.5}$ ( $\mu\text{g}/\text{m}^3$ ) <sup>a</sup>                 | 22 (20–24)                  | 20 (14–25)                             | 23 (22–24)                              |
| Physical activity (min/week)  | 77 (0–0)                    | 68 (0–0)                               | 102 (0–0)                               |
| Population density in Census tract<br>(people/ $\text{km}^2$ ) <sup>c</sup> | 22,400 (7,800–28,400)       | 3,100 (600–5,200)                      | 53,500 (31,900–61,600)                  |
| Intersection density (1-km network buffer) <sup>c</sup>                     | 57 (27–82)                  | 11 (2–20)                              | 109 (86–114)                            |
| Land use mix (1-km network buffer) <sup>c</sup>                             | 0.37 (0.25–0.49)            | 0.13 (0–0.23)                          | 0.59 (0.50–0.66)                        |

All continuous variables in high-walkability neighborhoods have statistically significant differences (for all variables  $p < 0.001$ ) compared with low-walkability neighborhoods (two-tailed *t*-test).

<sup>a</sup>Home-location annual-average concentrations. <sup>b</sup>Home-location annual average of daily 1-hr maximum concentrations.

<sup>c</sup>This land use variable was used to define walkability.



**Figure 2.** Spatial variation of air pollution exposure and physical inactivity. Physical activity estimates were derived from time-activity diaries, air pollution exposures were calculated from U.S. EPA monitoring data, and walkability was defined using publicly available land use variables. Icons for transport and recreational activities represent census tracts where > 25% of the survey respondents reported > 150 min/week of that activity type.



and risk factor. Physical inactivity was more strongly associated with IHD mortality (51 additional deaths/100,000/year overall) than were the other exposures, but IHD mortality attributable to physical inactivity was only slightly different between high- and low-walkability neighborhoods (7 fewer IHD deaths/100,000/year in high- vs. low-walkability). Conversely, overall estimated attributable IHD mortality due to exposure to  $PM_{2.5}$  was smaller (30 deaths/100,000/year), but the difference between neighborhoods was slightly larger than for physical inactivity (9 more IHD deaths/100,000/year in high- vs. low-walkability).  $O_3$  shows the reverse spatial pattern as  $PM_{2.5}$  (i.e.,  $O_3$  exposure is higher in low-walkability neighborhoods, whereas  $PM_{2.5}$  is lower) but a smaller difference in mortality between neighborhoods (3 fewer IHD deaths/100,000/year in high- vs. low-walkability). Attributable IHD mortality rates for  $NO_x$  (represented by risk estimates for men 40–49 years of age; not shown in Figure 4) were 13 (28) IHD deaths/100,000/year for low- (high-) walkability neighborhoods. Attributable risk estimates for physical inactivity,  $PM_{2.5}$ , and  $O_3$  showed similar patterns when neighborhoods were classified according to deciles of walkability scores [Supplemental Material, Figure S5 (<http://dx.doi.org/10.1289/ehp.1103806>)].

**Sensitivity analysis 1: scaling method for minutes of physical activity.** Results [see Supplemental Material, pp. 8–9 (<http://dx.doi.org/10.1289/ehp.1103806>)] indicate that our alternative assumptions reduce the variability in physical activity among neighborhoods. Specifically, the Monte Carlo simulation increases the share of nonsedentary individuals (subsequently reducing average risks from physical inactivity) but also yields reductions in estimated IHD mortality differences among neighborhoods. Our core conclusions are similar among the Monte Carlo simulations.

**Sensitivity analysis 2: air pollution model.** Central tendencies varied by pollutant and model; however, trends in the core conclusions

(i.e., shifts in exposure and risk by neighborhood type) were similar where it was possible to compare [see Supplemental Material, pp. 9–10 (<http://dx.doi.org/10.1289/ehp.1103806>)]. In general, differences in estimated IHD mortality rates between high- and low-walkability neighborhoods were larger when using the alternate models; therefore, base-case results reported above may be conservative estimates (i.e., underestimates) of air pollution spatial variability.

**Sensitivity analysis 3: physical activity dose-response.** Our results did not change appreciably when using the linear dose-response curves [see Supplemental Material, pp. 10–11 (<http://dx.doi.org/10.1289/ehp.1103806>)].

We also estimated RRs according to neighborhood type (high- or low-walkability) within strata of age (0–25 years, 26–50 years, > 50 years) and according to income and ethnicity [high income (> \$75,000) and white vs. low income (< \$35,000) and nonwhite]. The results reveal similar trends in risk differences between neighborhoods for each strata, suggesting that our results are robust to accounting for differences in income, ethnicity, and age. Details are in the Supplemental Material [pp. 11–14, Table S6 (<http://dx.doi.org/10.1289/ehp.1103806>)]. Prior literature further explores socioeconomic aspects of this topic (e.g., Ewing 2005; Frank et al. 2007; Sallis et al. 2009).

