FINAL REPORT

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Determination of Reactive Oxygen Species Activity in PM and Enhanced Exposure Assessment for the NIH, NIEHS Study Entitled: Ultrafine Particulate Matter and Cardiorespiratory Health

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ABSTRACT

This is the first study conducted in California among vulnerable individuals with coronary artery disease on the acute cardiovascular health effects of exposures near subject residences to size-fractionated particles and to particle characteristics linked to mobile sources. We conducted a comprehensive particulate matter (PM) monitoring effort for a repeated-measures panel study aimed at evaluating acute cardiovascular health effects of exposure to PM. We followed 64 nonsmoking elderly individuals with coronary artery disease living in retirement communities in the Los Angeles Air Basin of California. Subjects were followed with 12 weekly blood draws for biomarkers and over 10 days with ambulatory electrocardiographs and blood pressure monitors. This project supplements the exposure assessment for an NIH-funded study. We found the contribution of mobile sources to indoor PM levels was similar to their corresponding outdoor estimates. Analysis of the relation between PM redox activity and blood biomarkers was largely nonsignificant. However, analysis of health outcomes and direct air measurements revealed that primary combustion markers [elemental-black carbon (EC-BC), primary organic carbon, CO, NO_x-NO_2] were positively associated with blood pressure, electrocardiographic ST segment depression (an indicator of cardiac ischemia), biomarkers of systemic inflammation, and platelet activation, and were inversely associated with erythrocyte antioxidant enzymes. Particle number (PN) and particles<0.25 μm were more strongly associated with biomarkers than particles 0.25-2.5 μm. Biomarker associations were stronger for indoor exposures to EC and PN of outdoor origin than uncharacterized indoor exposures. Overall results suggest that current regulations of particle mass may not completely represent particle size fractions and components important to protect public health of vulnerable populations. This likely includes particles <0.25 μm and pollutant components linked to fresh traffic emissions, including indoor infiltrated particles from mobile sources.

EXECUTIVE SUMMARY

Background:

Numerous epidemiologic time series studies have shown generally consistent associations of outdoor particulate matter (PM) air pollution with cardiovascular hospital admissions and mortality. However, the pathophysiological mechanisms and causal pollutant components driving these associations are unclear. The present research is driven by the possibility that the time series associations may be due to airway deposition of airborne ultrafine particles and traffic-related pollutant components, followed by an increase in thrombogenic and inflammatory activity in the blood, and by adverse effects on cardiovascular function. This research relates to the Board's function in establishing air quality standards to protect human health. There have been no other studies to our knowledge conducted in California among vulnerable individuals on the acute cardiovascular health effects of exposures near subject residences to size-fractionated particles and to particle characteristics linked to general air pollutant sources and components.

Methods:

We conducted a comprehensive exposure assessment study and PM monitoring effort for a repeated measures panel study aimed at evaluating acute cardiovascular health effects of exposure to ultrafine PM. This project is to largely supplement the exposure assessment for an NIH, NIEHS funded study (grant no. ES-012243) entitled "Ultrafine Particulate Matter & Cardiorespiratory Health." Indoor and outdoor air pollution monitors were deployed under this CARB-AQMD funded exposure assessment effort to provide continuous air pollutant concentrations, as well as data on PM composition and redox activity. Modeling efforts specific to this proposal include PM source characterization, and additional repeated measures statistical analyses of the relationship between health outcomes and supplemental air pollutant measurements.

Under funding from NIH, we followed 64 nonsmoking elderly individuals with coronary artery disease (CAD) living in four retirement homes in the Los Angeles Air Basin of California (2 studied in Jul 2005 through Feb 2006, and 2 studied in Jul 2006 through Feb 2007). Each subject was to be followed for a total of 12 weeks in two 6-week seasonal periods (warm and cold). Each Friday, blood samples were obtained for biomarkers of inflammation including plasma interleukin-6 (IL-6), tumor necrosis factor-α and its receptor (sTNF-RII), and C-reactive protein (CRP). We also measured a biomarker of platelet activation, soluble platelet selectin (sP-selectin). Biomarkers of erythrocyte antioxidant activity included glutathione peroxidase-1 and superoxide dismutase (funded by funds to the Southern California EPA PM Center, Project 4). Over 10 days, we also monitored subjects' cardiovascular function with ambulatory electrocardiographs (ECG, to assess possible cardiac ischemic with ST segment depression) and ambulatory blood pressure monitors.

Supplemental air pollutant measurements funded under this contract included concurrent hourly indoor and outdoor concentrations of $PM_{2.5}$ mass and $PM_{2.5}$ elemental and organic carbon (EC-OC), and pollutant gases (NO_2, NO_x, and CO). At outdoor sites only, we measured hourly black carbon (BC) and ozone (O_3). Additional data from the NIH-funded study included hourly indoor and outdoor particle number (PN) concentrations (dominated by ultrafine PM), and size fractionated PM: quasi-ultrafine mode <0.25 μm ($PM_{0.25}$), accumulation mode 0.25-2.5 μm ($PM_{0.25\text{-}2.5}$), and coarse mode 2.5-10 μm ($PM_{2.5\text{-}10}$). Using this and other data, we also estimated primary and secondary organic carbon (OC_{pri}, SOC), and indoor EC, OC_{pri}, SOC, and PN of outdoor origin. We present results of the assessment of health impacts of the NIH-funded PM exposures here only to provide a more comprehensive picture of associations. Under this contract, we also conducted *in vitro* testing to assess redox activity in concentrated fine ($PM_{2.5}$) and ultrafine ($PM_{0.25}$) particle suspensions collected at indoor and outdoor sites with biosamplers constructed specifically for this task.

We analyzed the relationship of 10-day ambulatory cardiovascular outcomes and 12-weekly systemic (blood) biomarkers of inflammation and erythrocyte antioxidant activity to indoor and outdoor concentrations of EC, total OC (and OC_{pri}, SOC fractions), $PM_{2.5}$ mass, PN, and criteria pollutant gases, and to redox activity of PM using *in vitro* bioassay results. We analyzed data with mixed effects models adjusted for potential confounders.

Results:

Exposure assessment work provided a comprehensive view of indoor and outdoor exposure relations. We found that vehicular sources showed the highest contribution among the apportioned sources for both indoor and outdoor particles at all sites. The contribution of mobile sources to indoor levels was similar to their corresponding outdoor estimates, thus illustrating the significance of these sources on indoor PM concentrations.

The *in vitro* redox assay results revealed considerable differences between individual samples collected at any given site during a given weekly 2-day sample collection period. There were also differences between seasonal phases and community sites, but this was significant only for ultrafine PM, not fine PM. Differences between mean indoor and outdoor DTT and DHBA activity were found between seasons and sites. An analysis of the relation between DTT and DHBA activity and blood biomarkers was largely nonsignificant for $PM_{2.5}$ in year 1 subjects and $PM_{0.15}$ in year 2 subjects.

The analysis of biomarkers revealed that primary combustion markers (EC-BC, OC_{pri}, CO, NO_x-NO_2) were positively associated with inflammatory biomarkers and platelet activation and inversely associated with erythrocyte antioxidant enzymes (N=578). PN and $PM_{0.25}$ were more strongly associated with biomarkers than $PM_{0.25\text{-}2.5}$. Biomarker associations were stronger during cooler periods when only OC_{pri}, PN, and NOx were higher, suggesting that pollutant components and/or nanoparticles that increase during colder weather and air stagnation are important. We found weaker associations for sTNF-RII and CRP among subjects taking the anti-cholesterol drug, statin, which is known to reduce systemic inflammation and oxidative stress. We found weaker associations for sP-selectin among subjects taking the platelet aggregation inhibitor, clopidogrel. Associations were stronger for indoor exposures to EC and PN of outdoor origin than uncharacterized indoor exposures, suggesting that outdoor air pollution was important.

We found positive associations of hourly ambulatory systolic and diastolic blood pressure with exposure to outdoor home $PM_{2.5}$, BC, EC, OC, and to a lesser extent with exposure to outdoor CO and NO_x, but not PN. The strongest association was for OC, especially estimated fossil fuel combustion fraction (OC_{pri}). Associations were increasingly stronger from last 4-hr out to 9-day average exposures. We also found positive associations of ECG ST segment depression with exposure to outdoor home $PM_{2.5}$, BC, EC, OC, and to a lesser extent with exposure to outdoor CO and NO_x, but not PN. Associations were seen from lag 1 to a 6-day average. As with blood pressure, the strongest association was for OC, especially estimated fossil fuel combustion fraction (OC_{pri}).

Conclusions: A major implication of the exposure assessment findings is that, even if people (particularly the elderly retired population of our study) generally spend most of their time indoors, a major portion of the outdoor PM to which they are exposed comes from outdoor mobile sources.

In the epidemiologic analysis, we found traffic-related air pollutants near the home are associated with increased systemic inflammation, increased platelet activation, and decreased erythrocyte antioxidant enzyme activity, which may be partly behind air pollutant-related increases in systemic inflammation and thrombosis. Differences in association by period and particle size suggest components carried by ultrafine particles are important. The *in vitro* redox assay of concentrated $PM_{2.5}$ and $PM_{0.15}$ was not associated with biomarkers. This null result is most likely due to the limited sampling periods, although other unmeasured factors influencing activity could have affected results.

The significance of the indoor-outdoor exposure assessment findings is supported by the finding that compared with uncharacterized indoor PM, indoor infiltrated PM from mobile sources were more strongly associated with biomarkers among the subjects living in the studied retirement communities. This may not be the case for other people with major indoor sources of toxic air pollutants.

Findings from the study of both ambulatory blood pressure and ECG-detected ST segment depression suggest that traffic-related air pollution exposures are related to this response. This may increase risk of acute myocardial infarction in individuals with underlying CAD. Overall results suggest that current regulations of fine particle mass may not completely represent particle size fractions important to protect the public health of vulnerable populations such as the one studied. This likely includes particles <0.25 μm and pollutant components linked to fresh traffic emissions. However, CO and NO_x-NO_2 (routinely measured in California) functioned as good surrogates for associations with outcomes, even though levels did not exceed regulatory standards.

BODY OF REPORT

1. INTRODUCTION

1.1. Scope and Purpose of the Project

The primary purpose of this project is to largely supplement the exposure assessment for an NIH, NIEHS funded study (grant no. ES-012243) entitled "Ultrafine Particulate Matter & Cardiorespiratory Health." Indoor and outdoor air pollution monitors were deployed under the CARB-AQMD funded exposure assessment effort to provide continuous air pollutant concentrations, as well as data on PM composition and redox activity. Modeling efforts specific to this proposal include PM source characterization, and additional repeated measures statistical analyses of the relationship between health outcomes (collected with NIH funding) and supplemental air pollutant measurements.

The following tasks were completed:

1) **Build for UCI another particle concentrator** (diffusion dryer, pump, chiller, virtual impactors & concentrator) for either the outdoor or indoor samples and provide cost sharing by availing the one concentrator that USC currently has;

2) **Collect fine (includes ultrafine) and ultrafine particle samples with biosamplers both indoors and outdoors (concurrently) for testing redox activity (Task 4).** Following deployment of the air monitoring trailer, it was determined that there was insufficient space to run two biosamplers. Therefore, the biosampler for ultrafine particles was run in year one and biosampler for fine particles was run in year 2 of data collection in the panel study.

3) **Measure concurrently hourly indoor and outdoor EC-OC concentrations.** CARB will provide two Sunset Labs continuous EC-OC monitors (one indoors and one in the outdoor trailer). We will use this novel data for general source apportionment (primary and secondary OC). The UCI investigators will use this data in assessing acute short-term cardiovascular effects of EC and OC in the NIEHS-funded epidemiologic study. To this, CARB staff added and Aethelometer to measure outdoor black carbon (BC).

4) **Conduct *in vitro* testing to assess the generation of ROS by fine and ultrafine PM collected with biosamplers in Task 2.** An *in vitro* chemical assay of collected samples will be employed which enables quantitative determination of redox activity using dithiothreitol (DTT). This assay quantitatively measures the redox activity of a given sample by its ability to catalyze the consumption of the reducing agent, DTT. To this task we have added two additional an *in vitro* chemical assays of collected samples for 1) the quantitative determination of transition metal-based redox activity using the reaction of salicylic acid with hydroxyl radical to form dihydroxybenzoate (DHBA) isomers; and 2) the presence of constituents with electrophilic properties in a subset of collected samples demonstrated by their ability to inhibit the thiol enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

5) **Measure concurrently hourly indoor and outdoor criteria pollutant gases [NO_2, O_3 (outdoor only), and CO] and outdoor hourly $PM_{2.5}$.** This required equipment that CARB provided, including equipment in their trailer.

6) **Analyze the relationship of cardiovascular outcomes to the production of ROS by PM using *in vitro* bioassays of concentrated particle suspensions collected at indoor and outdoor sites.** The *in vitro* assays are designed to test the following central hypothesis: Organic constituents associated with PM, including polar organic compounds and metals, are capable of initiating oxidative stress by generating ROS or acting as electrophilic agents. *We hypothesize*

that biomarker associations with ultrafine and accumulation mode particle mass and with particle number concentration (in the NIEHS-funded Aims) will be better explained by redox activity from in vitro assays of PM. This is supported by evidence that oxidative stress may have a central role in the cardiovascular effects of air pollution through its ability to initiate the inflammatory process, thrombogenic activity and immunomodulating effects (Dhalla et al., 2000).

7) **Analyze the relationship of cardiovascular outcomes to hourly indoor and outdoor EC-OC concentrations.** We will examine the magnitude of associations for different averaging times and different exposure windows preceding ambulatory ECG and blood pressure measurements. *We hypothesize that associations of ambulatory cardiovascular measurements with hourly EC-OC concentrations will be strongest for peak exposures and for averaging times more proximal to outcomes*. We will also examine the relationship of biomarkers of inflammation and thrombosis to recent hourly and longer-term average cumulative daily averages of the hourly EC-OC concentrations. BC data were examined in this manner as well.

8) **Analyze the relationship of cardiovascular outcomes to hourly indoor and outdoor criteria pollutant gases and particulate air pollutants.** We will examine the magnitude of associations for different averaging times and different exposure windows preceding ambulatory ECG and blood pressure measurements. We will also examine the relationship of biomarkers of inflammation and thrombosis to recent hourly and longer-term average cumulative daily averages of the hourly indoor and outdoor criteria gases, and outdoor (trailer) hourly $PM_{2.5}$ concentrations.

The Aerosol Laboratory of the Department of Civil and Environmental Engineering at USC carried out Tasks 1-3, and also directed and trained staff from USC on the environmental field-work, including indoor and outdoor home monitors. Air sampling equipment provided by CARB (Task 5), including the fully equipped trailer, was operated by CARB MLD staff.

Dr. John Froines and Arthur Cho at UCLA were responsible for Task 4. We collaborated with these investigators at the Southern California Particle Center to incorporate their *in vitro* methods (Kim et al., 2001a; 2001b; Li et al., 2002a; 2002b) into the epidemiologic design.

UCI investigators were responsible for Tasks 5-8; for assistance with monitoring work to set up sites (Tasks 3 and 5), and for the general oversight of the entire project and its integration into the NIEHS-funded epidemiologic study. UCI and USC staff operated the trailer with CARB staff assistance. We received some assistance from the SCAQMD staff to help with periodic repair and operational issues. Dane Westerdahl at CARB also helped coordinate and assist in issues related to the deployment of the trailer, including exposure assessment and interactions with the SCAQMD.

1.2. Background

Heart disease is the leading cause of death and hospitalization among adults 65 years of age or older (Desai et al., 1999), which makes the identification of preventable causes of heart disease morbidity and mortality a major goal of epidemiologic research. Numerous epidemiologic time series studies have shown positive associations of outdoor ambient particulate matter (PM) air pollution with cardiovascular hospital admissions and mortality (Pope and Dockery, 2006). However, the causal components driving the relationship of cardiovascular morbidity and mortality with PM remain to be identified. Historically, the difficulty in accomplishing this in epidemiologic studies has been that the common use of air pollution data from central regional ambient sites has led to both exposure misclassification and high correlations between different pollutants, including PM and criteria pollutant gases. Both of these problems can be addressed with measurements of personal and/or microenvironmental exposures (Sarnat et al., 2000; 2001).

Another problem is that the importance of particle size and chemistry has been limited by reliance

on government monitoring of particle mass at two size cuts, PM_{10} (PM < 10 μm in aerodynamic diameter) and $PM_{2.5}$ (PM < 2.5 μm). However, there is sufficient reason to believe that ultrafine particles (PM < 0.1-0.2 μm) are capable of inducing the greatest amount of pulmonary inflammation per unit of PM mass. This view is supported by major characteristics of ultrafine particles, including high pulmonary deposition efficiency, magnitudes higher particle number concentration than larger particles, and thus a much higher surface area (Elder and Oberdörster 2006, Li et al. 2003). The ultrafine particle's surface can carry large amounts of adsorbed or condensed toxic air pollutants (oxidant gases, organic compounds and transition metals). Many of these toxic air pollutants have been identified as having pro-inflammatory effects (Li et al. 2003; Ntziachristos et al. 2007), yet relevant exposure data is rarely available to epidemiologists. The putative ability of UFP to translocate systemically from pulmonary sites makes them particularly relevant to the cardiovascular effects of inhaled PM (Elder and Oberdörster 2006).

We aimed to improve the characterization of PM exposure in order to yield clues to potentially important pollutant sources and causal component mixtures. These characteristics may not be adequately represented by ambient $PM_{2.5}$ and PM_{10} mass, which have been regulated by the US Environmental Protection Agency (Delfino et al. 2005). For example, traffic (increases spatial variability of UFP and UFP is rich in redox active PM (Sioutas et al. 2005). Regional ambient data is thus likely to misrepresent personal exposure. In addition, ambient PM is made up of primary combustion aerosols, photochemically-produced secondary organic aerosols, and mechanically-generated crustal material. These particle types have different spatial and temporal variability. The organic component mix and size distribution differs as well between these classes of particles, with primary aerosols being more common in UFP (<0.1 μm) and secondary aerosols more common in the accumulation mode (0.1-2.5 μm). Finally, because most human exposure to PM occurs indoors, it is important to assess indoor exposure to PM of outdoor origin since considerable exposure error may occur when using ambient data alone (Meng et al. 2005).

1.3. Working Definitions of PM

Ambient urban particulate air pollution has been described in three size distributions, as follows:

1) nuclei mode (ultrafine particles) approximately < 0.1 μm in diameter and largely comprised of primary combustion products;
2) accumulation mode between 0.1 μm and 1.0 μm in diameter, from aggregation of ultrafine particles and vapors; and
3) coarse mode > 1.0 μm in diameter, largely mechanically generated particles. Particles collected at a 50% probability of collection with a cutpoint of 2.5 μm are referred to as $PM_{2.5}$ or fine particles, and include mostly ultrafine and accumulation mode particles, which can reach the alveoli and small airways of the lungs.

Larger particles are coarse mode particles. Particle samples with a 50% cutpoint of 10 μm are referred to as PM_{10} and include both fine and coarse mode particles.

Unlike the case of coarse and fine particles, which are more naturally divided by a cutpoint of 2.5 μm, there is no clear cutpoint that separates ultrafine from accumulation mode PM. This is because, unlike coarse and fine (accumulation plus ultrafine) PM, which have distinctly different origins, a major fraction of accumulation mode PM originates from the ultrafine mode. The distinction between the ultrafine and accumulation modes has varied from 0.1 to 0.2 μm, depending on locations and season (Sioutas et al. 2005).

In the context of the present study, we set the lowest cutpoint at 0.25 μm, although this is likely to include some accumulation mode particles. This is a useful cutpoint for the following reasons. If "ultrafine PM" are defined as those originating mostly from vehicular emissions and accounting for over 90% of the number-based particle concentrations, a cutpoint between the ultrafine and

accumulation modes should ensure the accuracy and integrity this definition. In our recent studies of the Southern California Particle Center and Supersite (SCPCS), we have found that the mass median diameter of elemental carbon (EC), an excellent surrogate of vehicular emissions in Los Angeles, is in the range of 0.15-0.2 μm (Singh et al. 2002; Kim et al. 2002). Moreover, number median diameters in the inland valleys (receptor areas) of the Los Angeles Basin are in the 90-150 nm range in the summer months (Kim et al. 2002; Fine et al. 2004b). We thus believe that setting the cutpoint at no less than around 0.15 μm provide a more unambiguous separation between the ultrafine and accumulation mode PM. Furthermore, since associations between Condensation Particle Counter (CPC) readings (based on particle number concentrations) and heath outcomes are part of the present scope of work, this definition of ultrafine PM ensures consistency in the monitoring of ultrafine PM based on both number and in part, mass concentration using the chosen cutpoint of 0.25 μm. Because the chosen cutpoint cannot be exclusively attributed to ultrafine particles, we have chosen to refer to particles in this size range as "quasi-ultrafine."

2. OVERVIEW OF THE PROJECT

2.1. Overview of the NIEHS Study Design and Population

The CARB/AQMD-funded exposure assessment work described here enhanced our ability to evaluate the main hypothesis for testing in the NIH, NIEHS-funded study (grant no. ES-012243) entitled "Ultrafine Particulate Matter & Cardiorespiratory Health." Funding expired on 7/31/2008 and the grant is in a no-cost extension. The final report to NIEHS will include an overview of results presented here and any new results obtained during the no-cost extension, expected to continue through 7/31/2010. The aims of the NIH, NIEHS-funded study are to test the hypothesis that deposition of airborne ultrafine particles in the lungs can lead to an increase in thrombogenic and inflammatory activity in the blood, and to a disturbance in cardiovascular function. This is expected to increase the risk of adverse cardiovascular outcomes such as myocardial infarction.

To test the NIEHS-funded hypothesis, we conducted a panel study involving an analysis of repeated measures of cardiovascular outcomes and systemic biomarkers of inflammation in relation to exposures to particulate air pollution. Subjects included nonsmokers age 65 and older diagnosed with coronary artery disease (CAD). They lived in four retirement communities in the South Coast Air Basin of California, an area with high concentrations of freshly emitted toxic air pollutants. The elderly study population with CAD is likely to be among the most vulnerable to the adverse effects of air pollutants (Goldberg et al. 2001). All subjects were in independent living facilities and were ambulatory, i.e., these were not residents of convalescent homes. They are thus unlikely to differ greatly from other people with CAD of the same age.

Specific Aims for the NIEHS-funded study are:

1. to examine relationships of circulating biomarkers of inflammation and thrombosis to ultrafine PM exposures.
2. to test the hypothesis that biomarker associations with particle mass in Specific Aim 1 will be better explained by certain PM components.
3. to examine relationships of systolic and diastolic blood pressure (SBP and DBP) to personal exposure to ultrafine PM.
4) to examine the relationship of adverse cardiac clinical outcomes to personal exposure to ultrafine PM.
5) to test the hypothesis that associations with personal particle mass in Specific Aims 3 and 4 will be better explained by certain PM components.
6) to assess whether exhaled nitric oxide (eNO) is associated with ultrafine PM exposure, and whether eNO levels, in turn, predict biomarkers of inflammation and thrombosis.

The NIEHS-funded study is supplemented by the present CARB-funded work and U.S. Environmental Protection Agency (EPA) STAR grant no. RD83241301 to the University of California,

Los Angeles, for the Southern California Particle Center. Several of these Aims 1-5 have been advanced in several ways following funding by the present CARB/AQMD contract and by the EPA grant. The Particle Center funding added funds to the NIEHS study primarily to measure oxidative stress responses. This includes biomarkers that will be presented here (erythrocyte antioxidant enzyme activities). CARB/AQMD funding allowed us to better test the importance of composition (Aims 2 and 5) using organic and elemental carbon as tracers for sources and composition, and PM-related redox activity from reactive oxygen species (see section 2.2 below). The overall study based on integrated funding sources is referred to as the Cardiovascular Health and Air Pollution Study (CHAPS).

CHAPS is a panel study with daily repeated measurements of health outcomes and exposures in elderly individuals with CAD. The design is well suited to the study of acute-on-chronic patterns of change in physiologic factors important to cardiovascular diseases. The main analytic focus is on within-subject exposure-response relationships, with each subject serving as his/her own control. Between subject differences were evaluated to understand susceptibility factors related to medication use and region of study within the Los Angeles air basin. The design is statistically efficient because: 1) multiple exposure conditions and time frames are studied in each subject, and 2) response variability due to between-subject characteristics is reduced by repeated measurements without reductions in the magnitude of exposure-response relationships, thereby enhancing power and precision (Weiss and Ware 1996).

Eligibility Criteria are as follows:

Inclusion criteria included the following: age $\geq$ 65, history of CAD diagnosis, which could include a history of myocardial infarction and bypass surgery but not within the preceding 12 months, and sufficiently ambulatory to perform sit-to-stand transfers over short distances.

Exclusion criteria included the following: employment outside of the monitored home; smoking within the preceding 12 months; exposure to environmental tobacco smoke in the home; psychiatric disorders; dementia; alcohol or drug abuse; Parkinson's or other debilitating neuromuscular diseases; dialysis treatment; daily oral corticosteroids; or medical conditions that would place the subject or staff at risk from the blood donation. To further limit exposure to environmental tobacco smoke, the study subjects has to live in retirement communities prohibiting indoor tobacco smoke at shared locations and in buildings with common ventilation systems.

Following approval by retirement community management and boards, we began recruitment on site using presentations by the principal investigator, and recruitment flyers and newsletter announcements by retirement community staff. UCI staff were available to assess eligibility and recruit subjects on site.

The Institutional Review Board of the University of California, Irvine approved the study protocol. We obtained informed written consent from subjects.

We completed follow-up in the four communities in the LA air basin, three in the San Gabriel Valley and one in Riverside. These communities are located in inland urban areas of the basin considered to be down-wind smog receptor sites with aged PM (especially Riverside), but also affected by local traffic with freshly emitted PM (especially San Gabriel Valley communities). Our research as well as others has shown that PM concentrations and components by size-fraction vary across the sites because of traffic density and transport, and between our two seasonal study periods described below (Zhu et al. 2002a; 2002b; 2004; Fine et al. 2004a; 2004b; Polidori et al. 2007).

Over a seven month period, subjects were followed in two 6-week seasonal blocks with blood draws at the end of each week for circulating biomarkers of inflammation, thrombosis, oxidative stress and antioxidant activity. During the 12 weeks of total follow-up, subjects completed daily diaries for activities, stress, and medication use. Modifying effects on exposure-response relationships by medications such as statins (Schwartz et al. 2005) were investigated.

By design, in each community, we collected 6 weeks of data during a warmer period of higher photochemical activity (Jul to early Oct), and 6 weeks of data during a cooler period of higher air stagnation (late Oct through Feb). This was intended to enhance contrasts in PM composition, number and size distribution in each community (Sioutas et al 2005). We planned *a priori* to test differences in regression models for exposure-response relationships between seasonal phases of

study. In 2005-2006, two retirement communities were followed in four alternating six-week phases. Again in 2006-2007, another two retirement communities were followed in four alternating six-week phases. Below we show this planned follow-up scheme graphically (Table 1). We also show the groups and phases by general location name and abbreviation used later on in the results for group and phase designations (Table 2). We are prohibited by the IRB from revealing the exact location or name of the retirement communities.

Subjects completed a baseline questionnaire with items from our previous work, the Multiethnic Study of Atherosclerosis, and the Atherosclerosis Risk in Communities study. This was used to assess demographic, lifestyle and environmental risk factors, personal and family history of cardiovascular and pulmonary conditions, other comorbidities, and home environment. Medication use was assessed longitudinally. A baseline clinical work-up included an intake history and physical by study cardiologists and 12-lead ECG. Confirmation of CAD diagnosis was made with a medical records review and discussions with the subject's cardiologist (e.g. positive stress test, MI history).

Table 1. Timetable for Panel Data Collection and Subject Involvement.

Jul to Aug 2005			Sep to Oct 2005			Nov to Dec 2005			Jan to Feb 2006			
			Retirement Community Groups of up to18 Subjects, July 2005 to Feb 2006									
group 1			group 2			group 1			group 2			
Week Number in First Monitoring Period* (Phase 1, Photochemical Activity Dominates)						Week Number in Second Monitoring Period (Phase 2, Air Stagnation Dominates)						
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	

Jul to Aug 2006			Sep to Oct 2006			Nov to Dec 2006			Jan to Feb 2007			
			Retirement Home Groups of 18 Subjects, July 2006 to Feb 2007									
group 3			group 4			group 3			group 4			
Week Number in Third Monitoring Period (Phase 1,Photochemical Activity Dominates)						Week Number in Fourth Monitoring Period (Phase 2, Air Stagnation Dominates)						total
25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	48 weeks

* Each week we conducted a five-day ambulatory monitoring period from Mon through Fri and involved up to three subjects (Holter electrocardiograph and ambulatory hourly blood pressure). Blood draws took place each Friday of the monitoring weeks in the late afternoon. Outdoor monitoring in the trailer and selected indoor samplers were run continuously except when moving from one home to the next. Monitoring starts on the intervening week when no follow-up is conducted to yield 9 lag days before the first blood draw.

Table 2. Group and phase designations.

Location	Group (G) number	Phase (P) number*	Abbreviation
East San Gabriel Valley 1	1	1	G1P1
		2	G1P2
West San Gabriel Valley	2	1	G2P1
		2	G2P2
East San Gabriel Valley 2	3	1	G3P1
		2	G3P2
Riverside	4	1	G4P1
		2	G4P2

* Phase 1: warmer period of higher photochemical activity (Jul to early Oct); Phase 2: 6 weeks of data during a cooler period of higher air stagnation (late Oct through Feb)

2.2 What the CARB/AQMD-Funded Study Adds

The exposure data that was added to the NIEHS-funded study is summarized in Table 3. All other measurements, including health outcomes, time-activity and questionnaire data were collected using other funds. Although epidemiologic analyses are part of the present task list, most of the work was funded by NIEHS and EPA, including statistical support from Dr. Dan Gillen, Dept. Statistics, UCI.

Under the present CARB/AQMD funding, we conducted supplemental air monitoring and assessment of PM exposures including measurements in real-time that yielded clues to particle composition (EC-OC and OC primary and secondary fractions). The time course of cardiovascular responses to PM is unknown and the relevance of short-term hourly as compared with daily cumulative exposures requires evaluation. Under the present CARB/AQMD funding, we also assessed PM-related redox activity from reactive oxygen species (ROS) for both fine and ultrafine PM. Under the combined funding sources, we aimed to generate epidemiologic data that compliments tests of causation by size fraction and by composition in experimental models.

Results of the present supplemental effort and the NIEHS-funded study are intended to advance knowledge regarding the acute effects of air pollutants on cardiovascular function and clinical outcomes, and on biomarkers of systemic and pulmonary inflammation. We aim to contribute needed information on the health impacts of ultrafine particles, which is the focus of the NIEHS-funded study. The combined results of the NIEHS-funded study and the present supplement are intended to clarify findings in the literature of associations between ambient PM and cardiovascular mortality and hospital admissions. Gaps in the current literature addressed in the present project under the combined funding sources include:

1) the importance of particle composition, toxicity and related source characteristics to associations of PM with cardiovascular function and biomarkers of systemic inflammation in humans;
2) the relative magnitudes of association by recent shorter-term (hourly) vs. longer-term (daily) averaging times of home particle number, EC-OC, and BC;
3) the relationships of biomarkers of systemic inflammation and cardiovascular outcomes to hourly home criteria pollutant gases;
4) the potential magnitude of error in exposure-response relationships using outdoor measurements of particles to represent exposure as compared with indoor particles of outdoor origin.

We aim to generate much needed new information on the relationship between cardiovascular outcomes and exposures to chemical components of particles. Although not directly measured under the CARB-AQMD funding, this includes the putative pro-inflammatory effects of certain organic compounds such as polycyclic aromatic hydrocarbons (PAH), and the ability of particle mixtures of organics and transition metals to induce oxidative stress through ROS. We primarily aim to identify exposure to traffic-related PM and its effects. This has considerable relevance to the type of air pollution of concern to Californians.

The present research is also among the first epidemiologic studies to examine the effect on cardiovascular outcomes by real-time indoor and outdoor EC-OC (as part of the CARB/AQMD funding), and particle number concentration (as part of the NIH-funded project). We also evaluated effects of hourly criteria pollutant gases (O_3, CO, and NO_2) focusing on outdoor home measurements (CARB/AQMD funding). Additional outdoor home data in a CARB-supplied trailer included hourly $PM_{2.5}$ concentrations. The hourly data on gases and particulate air pollutants provided information needed to support short-term air regulatory/standards for consideration.

There are two major strengths in enhancing the indoor-outdoor monitoring effort of the NIEHS-funded study with additional hourly exposure data provided with the present air monitoring:

1) the ability to detect short-term health impacts; and
2) the ability to determine the relevance of outdoor air pollutant concentration to health effects from what are largely indoor exposures.

We compared indoor with outdoor source concentrations of PM since concentrations in outdoor air variably impact indoor air, where people spend most, but not all of their time. In the NIH-funded study, we are assessing the magnitude of indoor penetration of outdoor sources using gravimetric particle measurements (under current investigation). This CARB-funded project supplements the filter-based PM data and continuous particle number concentrations that were measured in the NIH-funded study by also collecting continuous EC-OC at both indoor and outdoor home sites. This effort included adding CARB's air monitoring trailer as an important addition to support the study because it is well instrumented and mobile.