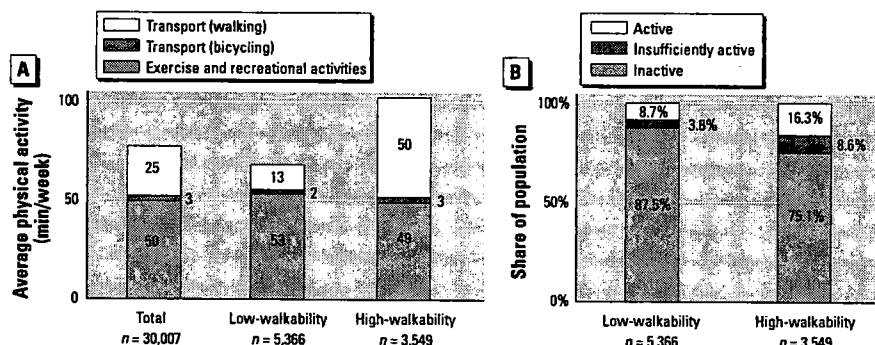
## Discussion

Our analysis summarizes between-neighborhood variations in two risk factors (exposure to air pollution, physical inactivity) using a time-activity travel diary for one region. We found risks were differential when stratified by neighborhood walkability. Specifically, when comparing estimated IHD mortality rates among neighborhoods, differences attributable to physical inactivity were modest and comparable to differences attributable to individual air pollutants. Because of spatial patterns associated with each pollutant, urban residents were often highly exposed to at least one but

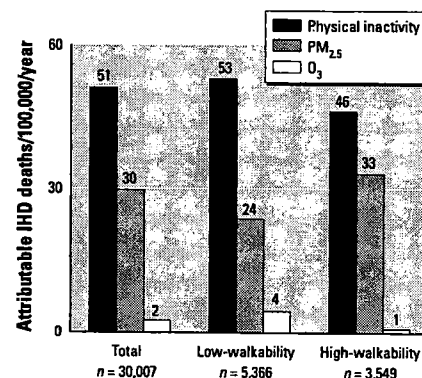
not all pollutants (e.g., high exposure to  $O_3$  in low-walkability neighborhoods or high exposure to  $PM_{2.5}$  in high-walkability neighborhoods). This trade-off suggests that the net health impact of neighborhoods may depend in part on spatial patterns of air pollution.

Recent health comparisons between air pollution and exercise (Carlisle and Sharp 2001; de Hartog et al. 2010) emphasize the greater health importance of exercise relative to air pollution. This prior research considered only people who exercise (Carlisle and Sharp 2001; de Hartog et al. 2010); here, we consider the entire population—nonsedentary plus sedentary individuals. Only a subset of a given population is physically active, and only a subset of that physical activity is influenced by neighborhood design; here, the net result is that spatial differences in attributable IHD mortality risks are of similar magnitude for physical inactivity as for air pollution. Our results indicate a doubling in the share of nonsedentary people in high- versus low-walkability neighborhoods (24.9% vs. 12.5%); however, all individuals—inactive and active—experience changes in air pollution exposures. For this study population, physical activity rates were higher (and exercise-attributable IHD mortality rates lower) in high- than in low-walkability neighborhoods. However, because variations in air pollution risk are similar to variations in physical inactivity risks, when comparing high- versus low-walkability neighborhoods, health benefits from increased physical activity may be offset by health risks from air pollution exposure.

Our study uses self-reported rather than objectively measured physical activity. Previous studies that have used objectively-measured physical activity to investigate effects of urban form on physical activity (Table 3) have reported mixed results: two studies reported



**Figure 3.** Differences among neighborhoods. (A) Average active transport (minutes walking and bicycling per person) and recreational activities. (B) Physical activity levels. The between-neighborhood difference in total physical activity is statistically significant ( $p < 0.001$ , two-tailed  $t$ -test).



**Figure 4.** Estimated attributable IHD mortality rates for each risk factor and neighborhood type. Rates were calculated using means of individual RRs and prevalence of exposure within neighborhood type [referent, > 150 min/week of physical activity; 10th percentile of air pollution exposure (13.6  $\mu\text{g}/\text{m}^3$  for  $PM_{2.5}$ , 39.8  $\mu\text{g}/\text{m}^3$  for  $NO_x$ , and 80.3  $\mu\text{g}/\text{m}^3$  for  $O_3$ )]. The overall incidence of IHD mortality in California is 191 deaths/100,000/year (CDC 2011).