This research is of paramount importance to regulatory agencies. The ultimate goal of any effective pollution control strategy is to tackle pollutant sources that have a major contribution to personal and indoor PM levels. Thus, the findings of this study have direct application to evaluations of air quality standards for PM and pollutant gases. Results are expected to advance understanding of the adverse effects of particulate air pollutants on the cardiovascular health of high-risk individuals.

Finally, we are interested in methods that can be used in epidemiologic research to better understand the influence of ROS on the cardiovascular health effects of PM. This was done in the CARB funded study using concentrated fine and ultrafine PM measured indoors and outdoors at each study site. Samples were analyzed for redox activity. This was not proposed to NIEHS. Table 3 summarizes the enhanced exposure assessment by comparing the NIH-funded study to the present proposal.

Table 3. Supplemental exposure assessment for the NIEHS study funded by CARB/SCAQMD.

	NIEHS funded study	Additions to NIEHS study
Funding (direct and indirect)	$2,939,182	$676,814 CARB/SCAQMD
Seasonality evaluated?	Yes-photochemistry & stagnation	
Target Exposure Sample size	Four sites, 240 days of indoor-outdoor sampling (48 weeks, five 24-hr periods/wk, or Sun pm to Fri pm).	Increased with trailer to 336 days (48 weeks, 7 d/wk) of outdoor criteria pollutant gases and I-O hourly BAM $PM_{2.5}$ & EC-OC monitoring with weekend sampling collected before each 5-day subject follow-up (Mon-Fri) and blood draw (Fri)
Reactive oxygen species: **DTT, DHBA**	None	Yes, using concentrated PM from Biosamplers at indoor & outdoor sites.
	Outdoor home air monitoring:	
ARB trailer added: Set up, operated, and level 1 + 2 QA by ARB staff* (except EC-OC)		Adds on-site metrics, monitors provided by CARB: continuous. BAM $PM_{2.5}$, Aethalometer black carbon, Sunset Labs EC-OC, particle number concentrations, criteria gases (O_3, NOx, CO) & met data
Home sample frequency	Mon-Fri	
PM mass	3-stage MOUDI mass (24hr) five 24-hr periods/wk	Continuous BAM $PM_{2.5}$ in trailer (paired samplers for reliability checks)
Carbon	None	Continuous EC-OC in trailer 7 days/week (USC effort)
PM organic chemistry	Yes-5 day MOUDI filter-based composites (ultrafine & fine PM size modes) for source tracers (primarily vehicular and photochemical)	Quinones and PAHs using VACES concentrated PM from the outdoor Biosampler (USC/UCLA effort).
PM elemental	Yes, (5-day composites, ultrafine, fine & coarse particles): ICP-MS Fe, V, Zn, Cr, Ni, Cu, Pb & Mn	
Particle number concentration	Yes, Condensation Particle Counter (CPC) outside communities	Added with trailer + one backup CPC provided by CARB
	Indoor air monitoring:	
Criteria Gases	Un-funded effort	NOx and CO monitors were set up, operated, and level 1 + 2 QA by ARB staff*
PM mass	3-stage Sioutas Sampler mass (24hr) five 24-hr periods/wk	Hourly BAM $PM_{2.5}$ (paired samplers for reliability checks) were set up, operated, and level 1 + 2 QA by ARB staff
Carbon	None	Continuous EC/OC 7 days/week (USC effort)
PM organic chemistry	Yes, 5-day Sioutas Sampler filter-based composites (ultrafine and fine PM size modes) for source tracers (primarily vehicular and photochemical)	
PM elemental	Yes, 5-day filter-based composites (ultrafine, fine & coarse particles): ICP-MS Fe, V, Zn, Cr, Ni, Cu, Pb & Mn	
Particle number concentration	Condensation Particle Counter	

* Data logger downloads and calibrations were done weekly by MLD staff and placed on the web for viewing preliminary data. Monthly QA'ed data were sent to USC and UCI monthly

3. TASKS 1-2. Build for UCI another particle concentrator and collect fine (includes ultrafine) and ultrafine particle samples with biosamplers both indoors and outdoors (concurrently) for testing redox activity (Task 4).

3.1. Materials and Methods

Concentrated indoor and outdoor fine and ultrafine ambient particles were collected by means of new and improved portable concentrators developed by the University of Southern California. These portable Versatile Aerosol Concentration Enrichment Systems (VACES) are based on technologies developed and published by Kim et al. (2001a and 2001b). They are capable of enriching the concentration of particles in the range of 0.01-10 μm by a factor up to 40, depending on the output flow rate. Their small size makes them also ideal for application to studies using mobile exposure platforms. By incorporating size-selective inlets, the VACES can provide concentrated ambient particles (CAPs) in carefully defined size ranges. They can be readily adapted to accommodate higher output flow rates that are desirable in conducting human exposure studies. The performance of these systems is described in greater detail by Kim et al. (2001a and b). Two VACES were used for size fractionated sample collection, one for indoor and one for outdoor microenvironments, in each of the four retirement communities. We requested funds for one of these two systems, as the other was provided by USC as part of our cost sharing to this project.

There was not enough space in either the indoor or outdoor locations for any community to perform concurrent ultrafine and fine PM sampling. Therefore, indoor and outdoor fine particles were collected in year 1 of the study and indoor and outdoor ultrafine particles were collected in year 2 of the study. Sampling was conducted on the Thursday and Friday of every week of sampling just prior to the beginning of the weekly Friday blood draws at 2:00 pm. Sampling took place between 3 and 6 hours per day, resulting in between 8 and 10 hours of sampling per sample.

Particles were concentrated by drawing air samples through pre-impactors with either a 2.5 μm cut-point (for fine particles) or 0.15 μm cut-point (for ultrafine particles) to remove larger particles. These particles are drawn through a saturation-condensation system that grows particles to 2-3 μm droplets, which are subsequently concentrated by virtual impaction. Highly concentrated particle suspensions were obtained by connecting the concentrator output to a sterilized liquid impinger (BioSampler, SKC West Inc., Fullerton, CA; Willeke, et al. 1998). Aerosols were collected using ultra pure (Milli-Q) deionized water (resistivity 18.2 megaohm; total organic compounds <10 ppb; particle-free; bacteria <1 colony forming unit/ml) as the collection medium. Previous studies have shown that the concentration enrichment process does not alter the physical, chemical and morphological properties of the particles (Kim, et al. 2001a, 2001b). The total amount of particulate matter loading in the collection medium were determined by multiplying the ambient concentration of each PM mode by the total air sample volume collected by each concentrator line. The particle concentrations in the aqueous medium were calculated by dividing the particle loading by the total volume collected in that period.

In the sampling line of the concentrator, fine or ultrafine PM was concentrated from a flow of 120 liters per minute (lpm) to a flow of 6 lpm, thus ideally enriched in concentration by a factor of 20. Of the 6 lpm of the concentrated flows of fine or ultrafine PM samples, 4 lpm was drawn through the BioSampler connected to the respective minor flow, while 2 lpm passed through a diffusion dryer (for fine or ultrafine PM) to remove excess water and dry the aerosol.

For mass concentration measurements, the filter substrates were first weighed before and after each field test using a Mettler 5 Microbalance (MT 5, Mettler-Toledo Inc., Highstown, NJ), under controlled relative humidity (e.g. 40-45%) and temperature (e.g., 22-24 °C) conditions. At the end of each experiment, filters were stored in the controlled humidity and temperature room for 24 h prior to weighing in order to ensure removal of particle-bound water.

3.2. Results and Discussion

Sample collection results are shown in Appendix Table A1.

4. TASK 3. Measure concurrently hourly indoor and outdoor EC-OC concentrations.

4.1. Materials and Methods

4.1.1 Sampling sites and schedule

The following describes the sampling setup for all air monitors, including that for EC-OC (Task 3) described in the next section. We made indoor and outdoor air pollutant measurements at four retirement communities in the Los Angeles air basin between 2005 and 2007. Two 6-week sampling campaigns were conducted at each location, one during summer and early fall (warmer phase) and one throughout late fall and winter (colder phase). Three of the communities were in the San Gabriel Valley, CA (San Gabriel groups 1, 2 and 3, Table 2) and the fourth in Riverside, CA (group 4, Table 2). San Gabriel Valley group 1 was located in a residential area about 50 km east of downtown Los Angeles, approximately 3 km away from a major freeway. San Gabriel Valley group 2 was about 8 km east of Los Angeles, approximately 300 m away of a major freeway. San Gabriel Valley group 3 was located about 55 km east of downtown Los Angeles, 2.5 km from two busy freeways and 150 m away from a major street. Riverside group 4 was located about 110 km east of Los Angeles, 15 km southeast of downtown Riverside, 3 km away from the closest freeway and 1 km from a major street (downwind of the site).

Two identical sampling stations were installed at each site, one indoors and one outdoors. The indoor sampling station at site San Gabriel Valley group 1 was set-up in a recreational area of the community's main building, adjacently to a construction site. The San Gabriel Valley group 2 indoor station was located in the dining room of the community's central building (see Polidori et al., 2007, for further details on groups 1 and 2). The indoor station at San Gabriel Valley group 3 was in a recreational area of the main retirement community complex. The indoor station at the Riverside site was in the hallway of the main building with a dining room, activity room and numerous apartment units nearby. Outdoor sampling equipment was set-up inside a movable trailer, positioned within 300 m from the indoor station at all sites. Photos of an outdoor and indoor site are shown below.

4.1.2 Pilot Testing

To pilot test the instrument performance and setup, two semi-continuous EC-OC field analyzers (Sunset Laboratory Inc.) were installed in the Particle Instrumentation Unit trailer at USC. They were configured to obtain hourly data of particulate elemental carbon (EC) and organic carbon (OC) side-by-side, sampling for 45 minutes followed by about 13 minutes of analysis. An Aethalometer, measuring black carbon (BC), was installed to sample at the same time. BC measurements were done by Aethalometer every 5 minutes and hourly average of these measurements was used for comparison with the EC-OC field analyzers. The instrumental setup is shown in Figure 1.

Detailed results of this pilot testing were published (Arhami et al. 2006).

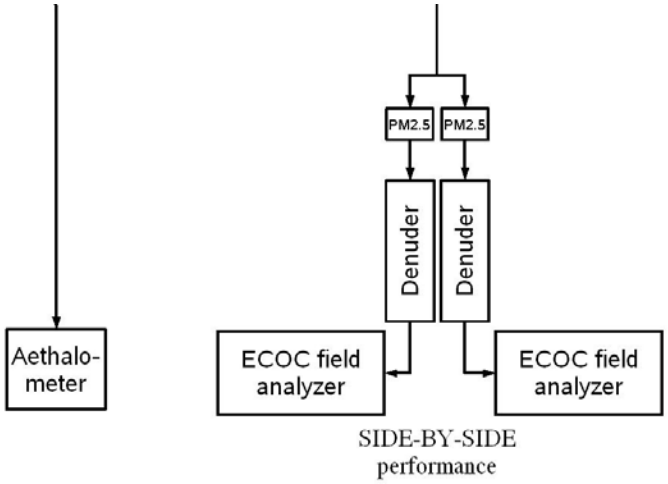


Figure 1. ECOC field analyzer and Aethalometer set-up. A $PM_{2.5}$ cyclone is installed upstream of each ECOC instrument's sampling inlet. Optional denuders can be installed between $PM_{2.5}$ cyclone and sampling inlet to remove OC in gas phase.

Side-by-side comparison of the two ECOC field analyzers showed very good agreement. Figure 2 shows a comparison for OC measured without denuders. The two instruments are denoted by OCEC1 and OCEC2.

The ECOC field analyzers also measure optical EC (similar to BC) in addition to thermal EC, OC, and total carbon (TC=EC+OC). TC minus optical EC is referred to as optical OC. A comparison between thermal and optical OC also showed good agreement between these two measurement methods (see Figure 3).

The measurement of particulate OC is affected by a positive artifact caused by adsorption of gas-phase OC onto the filter. The positive artifact is enhanced for short sampling times, when adsorption has not reached equilibrium. Therefore, the ECOC field analyzers, with sampling times of less than an hour, are particularly affected when compared to conventional filter sampling (e.g. 24 h samples). The denuder (see Figure 1) can be used to remove the gas-phase OC, and hence the positive artifact. However, this creates a negative artifact as now the equilibrium gas/particle partitioning is disturbed and part of the collected OC can evaporate from the filter.

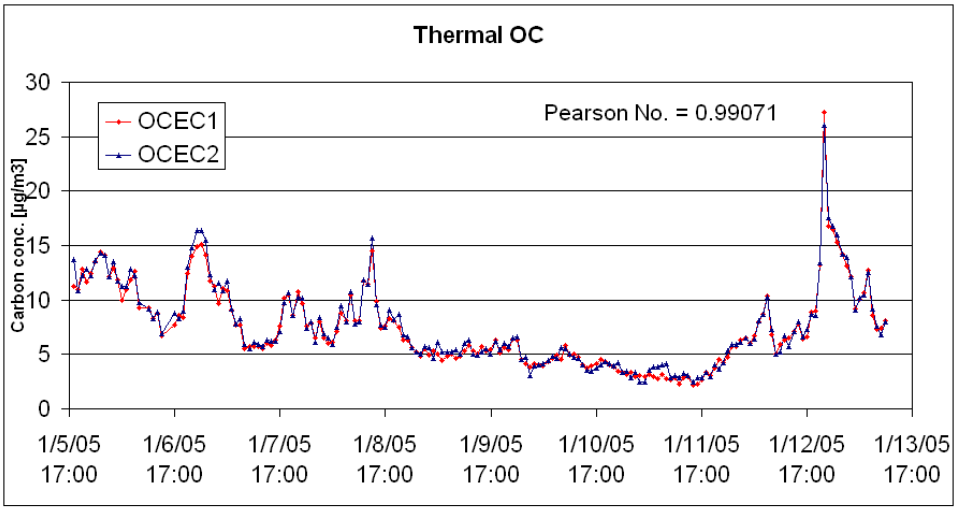


Figure 2. Thermal OC measured by OCEC1 compared with OCEC2.

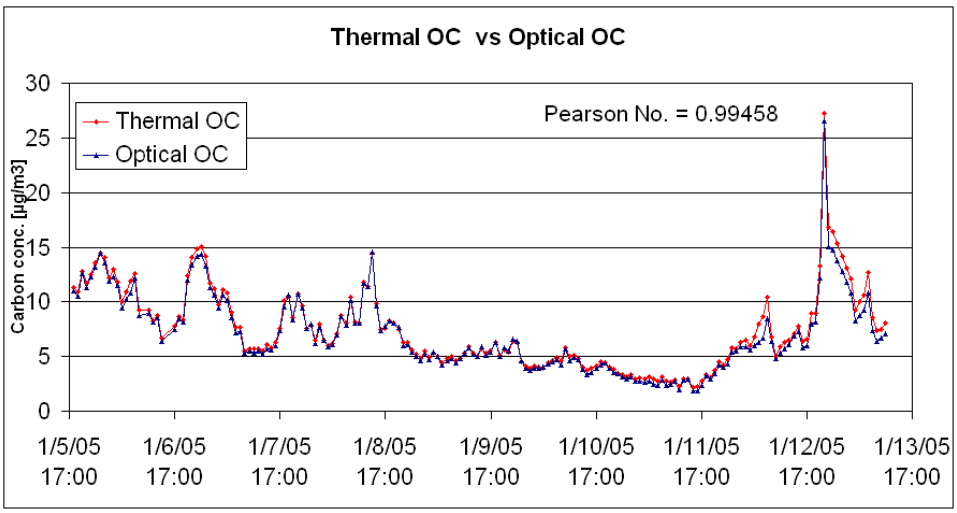


Figure 3. Thermal OC compared with optical OC (for instrument OCEC1).

Positive and negative artifacts were studied by measuring OC with the two instruments by changing the inlet configurations of one of the monitors (adding a denuder, a Teflon filter, or both) to study systematically these artifacts. The positive artifact was found to be relatively large (7.59 µg/m^3 on average), more than 50% of measured OC, but it was practically eliminated with a denuder. The negative artifact was much smaller (less than 20% of the positive artifact) and may be neglected in most cases.

This provided useful experience in the handling and maintenance of the ECOC field analyzers. Aspects such as filter change frequency and efficiency and duration of denuders were addressed as well. The effect of using a week-old filter versus a fresh filter on the measured OC and EC was shown to be negligible. However, the filters were changed once a week as recommended by the manufacturer. The denuder breakthrough was minor and specific to this type of denuder, which was manufacturer suggested denuder with carbon strips. The results did not show a significant change for more than two-month old (and in use) denuder and a fresh denuder; however the denuder strips were deployed a maximum of three months before replacement with fresh strips.

Further tests with the ECOC field analyzers included effects of different measurement protocols (NIOSH, IMPROVE and FAST-ramp) on measured EC and OC. EC and OC measurements using

different temperature profiles for analyzing the samples were highly correlated with one another. The FAST-ramp method we decided to use offers the potential for reducing the time and increasing the sensitivity of the analysis step, thus allowing for more continuous measurements and shorter sampling periods.

The EC concentrations from both ECOC field analyzers (measured thermally) were also compared to BC measurements made with the Aethalometer. The R^2 and slope of correlation between BC and EC were 0.96 and 1.39 $\pm$ 0.06, respectively, for one unit and 0.95 and 1.17 $\pm$ 0.05 for the other. This indicates a high correlation between EC and BC measurements, but systematically lower EC measurements than BC.

We also tested measurements of EC and OC in the ultrafine fraction of particulate matter with the help of ultrafine impactors developed by USC, which replaced the $PM_{2.5}$ cyclone used for the overall CHAPS study. Measurements of $PM_{2.5}$ and ultrafine fractions in parallel were intended to provide insight into size fractionated EC and OC content of particles and their sources. Considerably higher EC to OC ratios in the UF mode compared to the fine mode were found, which is due to the different sources and formation process of the two particle size ranges. EC from mobile sources (in the form of soot) is emitted primarily in smaller particles, and while OC is also emitted in smaller particles from mobile sources, a portion of accumulation mode OC is formed by the condensation of organic gases, which were either directly emitted from mobile sources or formed by photochemical secondary reactions (Kleemann 1999). Higher OC volatility in the fine mode compared to UF mode particles was found. This is consistent with OC condensation in larger mode since both photochemical products and condensable vapors from vehicles are often semi-volatile species which will partition to pre-existing particle surface area (Kleemann 1999). Details of the above findings are published (Arhami et al. 2006).

4.1.3. Estimation of primary and secondary OC

We estimated indoor and outdoor secondary OC (SOC) and primary OC (OC_{pri}) from total OC as detailed in our recent publication (Polidori et al. 2007) and summarized here. OC_{pri} is representative of particles emitted directly from combustion sources (mostly fossil fuels in the Los Angeles basin) while SOC represents semi-volatile and low-volatile products of photochemical reactions involving reactive organic gases from anthropogenic and biogenic sources. SOC is specific to our application but is also more generally referred to as secondary organic aerosol (SOA). Outdoor primary OC particles are mainly emitted from motor-vehicle exhaust, are mostly found in the ultrafine mode, and are comprised of well known carcinogenic species such as diesel particles and PAHs. Given their small size, they are more likely to deposit in the airways than coarse particles. There is little evidence linking exposure to SOA with respiratory inflammation (e.g. Baltensperger et al. 2008).

EC tracer method:

The contributions of SOC and OC_{pri} to measured outdoor OC were estimated from collected OC and EC concentrations using EC as a tracer of primary combustion generated OC (i.e., "EC tracer method") (Cabada et al. 2004; Lim et al. 2003; Polidori et al. 2006; Turpin et al. 1995). This method assumes that OC_{pri} and EC are emitted from the same combustion sources. Data points characterized by high CO and NO peaks, mainly observed during rush hour traffic, were used to identify periods dominated by primary sources, when SOC is less likely to be formed. By regressing the OC and EC data collected during these periods, the characteristic primary OC/EC ratio for each month of study was determined. Because a conventional linear least-squares regression assumes that there are uncertainties only in the dependent variable, a Deming linear least-squares regression (Cornbleet and Gochman 1979; Deming 1943) was used instead, and the uncertainties in OC and EC were assumed equal. Thus, OC_{pri} and SOC can be estimated by the following expressions:

$$OC_{pri} = a \times EC + b \quad (1)$$

$$SOC = OC - OC_{pri}, \quad (2)$$

where a = $(OC/EC)_{pri}$, which is the characteristic primary OC/EC ratio for the study area, and b = non-combustion primary OC. Typically, the SOC values estimated through this method vary with season and location and are generally higher during the afternoon hours of summertime photochemical smog episodes (e.g., in the Los Angeles basin) and at locations that are recipients of long-distance transport (e.g., the Eastern United States).

4.1.4. Estimation of indoor PM of outdoor origin

Air exchange rates and infiltration factors (F_{inf}) at each site were determined. Estimated F_{inf} and measured particle concentrations were then used in a single compartment mass balance model to assess the contributions of indoor and outdoor sources to measured indoor EC, OC_{pri}, SOC, and PN as detailed in our recent publication (Polidori et al. 2007) and summarized here. Indoor exposures to PM of outdoor origin are relevant to personal PM exposures given that people generally spend most of their time indoors.

Single compartment mass balance model:

A single compartment mass balance model (Meng et al. 2005; Polidori et al. 2006; Wallace 1996) was used to assess the mean contributions of indoor and outdoor sources to measured indoor OC, EC, $PM_{2.5}$ and PN concentrations. Under the assumption of perfect instantaneous mixing and the assumption that the factors affecting the indoor concentrations are constant or change slowly with time, the steady state indoor concentration of any particulate species can be described by the following equation:

$$C_{in} = \frac{P(AER)C_{out}}{AER + k} + \frac{Q_i / V}{AER + k} = F_{inf}C_{out} + C_{ig} = C_{og} + C_{ig}$$

where, C_{in} is the indoor concentration of the species of interest ($\mu g/m^3$), C_{out} is the corresponding outdoor concentration ($\mu g/m^3$), F_{inf} is the corresponding infiltration factor (dimensionless), C_{ig} is the indoor-generated concentration for the same species found indoors and C_{og} is the outdoor-generated concentration for the same species found indoors. Typically, in the mass balance model C_{ig} is expressed by Qi / $V(a+k)$, where Q_i is the indoor source strength ($\mu g/h$), and V is the house volume (m^3).

Estimation of the infiltration factor (F_{inf}):

The infiltration factor (F_{inf}), defined as the equilibrium fraction of ambient particles that penetrate indoors and remain suspended, is a key determinant of the indoor concentrations of particulate species. F_{inf} is described by the following equation:

$$F_{inf} = P(\text{AER})/(\text{AER}+k) \qquad (1)$$

where, P is the penetration coefficient (dimensionless). F_{inf} for particles varies with particle composition, particle size and volatility, surface to volume ratio of the indoor sampling location and indoor air-speed. F_{inf} is typically highest for non-volatile species such as EC (Lunden et al. 2003; Sarnat et al. 2006). F_{inf} for OC, EC, and PN were estimated from the corresponding indoor/outdoor concentration ratios. In particular, hourly indoor/outdoor ratios (I/O) for each particulate species were determined at times when no indoor particle sources, such as cooking or cleaning, were likely to be present (i.e. only I/O ratios ≤ 1 were considered). Daily F_{inf} estimates were then obtained by averaging these segregated hourly I/O ratios. Mean F_{inf} for each group and phase of the study were also determined by averaging the corresponding daily values. To verify these results, the same analysis of the I/O concentration ratios was then repeated by using only nighttime data (from 00:00 to 06:00 am), for at this time resident activities causing indoor particle generation were expected to be minimal.

Estimation of the Air Exchange Rate (AER):

The indoor-outdoor air exchange rates (AER; h^{-1}) at each community site were estimated from indoor CO measurements collected during periods affected by a dominant indoor source. We considered in our calculations only time-periods when the CO concentration peaked at values significantly higher than the background CO level and that was followed by a non-source period (mostly observed in the morning and probably associated with cooking activities). Assuming an exponential decay of particles, that AER and outdoor concentrations are constant during the decay period, and that indoor concentrations are well mixed, then:

$$C_t = e^{-(AER+k)t} C_0 \quad \textbf{(1)}$$

or

$$\ln C_t = -(AER+k)t + \ln C_0 \quad \textbf{(2)}$$

where, C_t is the indoor CO concentration after time t (after the decay period), C_0 is the initial peak CO concentration (right after CO emission) and k is the indoor loss rate for particles or gases (h^{-1}) (Abt et al 2000). Since k is rather negligible for CO, it was possible to estimate the AERs for the sites directly from the above-mentioned eq (2) by regressing $ln\ C_t$ over $ln\ C_0$.

4.2. Results and Discussion

4.2.1. Estimation of primary and secondary OC

Analysis of exposure data by USC investigators is presented below. The data provides new insight into the relative importance of indoor and outdoor PM sources to measured indoor OC, EC, $PM_{2.5}$ and PN concentrations. The results obtained here were used to examine the relationships between PM of ambient origin and cardiovascular outcomes.

Year 1:

A comprehensive dataset was constructed to enable a preliminary analysis of the relationships between indoor and outdoor $PM_{2.5}$ continuous measurements for the first of two years of data (Delfino et al. 2008). In the following, we describe the estimation of primary and secondary OC for year 2 for retirement community group 1 (G1, East San Gabriel Valley site) and retirement community group 2 (G2, West San Gabriel Valley site) for phase 1 (P1) from July into Oct 2006, and phase 2 (P2) from the end of Oct through Feb 2007.

The most important results obtained at each community in study year 1 are reported below. The contributions of primary and secondary organic aerosol (SOA) to outdoor OC were estimated from measured outdoor OC and EC concentrations using EC as a tracer of primary combustion-generated OC ("EC tracer method") as described above.

Community group 1:

Indoor OC was slightly higher than outdoor OC indicating a moderate influence of OC sources at this indoor site (a "recreational area"). Typically, indoor and outdoor EC tracked each other well suggesting that the EC measured indoors was mostly of outdoor origin. At times, especially in the early afternoons of the first phase of the study (from 07/06/05 to 08/22/05), indoor EC was slightly higher indoors than outdoors. This might be due to the vicinity of a major construction site to the indoor sampling areas (as close as 20 feet) rather than to the presence of indoor sources of EC. As expected, the indoor and outdoor concentrations of important gaseous species such as CO, NO and

NO_x were comparable and track each other well. The average SOA concentration outdoors represented 40-43% of measured outdoor OC from 07/06/05 to 08/19/05 (group 1, phase 1) and 38-45% of measured outdoor OC from 10/17/05 to 12/09/05 (group 1, phase 2). These values are similar to previous summertime estimates for Pittsburgh and Atlanta, and larger than previous SOA estimates in the Los Angeles Basin. The estimated outdoor SOA concentrations along with the correspondent percentage of measured outdoor OC that was SOA are reported in Table 4 for each group and for each phase of the study.

The F_{inf} for OC, EC, particle number and $PM_{2.5}$ concentrations were 0.61, 0.68, 0.54 and 0.64, respectively, consistent with other estimations obtained during previous studies. The AERs calculated between 07/06/05 and 08/19/05 (group 1, phase 1), and between 10/17/05 and 12/09/05 (group 1, phase 2), were 0.31 (± 0.08) and 0.36 (± 0.09), respectively, consistent with AER estimations obtained in other Southern California residences. By using a single compartment mass balance model and the estimated F_{inf} and AER values, we are evaluated the strength of the indoor sources of OC, EC, particle number and $PM_{2.5}$.

Community group 2:

This site was characterized by distinct morning OC peaks typically occurring at 7:00 am, most likely due to cooking (indoor samples were collected in the dining area located next to the kitchen). Outdoor EC was typically higher than indoor EC, and indoor and outdoor EC tracked each other well. During the wintertime, the OC, EC, CO and NO_x concentrations peaked at night between 20:00 pm and 02:00 am probably because of a temperature inversion. As expected, the indoor and outdoor concentrations of important gaseous species such as CO, NO and NO_x were comparable and track each other well. The average SOA concentration outdoors represented 30-42% of measured outdoor OC from 08/23/05 to 10/14/05 (group 2, phase 1) and 40-42% of measured outdoor OC from 01/05/06 to 02/16/06 (group 2, phase 2). Wintertime values are higher than previous summertime estimates for Pittsburgh and for the Los Angeles Basin.

The F_{inf} for OC, EC, particle number and $PM_{2.5}$ concentrations were 0.56, 0.72, 0.46 and 0.58, respectively, consistent with other estimations obtained during previous studies. The AERs calculated between 08/23/05 and 10/14/05 (group 2, phase 1), and between 01/05/06 and 02/16/06 (group 2, phase 2), were 0.35 (± 0.09) and 0.30 (± 0.11), respectively, consistent with AER estimations obtained in other Southern California residences. By using a single compartment mass balance model and the estimated F_{inf} and AER values, we are currently evaluating the strength of the indoor sources of OC, EC, particle number and $PM_{2.5}$.

Table 4. Descriptive analysis of carbon data. Primary OC/EC ratios $(OC/EC)_{pri}$, non-combustion (primary) OC (μg/m^3), correlation (R^2) between all OC and EC data during periods dominated by primary emissions, outdoor SOA concentrations (μg/m^3), and the correspondent percentage (%) of measured outdoor OC that was SOA measured or estimated during CHAPS.

Group/Phase	From	To	(OC/EC)pri	Non-combustion OC	R2	SOA (ug/m3)	SOA (%)
G1P1	07/06/05	07/31/05	1.68	0.59	0.80	2.46	43
G1P1	08/01/05	08/19/05	2.48	0.07	0.80	2.40	40
G2P1	08/23/05	09/30/05	1.76	1.31	0.85	3.17	42
G2P1	10/01/05	10/14/05	2.10	1.47	0.94	2.56	30
G1P2	10/17/05	10/31/05	2.55	0.04	0.80	2.65	45
G1P2	11/01/05	12/09/05	2.03	0.49	0.79	2.27	38
G2P2	01/05/06	01/31/06	2.25	0.08	0.91	3.24	42
G2P2	02/01/06	02/16/06	2.09	0.62	0.94	3.66	40

Year 2:

In the following, we describe the estimation of primary and secondary OC for year 2 for retirement community group 3 (East San Gabriel Valley site) and retirement community group 4 (Riverside site) for phase 1 from July into Oct 2006, and phase 2 from the end of Oct through Feb 2007.

As above, we estimated outdoor concentrations of secondary organic aerosol (SOA) and primary OC (OC_{pri}) using EC as a tracer of combustion-generated OC ("EC tracer method") as described in detail in the Methods section above. The time averaged diurnal pattern for estimated primary OC and SOA concentrations during year 2, and the corresponding measured CO and O_3 concentrations, are reported in Figures 4 to 11. The results replicate what we found in year 1, which is published in Polidori et al (2007). The percentage contributions of outdoor SOA to particulate OC were 46, 39, 30, and 27% during group 3 phase 1 (Figure 4), group 4 phase 1 (Figure 8), group 3 phase 2 (Figure 6), and group 4 phase 2 (Figure 10), respectively. The higher summertime estimates are consistent with higher photochemical activities during this period and with the summertime results obtained during year 1. Wintertime values of ~30% are close to the results reported by Strader et al. (1999). Under suitable conditions (clear skies, low horizontal winds, and low mixing height) elevated SOA concentrations (as high as 15-20 μgC/m^3) could be produced in the wintertime, mainly due to the oxidation of aromatics species such as toluene, xylenes, trimethylbenzenes, naphthalenes, and 1,3,5-trimethylbenzene.

With the exception of the summertime data (group 3 phase 1; Figures 4-5), the average concentrations of SOA and O_3, and those of the corresponding primary OC and CO did not track one another well across the entire day, although concordance was usually good during parts of the day when the paired variables are expected to peak. Ozone and CO are tracers of primary combustion and photochemical activities, respectively. We suspect that the weaker correlation between SOA and O_3 observed during year 2 as compared with year 1 (especially at site group 4) was due to a substantial contribution of biogenic SOA from the oxidation of Terpenes (a large and varied class of hydrocarbons, produced primarily by a wide variety of plants, particularly conifers) emitted from the nearby vegetation.

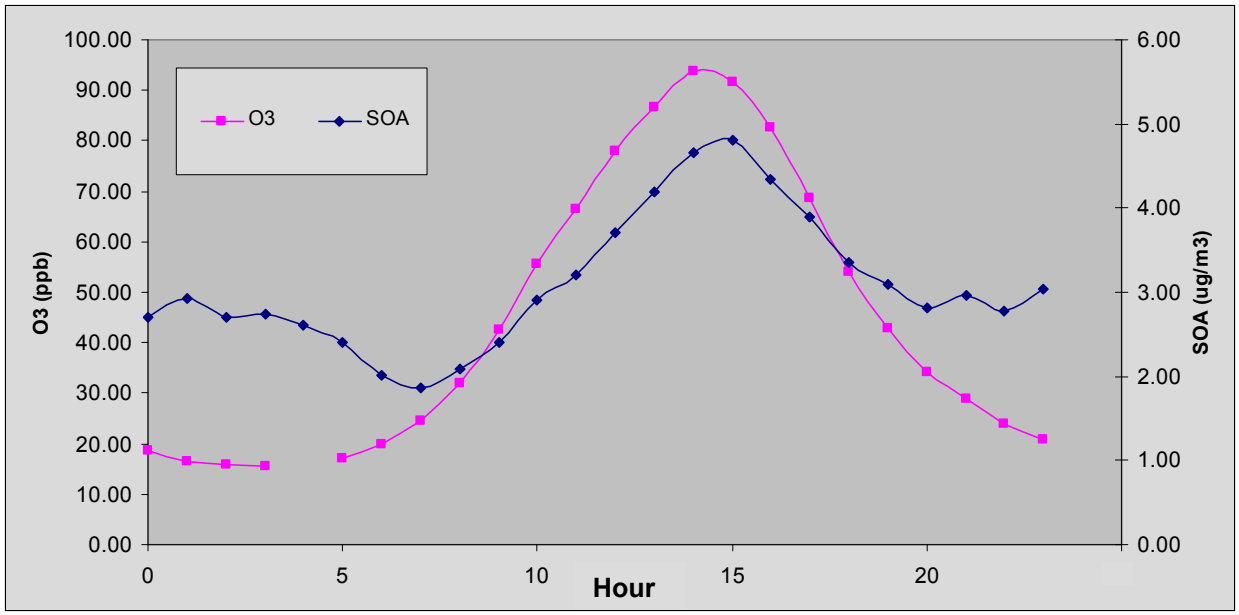


Figure 4. Time averaged diurnal relationship between O_3 and estimated secondary organic aerosol (SOA) for the 6-week follow-up during group 3 phase 1. O_3: ozone, SOA: secondary oprganic aerosols.