**Table 3.** Comparison of results from studies using objective measures of physical activity with results from the present study.

| Study               | Location                       | Measure of physical activity             | Measure of urban form  | Core result  |
|---------------------|--------------------------------|--|--|--|
| Sallis et al. 2009  | Seattle, WA, and Baltimore, MD | Objective: 7-day accelerometer           | Walkability (net residential density, intersection density, land use mix, retail floor area ratio) | 41 min/week increase in physical activity between high- vs. low-walkability neighborhoods  |
| Frank et al. 2005   | Atlanta, GA                    | Objective: 2-day accelerometer           | Walkability (net residential density, intersection density, land use mix)                          | Two-fold increase in meeting physical activity recommendations in high- vs. low-walkability neighborhoods  |
| Forsyth et al. 2008 | St. Paul, MN                   | Objective: 7-day accelerometer           | Population density, block size (street pattern)  | Significant increase in transport-related physical activity (high- vs. low-walkability neighborhoods) but no difference in total physical activity       |
| Present study       | South Coast Air Basin, CA      | Self-report: one-day time activity diary | Walkability (population density, intersection density, land use mix)                               | 34 min/week increase in physical activity between high- vs. low-walkability neighborhoods (2-fold increase in meeting physical activity recommendations) |

differences in physical activity by neighborhood type (Frank et al. 2005; Sallis et al. 2009), and one indicated shifts in the purpose (transport vs. fitness) but not the amount of physical activity (Forsyth et al. 2008). These findings suggest that urban-scale differences in physical activity rates are similar between objectively measured physical activity and our self-reported measures of activity. For example, differences in per capita physical activity between high- and low-walkability neighborhoods in Seattle, Washington, and Baltimore, Maryland, were similar to differences in our southern California population [41 min/week (Seattle, Baltimore) versus 34 min/week (southern California) (Sallis et al. 2009)].

Our study limitations include those associated with travel surveys and self-reported information in general. For example, travel surveys typically undercount trips by all modes (Bricka and Bhat 2006), affecting estimates of travel time (Wolf et al. 2003). The SCAG survey suggests that vehicle undercount rates may approach 20–25% but gives little information regarding non-motorized trips (SCAG 2004). Undercount rates may be differential by trip length (SCAG 2004), mode, or neighborhood. Comparisons with studies using objectively measured physical activity (see preceding paragraph) suggest that our core findings are robust to trip undercounting and other problems with self-reported travel data.

Our work is motivated by the goal of understanding and designing clean, healthy, sustainable cities (Giles et al. 2011). Our investigation explores only one location (Los Angeles), one health outcome (IHD), one cohort, a small number of pollutants ( $\text{NO}_x$ ,  $\text{PM}_{2.5}$ ,  $\text{O}_3$ ), and physical inactivity. Clearly, further analyses incorporating other risk factors (e.g., noise, transport injury) linked to the built environment are warranted. Interaction between physical activity and air pollution may vary on an even smaller scale than we have investigated in the present study (i.e., within neighborhoods). Future analyses could use age-specific risks of IHD mortality for air

pollution and physical inactivity. Our analysis is descriptive (i.e., cross-sectional) in nature; more research is needed to explore causality between urban form and health risks (especially for physical activity, because ambient air pollution exposure is largely determined by geographical location).

Despite these limitations, our results are relevant to health officials, sustainability scientists, and urban planners. To our knowledge, ours is the first analysis that directly compares health risks for both air pollution and physical inactivity among neighborhoods based on activity patterns for a random sample of residents in an urban area, and thus is the first to quantify relationships between urban form and the health impacts of physical activity and air pollution. We found that attributes of the built environment were associated with both air pollution exposure and physical inactivity. These results emphasize that to be health protective, neighborhoods designed to decrease risks from one factor must avoid unintentionally increasing risks from other factors.

## Conclusion

We compared the health impacts attributable to air pollution and physical inactivity among neighborhoods for one cohort (~ 30,000 individuals in Southern California). A larger proportion of our Southern California study population was classified as nonsedentary in high- versus low-walkability neighborhoods (25% vs. 13%). However, because only a small share of the total population was classified as physically active, we estimated only moderate differences in IHD mortality rates attributable to physical inactivity between neighborhood types. Spatial patterns of estimated attributable IHD mortality rates varied by pollutant: estimated mortality due to increased  $\text{PM}_{2.5}$  and  $\text{NO}_x$  were greater in high- than in low-walkability neighborhoods, whereas estimated IHD mortality due to increased  $\text{O}_3$  was greater in low- than in high-walkability neighborhoods. In general, differences in estimated IHD mortality between neighborhoods were comparable for exposure to air pollutants and

physical inactivity. Our results suggest complex within-urban spatial trade-offs in health risks associated with air pollution and physical inactivity. Efforts to design healthy neighborhoods should account for many factors, including air pollution and physical inactivity, and not address one concern at the expense of others.

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