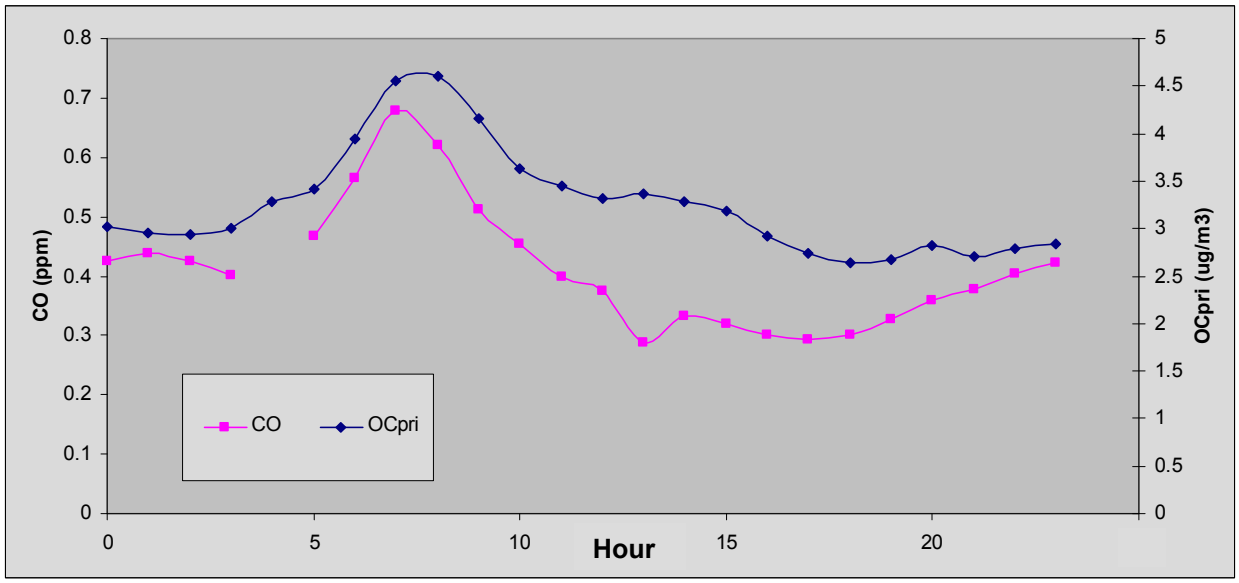


Figure 5. Time averaged diurnal relationship between CO and estimated primary OC for the 6-week follow-up during group 3 phase 1. CO: carbon monoxide, OCpri: primary organic carbon.

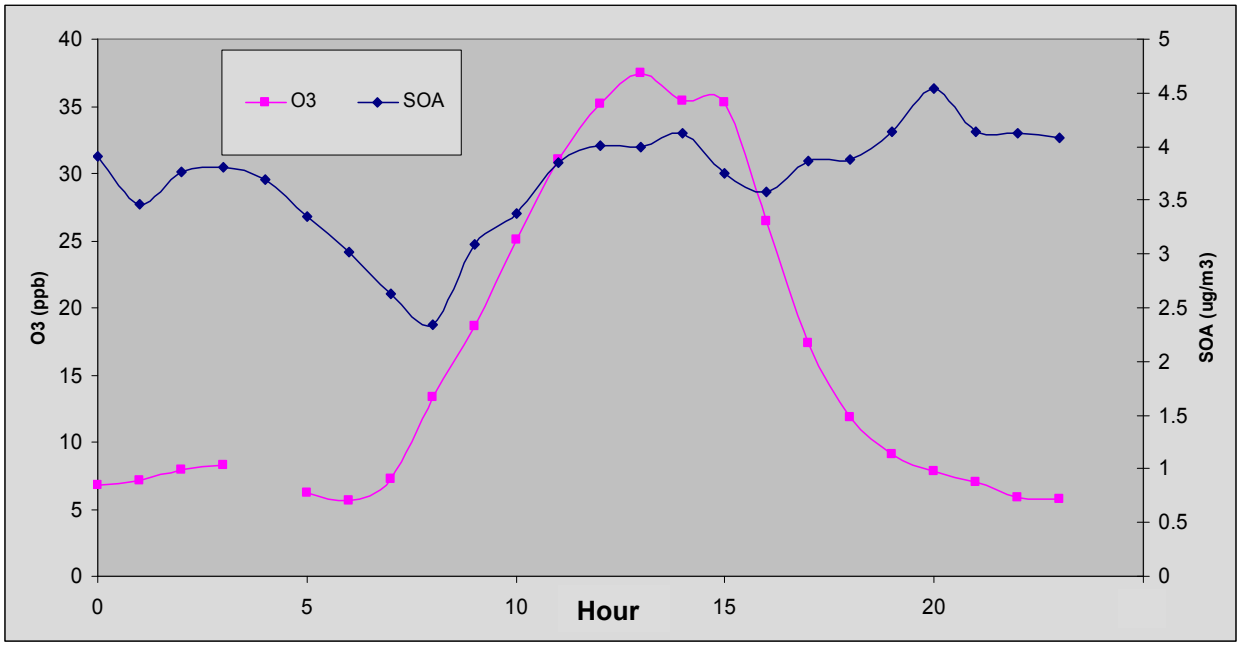


Figure 6. Time averaged diurnal relationship between O_3 and estimated secondary organic aerosol (SOA) during for the 6-week follow-up during group 3 phase 2. O_3: ozone, SOA: secondary oprganic aerosols.

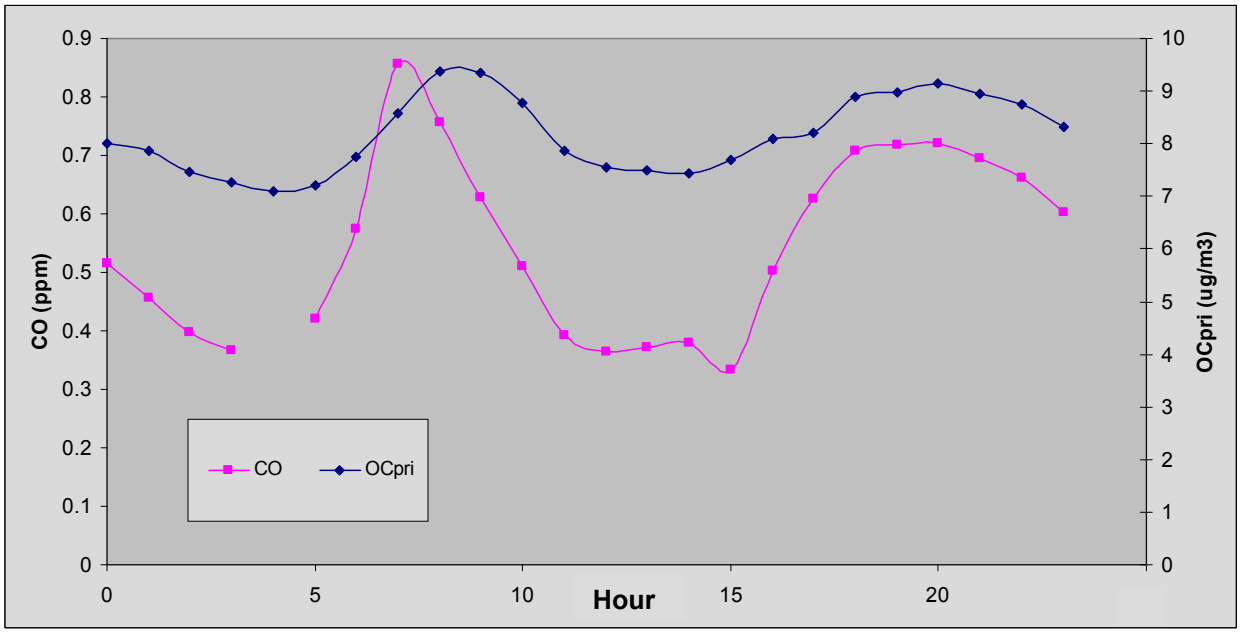


Figure 7. Time averaged diurnal relationship between CO and estimated primary OC for the 6-week follow-up during group 3 phase 2. CO: carbon monoxide, OCpri: primary organic carbon.

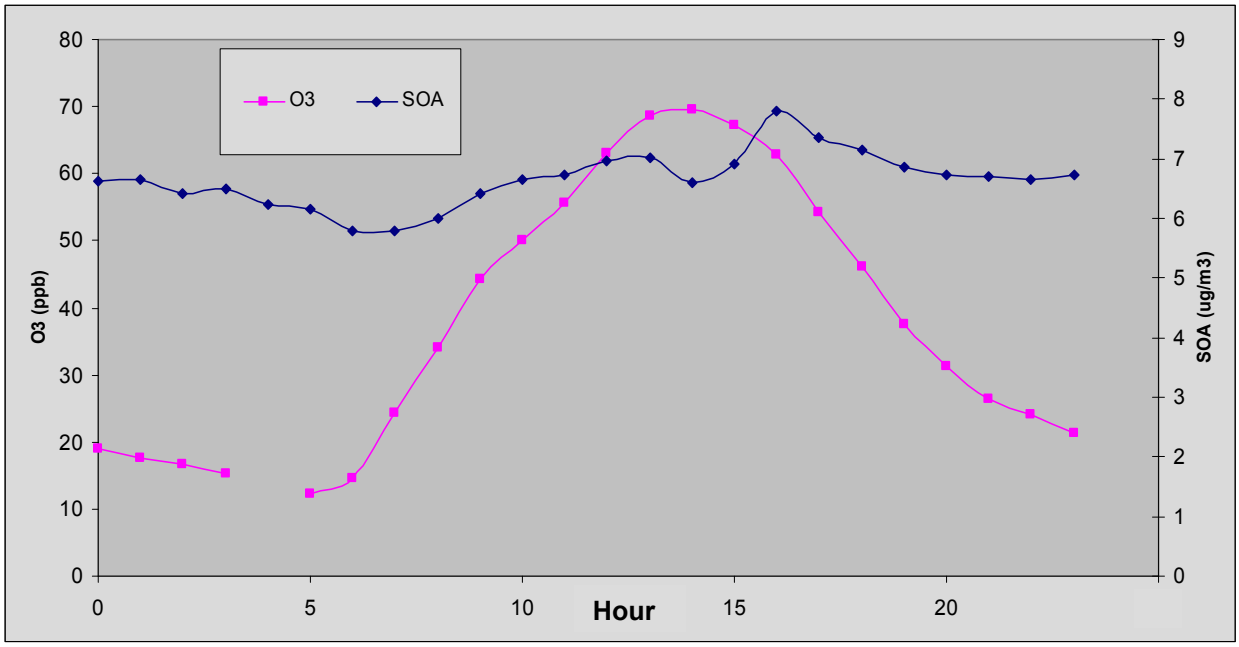


Figure 8. Time averaged diurnal relationship between O_3 and estimated secondary organic aerosol (SOA) for the 6-week follow-up during group 4 phase 1. O_3: ozone, SOA: secondary oprganic aerosols.

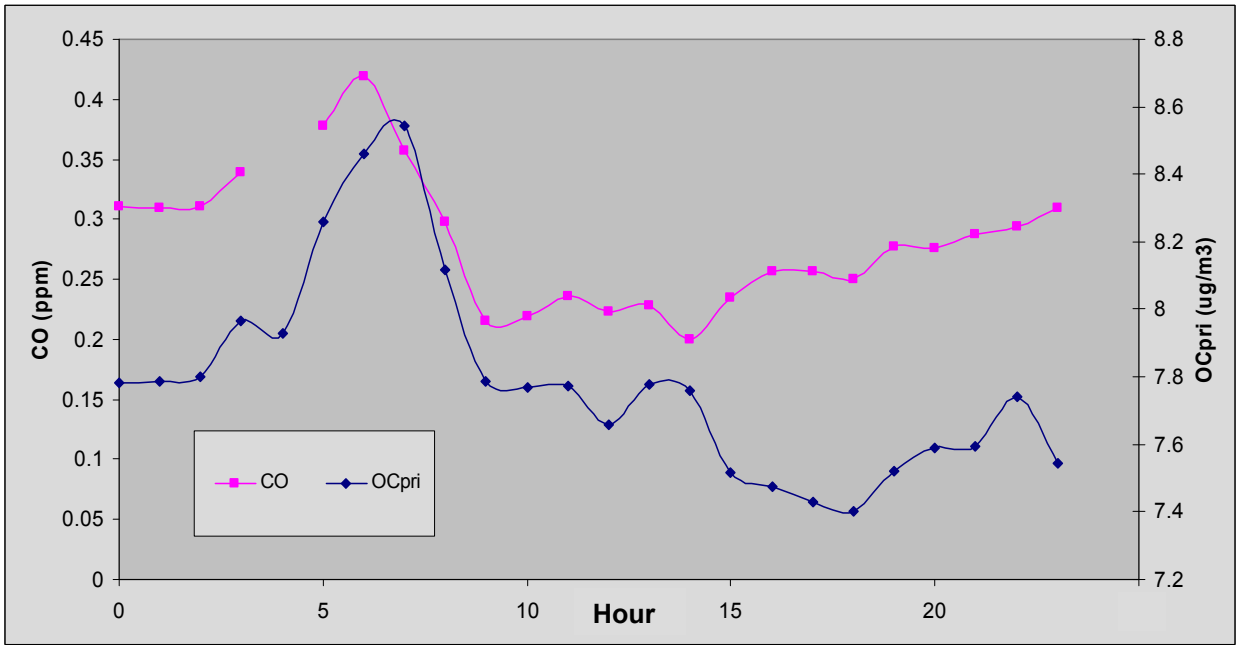


Figure 9. Time averaged diurnal relationship between CO and estimated primary OC for the 6-week follow-up during group 4 phase 1. CO: carbon monoxide, OCpri: primary organic carbon.

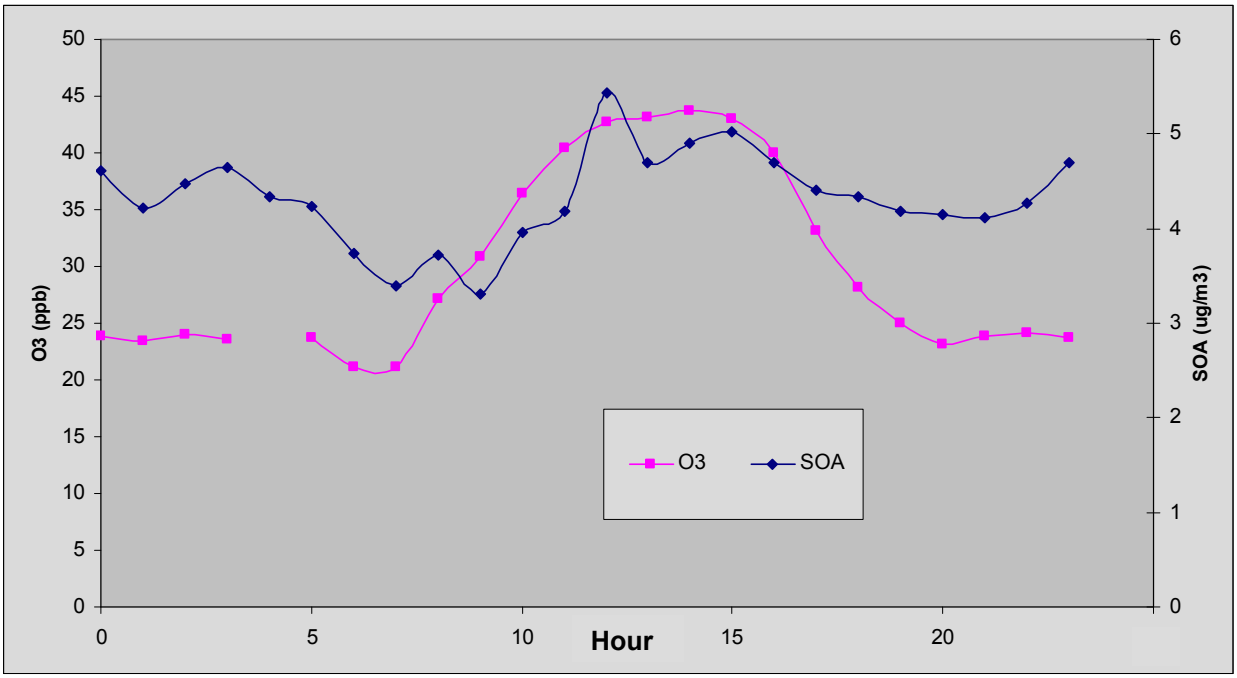


Figure 10. Time averaged diurnal relationship between O_3 and estimated secondary organic aerosol (SOA) for the 6-week follow-up during group 4 phase 2. O_3: ozone, SOA: secondary oprganic aerosols.

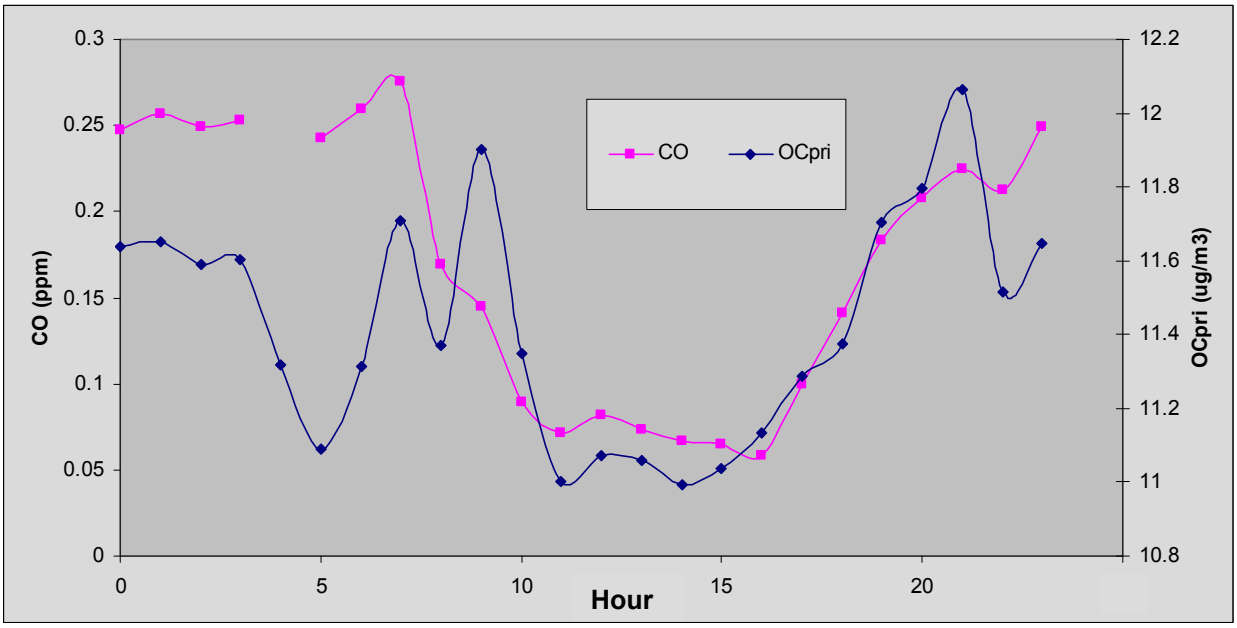


Figure 11. Relationship between CO and estimated primary OC during for the 6-week follow-up during group 4 phase 2. CO: carbon monoxide, OCpri: primary organic carbon.

4.2.2. Estimation of indoor PM of outdoor origin

Year 1:

By using a mass balance approach (described above under Methods) and using the first year of data, we estimated the amount of outdoor SOA and outdoor primary OC that penetrated inside groups 1 and 2 sites. As illustrated in Figure 12, the average percentage contribution of indoor SOA of outdoor origin to measured indoor OC varied from 24% (1.36) to 27% (1.21) for group 1 phase 1 (G1P1) and group 1 phase 2 (G1P2), respectively, and from 32% (1.79) to 37% (1.80) for group 2 phase 1 (G2P1) and group 2 phase 2 (G2P2), respectively (the corresponding average concentrations in μgC/m^3 are reported in parenthesis).

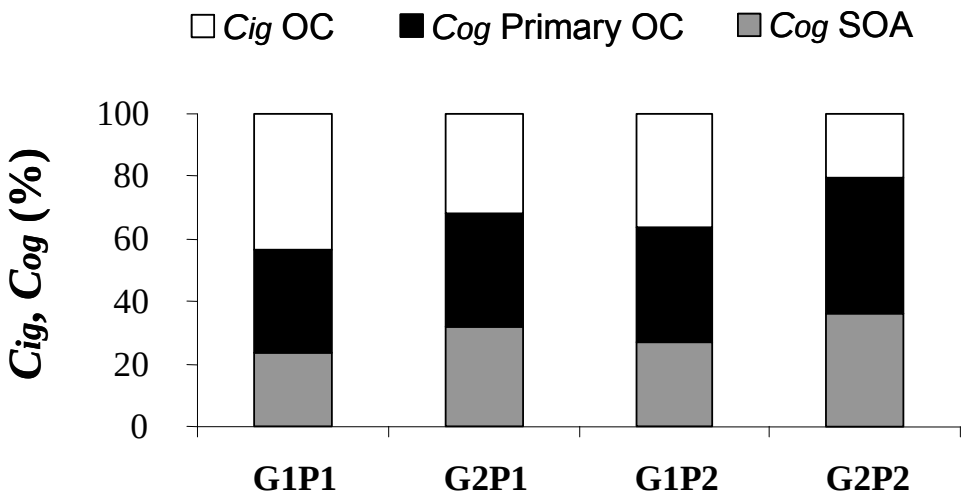


Figure 12. Estimated indoor primary organic carbon (OC) and indoor secondary prganic aerosol (SOA) concentrations of outdoor origin ("C_{og} Primary OC" and "C_{og} SOA", respectively). Data are expressed as a percentage of the corresponding measured indoor concentrations (C_{in}), and averaged throughout G1P1 (group 1 phase 1), G2P1 (group 2 phase 1), G1P2 (group 1 phase 2) and G2P2 (group 2 phase 2). Estimated indoor OC concentrations of indoor origin (C_{ig} OC) are also reported.

Figure 12 also shows that on average 33% (1.78), 36% (2.16), 37% (1.79) and 43% (2.07) of measured indoor OC was comprised of outdoor-generated primary OC during group 1 phase 1 (G1P1), group 2 phase 1 (G2P1), group 1 phase 2 (G1P2) and group 2 phase 2 (G2P2), respectively (average concentrations in μgC/m^3 in parenthesis). To the best of our knowledge, these results are among the first to quantify the contributions of outdoor-generated SOA and primary OC to indoor OC and to demonstrate their importance in indoor environments. These outcomes have been used by CHAPS investigators to clarify the links between exposure to $PM_{2.5}$ of indoor and outdoor origin and its effects on cardiovascular outcomes (Tasks 7-8).

These calculations were based on the assumption that F_{inf} for SOA and primary OC were equal to F_{inf} estimated for total OC. This assumption is reasonable, although it is likely to underestimate F_{inf} for the SOA component and to overestimate F_{inf} for the primary-generated OC fraction. In fact, other studies have shown that the size distribution of SOA is generally concentrated in the accumulation mode (characterized by nighttime F_{inf} of ~ 0.7) while primary-generated OC particles show a distinct ultra-fine mode (F_{inf} is ~ 0.5) commonly associated with fresh emissions. However, when using a F_{inf} of 0.7 for SOA and a F_{inf} of 0.5 for primary OC the correspondent mass balance results for all groups

and phases of CHAPS varied by less than 5%.

Estimates of F_{inf} and of the background source strength (indoors) for OC, EC, $PM_{2.5}$ and PN during CHAPS year 1 are reported in Table 5.

Table 5. Estimates of F_{inf} and of the background source strength (indoors) for OC, EC, $PM_{2.5}$ and PN during study year 1.

	Species	F_{inf}	Background source*
G1P1	OC	0.62	1.17
	EC	0.68	0.02
	PN	0.60	-126.83
	$PM_{2.5}$	0.64	-0.17
G2P1	OC	0.59	1.07
	EC	0.72	0.05
	PN	0.49	849.46
	$PM_{2.5}$	0.62	1.27
G1P2	OC	0.65	0.06
	EC	0.79	0.05
	PN	0.60	1568.40
	$PM_{2.5}$	0.63	1.56
G2P2	OC	0.66	0.05
	EC	0.80	0.08
	PN	0.48	1264.40
	$PM_{2.5}$	0.60	0.85

* OC and EC in $\mu gC/m^3$; PN in $ptcl\#/cm^3$; $PM_{2.5}$ in $\mu g/m^3$ G1P1: group 1 phase 1, G2P1: group 2 phase 1, G1P2: group 1 phase 2, G2P2: group 2 phase 2.

By multiplying the measured outdoor 1-hr OC, EC, $PM_{2.5}$ and PN concentrations (*Cout*) by the correspondent estimated F_{inf}, we determined the indoor contribution of outdoor origin for each particulate species (*Cog*) and for each group (G) and phase (P) of CHAPS. The resulting indoor contributions of indoor origin (C_{ig}) were then estimated by subtracting C_{og} from C_{in} on a sample-by-sample basis. Figure 13 shows the calculated C_{ig} concentrations for OC (1a), EC (1b), $PM_{2.5}$ (1c) and PN concentrations (1d) expressed as a percentage of the corresponding measured indoor concentrations (C_{in}), and averaged throughout G1P1, G2P1, G1P2 and G2P2 (black columns). For comparison, the lowest possible C_{ig} estimations for OC, EC, $PM_{2.5}$ and PN concentrations (grey columns) were obtained by assuming that all outdoor particles penetrated through the building envelope and that there were no particle losses indoors (F_{inf} =1). This gives a reasonable range for the estimated C_{ig} results.

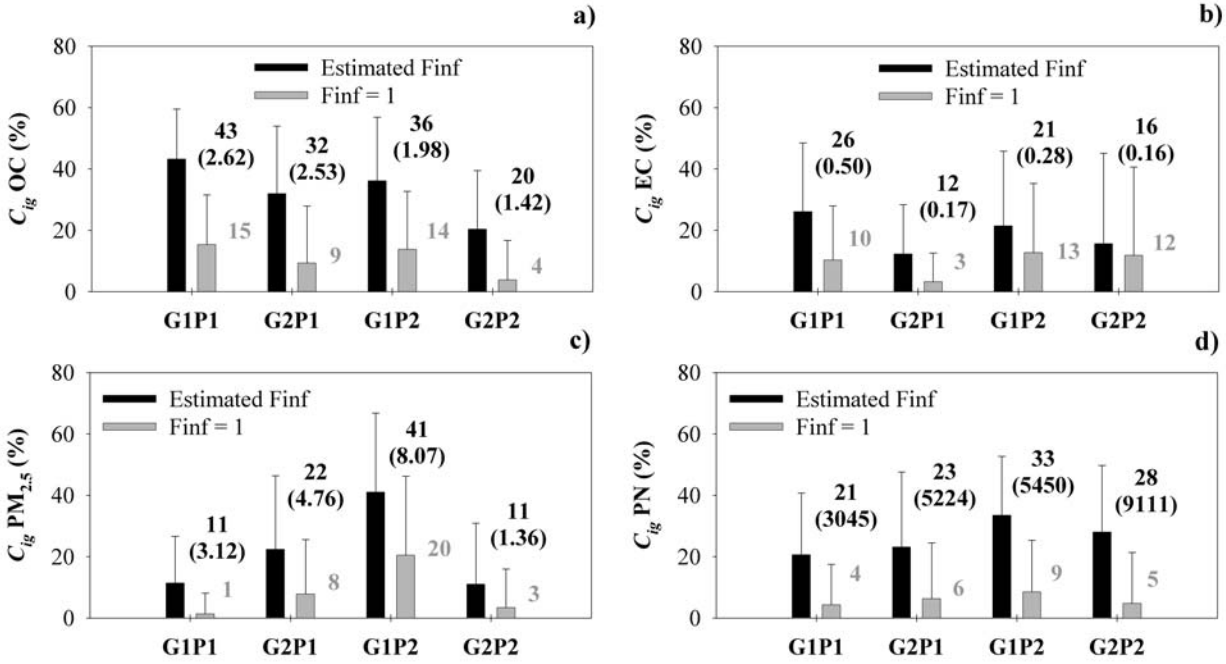


Figure 13. Calculated indoor concentrations of indoor origin (C_{ig}) for OC (1a), EC (1b), $PM_{2.5}$ (1c) and PN (1d) expressed as a percentage of the corresponding measured indoor concentrations (C_{in}), and averaged throughout group 1 phase 1 (G1P1), group 2 phase 1 (G2P1), group 1 phase 2 (G1P2) and group 2 phase 2 (G2P2) (black columns). The lowest possible C_{ig} estimations for the same species (grey columns) were obtained by assuming F_{inf} =1. Error bars represent + 1σ (1 standard deviation) of all C_{ig} estimates obtained within each group (G) and phase (P).

Our estimates indicate that, on average, 43% (2.62), 32% (2.53), 36% (1.98) and 20% (1.42) of measured indoor OC was emitted or formed indoors during G1P1, G2P1, G1P2 and G2P2, respectively (the correspondent average indoor-generated OC concentrations in μgC/m^3 are reported in parenthesis) (Figure 13a). These results suggest that although the G2 indoor site was characterized by higher indoor morning OC peaks due to cooking (see above for details), the overall contribution of indoor sources to measured indoor OC was actually higher at the G1 site. The rather low C_{ig} OC estimates obtained during CHAPS are consistent with the prevailing use of central air conditioning at both G1 and G2 indoor sites.

The average percentages of measured indoor EC that was generated indoors were 26% (0.50), 12% (0.17), 21% (0.28) and 16% (0.16) for G1P1, G2P1, G1P2 and G2P2, respectively (average indoor-generated EC in μgC/m^3 in parenthesis) (Figure 13b). These values are quite close to the detection limit for EC for semi-continuous carbon measurements, typically around 0.15-0.35 μgC/m^3, and suggest that indoor sources of EC were not an important contributor to measured indoor EC during CHAPS. These results are consistent with indoor/outdoor EC ratios close to or slightly lower than unity obtained in several previous studies conducted both in California and around the world.

The mass balance model results also showed that on average 11% (3.12), 22% (4.76), 41% (8.07) and 11% (1.36) of measured indoor $PM_{2.5}$ was emitted or formed indoors during G1P1, G2P1, G1P2 and G2P2, respectively (the correspondent average indoor-generated $PM_{2.5}$ concentrations in μg/m^3 are reported in parenthesis) (Figure 13c). These outcomes are somewhat difficult to interpret and suggest that the seasonal emission/formation of indoor $PM_{2.5}$ from indoor sources was highly variable. It is important to recognize that the $PM_{2.5}$ concentrations measured indoors during G2P2 were unusually low compared to the corresponding outdoor $PM_{2.5}$ concentrations and to the G2P1 $PM_{2.5}$ data. Whether or not this was due to seasonal changes in home dynamics and/or ventilation

conditions between G2P1 and G2P2 remains unclear.

The average percentage of measured indoor PN concentration that was emitted/formed indoors were 21% (3045), 23% (5224), 33% (5450) and 28% (9111) for G1P1, G2P1, G1P2 and G2P2, respectively (average indoor-generated PN/cm^3 reported in parenthesis) (Figure 13d). These results suggest that the PN concentration of indoor origin increased from summer to fall (at the G1 site) and from fall to winter (at the G2 site). The seasonal increase in C_{ig} for PN concentration was probably due to the use of indoors fan heaters during the wintertime. Other indoor activities such as cooking might have increased the indoor levels of PN concentrations by a substantial amount.

Year 2:

In the following, we describe the indoor PM data for year 2 for group 3 (G3, East San Gabriel Valley site) and group 4 (G4, Riverside site) for phase 1 (P1) from July into Oct 2006, and phase 2 (P2) from the end of Oct through Feb 2007.

Methods for estimating indoor PM of outdoor origin were described in the Methods section above. Here we use the "all hours" method to estimate the infiltration factor (F_{inf}) of $PM_{2.5}$ and of several $PM_{2.5}$ components. A single compartment mass balance model (also described in the Methods section above) was then used to estimate indoor and outdoor source contributions.

As illustrated in Figure 14, the average percentage contribution of indoor SOA of outdoor origin to measured indoor OC ("Cog SOA"), decreased from phase 1 to phase 2 at both sites. This is consistent with expectations, because the outdoor concentration of SOA (produced by photochemical reactions) typically decreases going from spring/summer to fall/winter. Conversely, the average percentage contribution of indoor primary OC of outdoor origin to measured indoor OC ("Cog primary OC") increased from phase 1 to phase 2 at both sites as a result of the increased production of outdoor primary-generated OC during the colder months (also consistent with expectations).

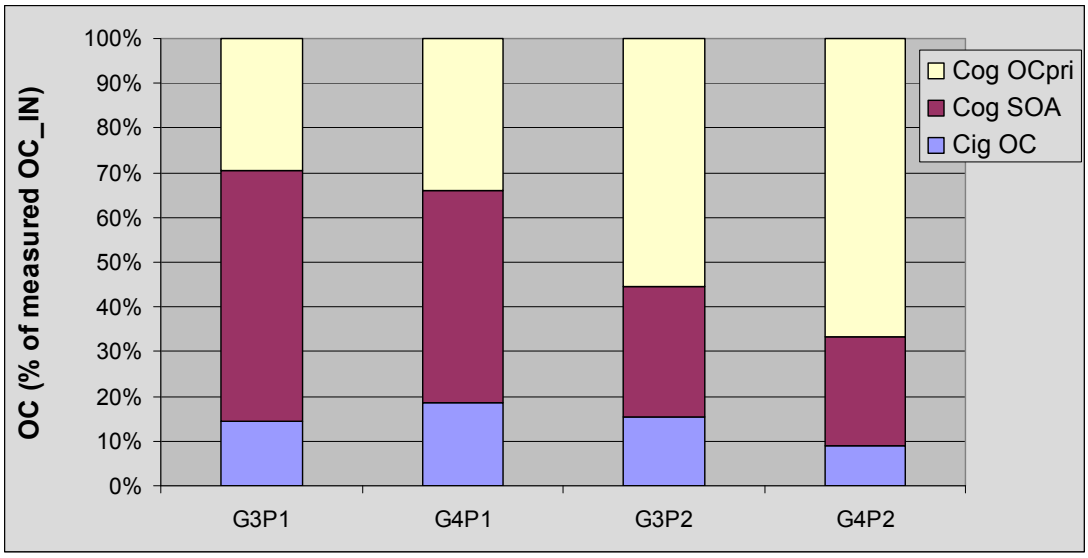


Figure 14. Estimated indoor primary OC and indoor SOA concentrations of outdoor origin ("C_{og} OC_{pri}" and "C_{og} SOA", respectively) expressed as a percentage of the corresponding measured indoor concentrations (C_{in}), and averaged throughout group 3 phase 1 (G3P1), group 4 phase 1 (G4P1), group 3 phase 2 (G3P2) and group 4 phase 2 (G4P2). Estimated average indoor OC concentrations of indoor origin ("C_{ig} OC") are also reported.

Figures 15 to 18 shows the calculated Cig and Cog concentrations for EC (Figure 15), PN (CPC; Figure 16), active surface area (NSAM described below; Figure 17), and $PM_{2.5}$ (Figure 18), expressed

as a percentage of the corresponding measured indoor concentrations (C_{in}), and averaged throughout G3P1, G4P1, G3P2 and G4P2. In all cases, the indoor contributions of outdoor origin (C_{og}) were substantially higher than the corresponding indoor contributions of indoor origin (C_{ig}), highlighting the dominant role of outdoor sources in determining the indoor concentrations of $PM_{2.5}$ and its components. For example, the mass concentration corresponding to the average percentages of measured indoor EC that was generated indoors (Figure 15) were as in year 1 quite close to the detection limit for EC for semi-continuous carbon measurements (typically 0.15 to 0.35 $\mu gC/m^3$). These outcomes are consistent with indoor/outdoor EC ratios close to or slightly lower than unity obtained in several previous studies. Despite the dominant role of outdoor sources in determining the measured indoor concentrations, the contribution of indoor sources to the measured indoor concentrations of PN, active surface area, and $PM_{2.5}$ (Figures 16, 17, and 18, respectively) were not negligible at both G3 and G4 sites.

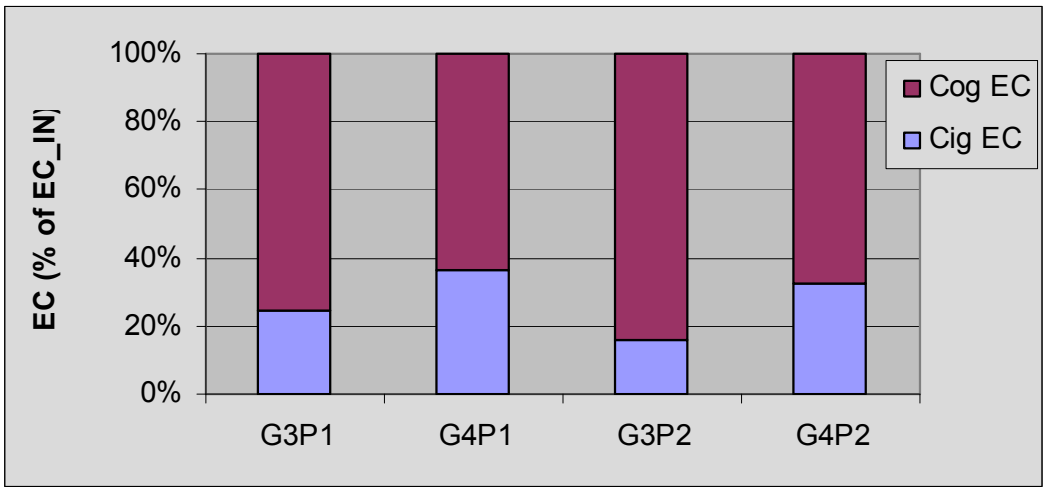


Figure 15. Calculated indoor concentrations of indoor and outdoor origin (C_{ig} and C_{og}, respectively) for EC expressed as a percentage of the corresponding measured indoor EC and averaged throughout group 3 phase 1 (G3P1), group 4 phase 1 (G4P1), group 3 phase 2 (G3P2) and group 4 phase 2 (G4P2).

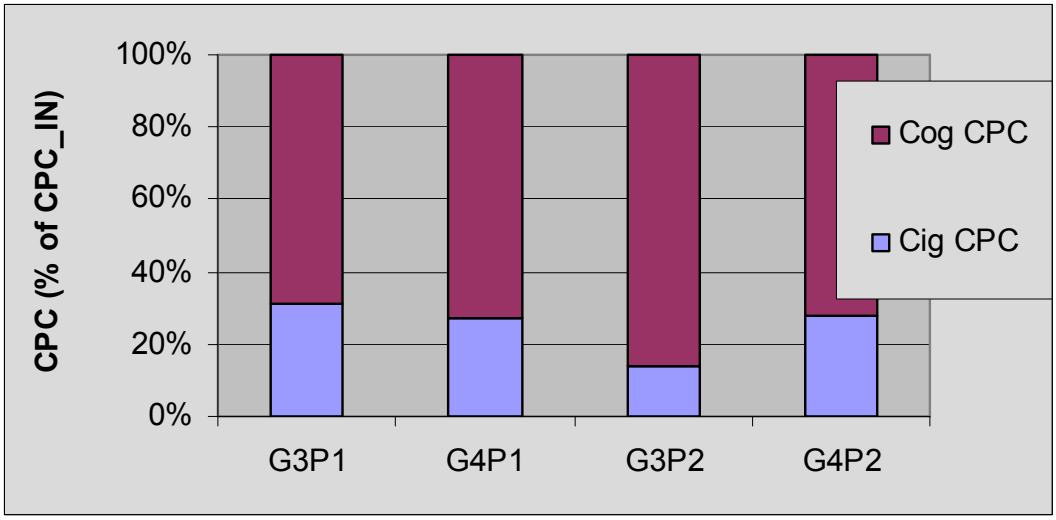


Figure 16. Calculated indoor concentrations of indoor and outdoor origin (C_{ig} and C_{og}, respectively) for PN (CPC) expressed as a percentage of the corresponding measured indoor PN and averaged throughout group 3 phase 1 (G3P1), group 4 phase 1 (G4P1), group 3 phase 2 (G3P2) and group 4 phase 2 (G4P2).

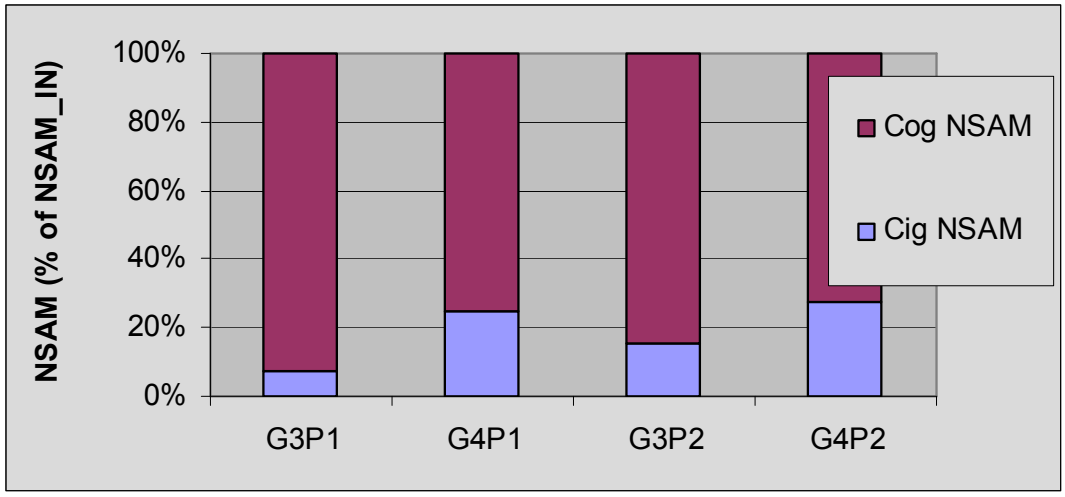


Figure 17. Calculated indoor concentrations of indoor and outdoor origin (C_{ig} and C_{og}, respectively) for active surface area (NSAM) expressed as a percentage of the corresponding measured indoor active surface area and averaged for group 3 phase 1 (G3P1), group 4 phase 1 (G4P1), group 3 phase 2 (G3P2) and group 4 phase 2 (G4P2).

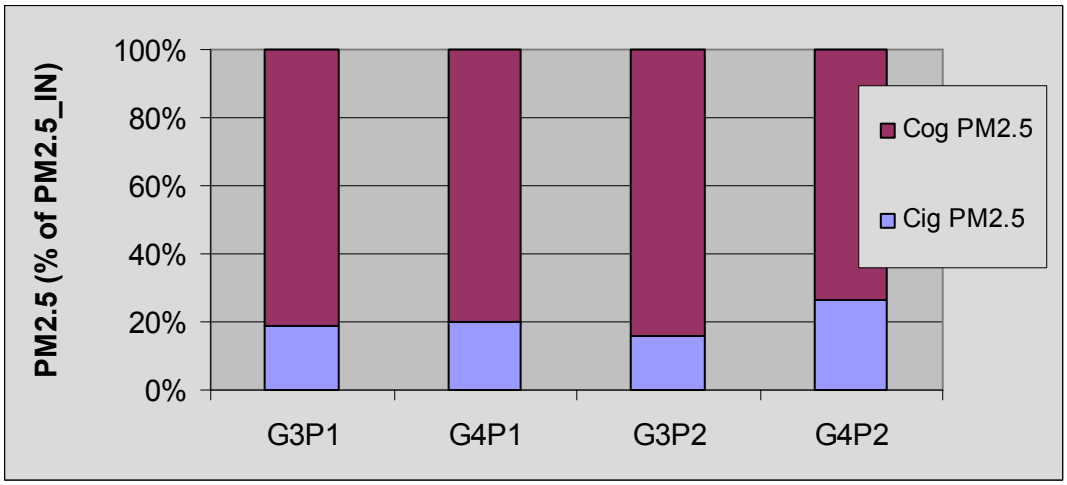


Figure 18. Calculated indoor concentrations of indoor and outdoor origin (C_{ig} and C_{og}, respectively) for $PM_{2.5}$ expressed as a percentage of the corresponding measured indoor $PM_{2.5}$ and averaged throughout group 3 phase 1 (G3P1), group 4 phase 1 (G4P1), group 3 phase 2 (G3P2) and group 4 phase 2 (G4P2).

4.2.3. Analysis of Nanoparticle Surface Area Monitors

This data was added to better understand the potential for PM to carry toxic components that are deposited on lung surfaces. It was not collected for use in the analysis of health outcomes as in Tasks 7-8 since the duration of data collection are not comprehensive.

Two identical Nanoparticle Surface Area Monitors (NSAM; TSI Inc.) were used to study the particle surface concentration at one of the retirement communities from January 5 through January 31, 2006 at G2P2 both indoors and outdoor. The NSAM signal was combined with Continuous Particle Counter (CPC; TSI Inc.) number concentration measurements to estimate the mean surface diameter of the collected $PM_{2.5}$. Table 6 presents the mean particle surface and number concentrations measured with the NSAM and CPC, respectively.

Table 6. Mean particle surface and number concentrations.

	Daily sampling duration	Total hourly samples	NSAM Surface (μm^2 cm^{-3})		CPC Concentration (cm^{-3})	
			Mean	Standard Deviation	Mean	Standard Deviation
Indoor	24h	557	45.2	26.1	12938	4094
Outdoor	24h	630	68.9	38.7	18448	6049

The total number of hourly samples is also reported. Mean values correspond to continuous 24 h sampling. The variance in the mean concentration is expressed by means of the standard deviation over the entire sampling period. The diurnal profile of surface and particle number concentrations is reported in Figures 19 and 20, respectively.

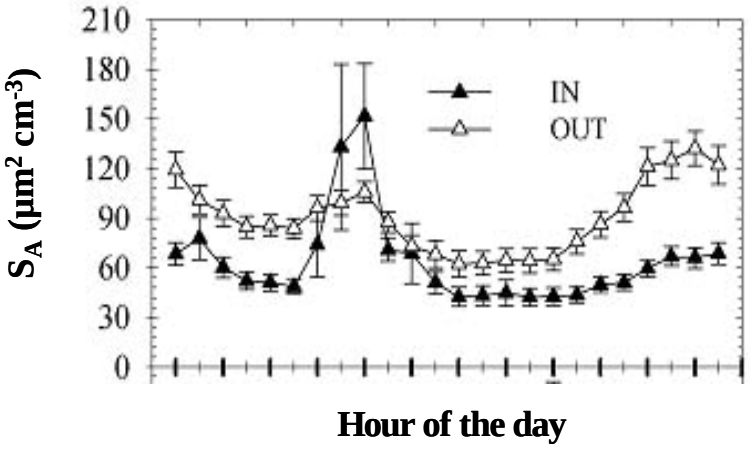


Figure 19. Temporal variation of the particle surface concentration measured with the NSAM Indoors (IN) and outdoors (OUT) of a retirement community.

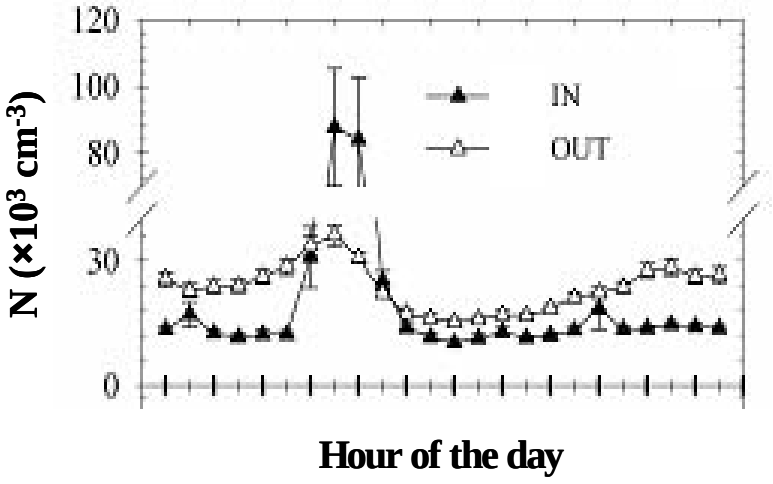


Figure 20. Temporal variation of the outdoor particle number concentration measured with the CPC.

Throughout the sampling period, the surface area peaked between 06:00 and 08:00 because of morning rush hour. The surface concentration then dropped during midday and increased again in the evening, forming a second local peak between 23:00 and 01:00. The particle number concentration seemed to follow a similar pattern. In the absence of any known urban sources whose activity increased during the overnight period, the surface increase was likely due to new particle formation by nucleation as well as condensational particle growth as the temperature drops and the atmospheric mixing height decreases during that period. Interestingly, this seemed to predominately affect only the outdoor site. However, at 6:00–9:00 in the morning, substantial peaks appeared in both number and surface of indoor particle concentrations, which by far exceed the outdoor increase. Those peaks are a strong indication of an indoor source, and most likely are related to morning cooking activities in the kitchen adjacent the indoor sampling site where all meals of the day were cooked at this time by using gas stoves/ovens. With the exception of these local peaks due to cooking, the magnitudes and trends of indoor and outdoor particle concentrations closely track each other, with indoor levels always lower than outdoor levels, which suggest that the majority of indoor particles in that site originate from outdoors. The diffusion charger response and the total particle number concentration measured by a CPC can be combined to estimate the mean surface diameter (*dS*) according to the following equation:

$$d_S = \left(\frac{S_A}{0.01keQN} \right)^{1/1.285}$$

The diurnal profile of the mean surface diameter (d_S) is shown in Figure 21.

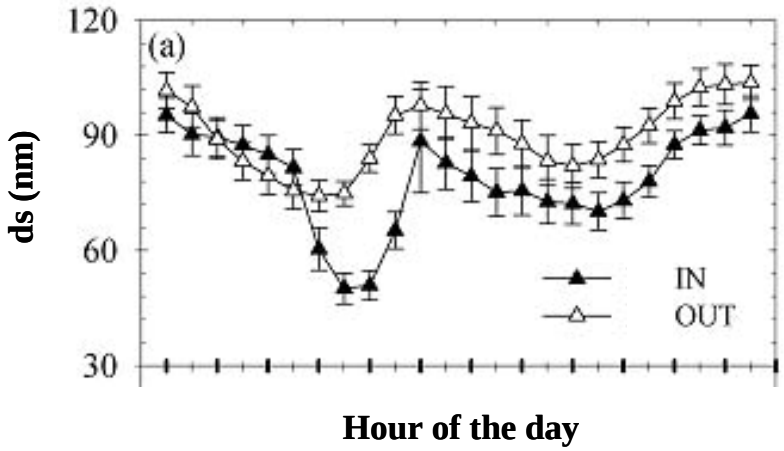


Figure 21. Temporal variation of the mean surface diameter obtained by combination of the NSAM and the CPC concentrations.

The mean diameters indoors and outdoors tracked each other well during the day, except when cooking activities were taking place (6:00–9:00). During that time, a large number of small particles were produced indoors, which lead to an increase in the surface concentration. In general, d_S increased both indoors and outdoors in the evening as the temperature drops. However, the mean indoor diameter was always slightly lower than the outdoor (except between 3:00–5:00), which may be due to some particle evaporation as the aerosol is transported in the warmer indoor environment. It is known that particulate species such as ammonium nitrate and organic compounds, which may account for 35–60% of outdoor $PM_{2.5}$ mass in the Los Angeles basin, volatilize as they enter indoors.

5. TASK 4. Conduct *in vitro* testing to assess the generation of ROS by fine and ultrafine PM collected with biosamplers in Task 2.

5.1. Materials and Methods

Dithiothreitol (DTT) assay

This procedure, measures the ability of PM or its constituents to catalyze the oxidation of DTT by oxygen (Kumagai et al., 2002). The assay measures the catalytic capacity of the PM sample by determining the rate of DTT consumption in the presence of a specified quantity of the sample. This catalytic reaction has been initially demonstrated for the highly redox active 9,10-phenanthroquinone, but other quinones such as the naphthoquinone are also capable of catalysis. Catalytic activity, expressed by the rate of DTT consumption per minute per microgram of sample, reflects the redox activity of the sample. In the assay, DTT consumption is followed by measuring the loss of DTT through its reaction with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). This disulfide reacts with the remaining DTT to generate the mixed disulfide and liberates 5-mercapto-2-nitrobenzoic acid, measured spectrophotometrically at 412 nm.

Redox samples, containing known masses of PM, are incubated with DTT (10 μM) in Tris buffer at pH 8.9 for times varying from 10 to 90 minutes. At pre-selected times, aliquots of the incubation mixture are added to another test tube containing buffer and DNTB, mixed and the absorption at 412 nm measured. The quantity of PM used depends on its catalytic ability so that some trial and error is necessary to select a concentration that will deplete < 20% of the DTT in the period of the reaction.

Samples were collected on two days (Thursdays and Fridays) for each of the 48 weeks of exposure assessment runs as described above for Task 2. The target number of samples to be collected on each of the two days was: 48 two-day composite samples x two sites (indoor + outdoor) x two PM size modes = 192. The ROS activity and related composition data was regressed on biomarkers of effect from blood draws on the end of Friday (Task 6).

Dihydroxybenzoic acid (DHBA) Assay

This assay determines the ability of the sample to catalyze the Fenton reaction, a reaction between hydrogen peroxide and a transition metal ion to generate hydroxyl. The hydroxyl reacts with salicylate to form dihydroxy benzoic acid isomers, which are determined by HPLC. Ascorbate is used as an electron source that reduces both oxygen and the transition metal to enable the reaction. The units are nmoles AA consoumed/min*micg and nmoles DHBA formed/min/μg.

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) Assay

Additional in-vitro toxicity assays were performed by UCLA investigators for six Riverside BioSampler collections collected under this contract. The assay utilized the thiol enzyme, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The rationale for the study was that some chemical species elicit their effects through covalent bond formation with nucleophilic functions in the cell. The properties were demonstrated by their ability to inhibit GAPDH. GAPDH is irreversibly inactivated by electrophiles under anaerobic conditions by covalent bond formation. This inactivation can be blocked by the prior addition of a high concentration of dithiothreitol (DTT) as an alternate nucleophile. Addition of DTT after the reaction between the electrophile and GAPDH, however, does not reverse the inactivation. This property was utilized to develop a procedure that provides a quantitative measure of electrophiles present in samples of ambient particles collected in the Los Angeles Air Basin and in diesel exhaust particles. The toxicity of electrophiles is the result of irreversible changes in biological molecules. Once a covalent bond is formed between an electrophile and a nucleophilic center on a biological target, recovery from the event requires resynthesis of the target. If the resynthesis is slow, the irreversible effects can be cumulative and manifest themselves

after chronic exposure to low levels of electrophiles.

5.2. Results and Discussion

Year 1, Fine PM:

Collection of aqueous particle suspensions with the VACES concentration system for use in *in vitro* toxicity assays were presented above (Task 2). Measurements in 7/14/2005 were not reliable because the sample is stored in glass bottle with undetermined background Fe content. DBHA analysis was not performed for the weeks 9-10 (first two weeks in West San Gabriel) ending Aug 11 and Aug 18 because this sample was combined with the remainder of previous samples to provide enough samples to do quinone analysis and possibly other toxicity experiments.

In Appendix A we show raw results for the dithiothreitol (DTT) and dihydroxybenzoic acid (DHBA) assays (Table A2). Here we show overall year 1 descriptive statistics for activity per m^3 of air (Table 7). In addition, the data from each collection period (by group and phase) were pooled and means and standard deviations determined (Table 8). There were no significant differences by two-sided t-tests ($p < 0.05$) in mean concentrations between phase in each group or for between groups for each phase. No problems were encountered in the assays; the values for the standards that are routinely analyzed in each assay were consistent with all previous assays. The assays performed were consistent, based on the control values for each assay. Note that the absolute values for each measurement in Tables 7-8 cannot be compared, i.e., DTT vs. DHBA.

Table 7. Overall indoor and outdoor DTT and DHBA activity (nmoles/min/m^3): Year 1 concentrated $PM_{2.5}$ data.

In vitro variable	mean ±SD	median	minimum/maximum
DTT indoor	0.514 ± 0.296	0.484	0.073/1.205
DTT outdoor	0.746 ± 0.408	0.638	0.154/1.679
DHBA indoor	0.252 ± 0.174	0.223	0.000/0.631
DHBA outdoor	0.352 ± 0.260	0.288	0.000/0.925

Table 8. Descriptive statistics for DHBA and DTT activity in ultrafine particles, year 1 concentrated $PM_{2.5}$ data by group and phase (mean nmoles/min/m^3 ± SD).

	G1	G1	G2	G2
Variable	**Phase 1**	**Phase 2**	**Phase 1**	**Phase 2**
DHBA outdoor	0.221 ± 0.229	0.302 ± 0.230	0.502 ± 0.304	0.358 ± 0.249
DTT outdoor	0.736 ± 0.203	0.616 ± 0.422	1.052 ± 0.442	0.580 ± 0.400
DHBA indoor	0.280 ± 0.265	0.291 ± 0.184	0.197 ± 0.094	
DTT indoor	0.557 ± 0.141	0.551 ± 0.296	0.548 ± 0.339	0.407 ± 0.386

Seasonal differences:

Small differences were noted for the metal based redox activity (DHBA) and redox activities as measured by DTT per m^3 air between seasons at the two communities (Table 9). T-tests results for differences in means in Table 8 between phases within group and between groups within phase showed none were significant. In general, for outdoor samples in San Gabriel Valley group 1 showed slightly higher values of outdoor DTT and DHBA in phase 2 seasons (late fall and winter) compared to phase 1 season (summer and early fall), but the opposite was seen in San Gabriel Valley group 2.

Table 9. Seasonal differences in DHBA and DTT ratios of mean nmoles/min/m^3, year 1 concentrated $PM_{2.5}$.

	Group 1	Group 2
ratios	**Phase 2/ Phase 1**	**Phase 2/ Phase 1**
DHBA out	1.36	0.71
DHBA in	1.04	1.22
DTT out	1.35	0.55
DTT in	0.99	0.74

Outdoor-indoor ratios:

Outdoor DTT and DHBA activity was higher per m^3 air than indoor activity in group 2. In group 1 there was less difference for DTT, and for DHBA there was lower outdoor activity in phase 1 and no difference in phase 2 (Table 10).

Table 10. Ratio of outdoor over indoor redox activities in mean nmoles/min/m^3, year 1 concentrated $PM_{2.5}$.

	Ratio outdoor/indoor			
	G1P1	**G1P2**	**G2P1**	**G2P2**
DHBA	0.79	1.04	2.55	1.48
DTT	1.32	1.12	1.91	1.42

Year 2, Ultrafine PM:

The ultrafine PM samples were subjected to DTT and DHBA assays by UCLA investigators for all year 2 sites (San Gabriel Valley group 3, and Riverside group 4) and two phases at each site. In Appendix A we show raw results for the dithiothreitol (DTT) and dihydroxybenzoic acid (DHBA) assays (Table A3). Here we show overall descriptive statistics here per m^3 of air (Table 11). Indoor DTT and DHBA activity was higher than outdoor DTT (mean ratio = 2.22) and DHBA activity (mean ratio = 3.89). We have no explanation for these results although other metals data (relevant to DHBA activity) from the NIH study shows in some cases higher indoor than outdoor concentrations of Cu and Zn in a few sites and seasons, but not more than an I/O ratio of 1.3 (Polidori et al submitted).

Collections consisted only of ultrafine particles. The samples therefore are distinct from those collected earlier in the project during year 1 in community groups 1 and 2 ($PM_{2.5}$ samples). Samples were collected outdoors and indoors at the same time. The data from each collection period (by

group and phase) were pooled and means and standard deviations determined (Table 12). No problems were encountered in the assays; the values for the standards that are routinely analyzed in each assay were consistent with all previous assays. The assays performed were consistent, based on the control values for each assay.

Table 11. Overall indoor and outdoor DTT and DHBA activity per ultrafine PM mass (nmoles/min/m^3): year 2 concentrated $PM_{0.15}$ data.

In vitro variable	mean ±SD	median	minimum/maximum
DTT indoor	0.158 ± 0.133	0.151	0.000/0.630
DTT outdoor	0.213 ± 0.135	0.216	0.000/0.520
DHBA indoor	0.109 ± 0.245	0.049	0.013/1.214
DHBA outdoor	0.070 ± 0.122	0.035	0.009/0.625

Table 12. Descriptive statistics for DHBA and DTT activity in ultrafine particles, year 2 concentrated $PM_{0.15}$ data by group and phase (mean nmoles/min/m^3 ± SD).

	G3	G3	G4	G4
Variable	**Phase 1**	**Phase 2**	**Phase 1**	**Phase 2**
DHBA outdoor	0.036 ± 0.023	0.068 ± 0.024*	0.021 ± 0.004	0.159 ± 0.230
DTT outdoor	0.127 ± 0.124	0.399 ± 0.114*	0.204 ± 0.044	0.168 ± 0.076**
DHBA indoor	0.039 ± 0.020	0.074 ± 0.020*	0.018 ± 0.004**	0.311 ± 0.457
DTT indoor	0.075 ± 0.125	0.237 ± 0.041*	0.145 ± 0.021	0.201 ± 0.212

* $p < 0.05$ for differences between phase in each group;
** $p < 0.05$ for differences between groups for each phase.

Seasonal differences:

Wide differences were noted for the metal based redox activity (DHBA) between seasons in Riverside group 4, but considerably less for San Gabriel Valley group 3. However, seasonal differences were not significant for group 4 likely due to the wide variance. Seasonal differences were significant for group 3 for both DTT and DHBA with higher activity in phase 2 (Tables 12-13). DTT was significantly higher in phase 2 at group 3 than at group 4. DHBA was significantly higher in phase 1 at group 3 than group 4 ((Table 12). In general, there was higher DTT activity per m^3 air in phase 2 seasons (late fall and winter) compared to phase 1 season (summer and early fall) in San Gabriel Valley group 3 (Tables 12-13), but not Riverside group 4.

Table 13. Seasonal differences in DHBA and DTT ratios of mean nmoles/min/m^3 year 2 concentrated $PM_{0.15}$ data.

	Group 3	Group 4
ratios	Phase 2/ Phase 1	Phase 2/ Phase 1
DHBA out	1.88	7.57
DHBA in	1.89	17.28
DTT out	3.14	0.82
DTT in	3.16	1.39

Outdoor-indoor ratios:

The metal based redox activities (DHBA) appeared to be similar outdoor and indoor in both San Gabriel Valley group 3 and Riverside group 4, except for phase 2 Riverside, which showed lower outdoor levels (Table 14). This is similar to year 1 fine PM data San Gabriel Valley group 1. There were some differences between the indoor and outdoor redox activities as measured by DTT. There was generally higher outdoor to indoor DTT activity per m^3 air, similar to year 1 fine PM data.

Table 14. Ratio of outdoor over indoor redox activities in mean nmoles/min/m^3 year 2 concentrated $PM_{0.15}$ data.

	Ratio outdoor/indoor			
	G3P1	G3P2	G4P1	G4P2
DHBA	0.92	0.92	1.16	0.51
DTT	1.69	1.68	1.41	0.83

GAPDH

A manuscript was published reporting the presence of constituents with electrophilic properties in ambient particles at the Riverside retirement community collected in this study and diesel exhaust particles collected under separate funding (Shinyashiki et al. 2008). The results of the GAPDH inactivation for our Riverside samples are shown in Table 15. The results show that the samples exhibited the ability to inactivate GAPDH under anaerobic conditions by a process that was blocked by allowing the suspension to react first with DTT at 1 mM. Table 15 summarizes the results and the properties of the individual samples used. Samples for September 14 and October 12, 2006 were particularly active when the results are normalized to mass or to volume of air sampled. However, inactivation per unit mass varied considerably, likely due to the varying chemical composition of PM in that site.

The study showed the presence of electrophilic substances in suspensions of ambient air particles that irreversibly inactivated GAPDH under anaerobic conditions by covalent bond formation. Although the assay does not identify the specific chemical species responsible for the inhibition of GAPDH, it does provide information on the potential for atmospheric particles and semi-volatile components to deliver electrophiles to target organs. Based on these preliminary results, an assay was developed that would allow multiple samples to be tested under conditions that compare electrophilic properties against a NEM standard, thereby providing the ability to compare sample electrophile content in a quantitative manner.

Table 15. Inhibition of GAPDH by suspensions of ambient air particles in Riverside, CA.

Sample date	% inhibition /aliquot [a]	PM concentration (µg/mL)	% inhibition/µg [b]	Vol air sampled (m^3)	% inhibition/m^3 [c]
Aug 31	14.6 ± 1.71	133.3	1.09 ± 0.128	4.8	228 ± 26.7
Sep 14	27.0 ± 1.62	32.08	8.41 ± 0.506	4.8	1080 ± 65.0
Sep 21	16.6 ± 2.44	41.44	4.00 ± 0.590	4.4	629 ± 92.8
Sep 28	21.7 ± 2.00	103.2	2.10 ± 0.194	5.5	375 ± 34.5
Oct 05	10.8 ± 0.603	34.82	3.11 ± 0.173	4.2	578 ±32.2
Oct 12	13.2 ± 1.20	19.81	6.66 ± 0.604	5.0	826 ± 74.9

Data are shown as % inhibition compared with control (mean ± SE, n = 3). Values were normalized to volume (100 µL) of aliquot added to incubation (a), mass of particles (b) and equivalent of original air volume (c).

6. TASK 5. Measure concurrently hourly indoor and outdoor criteria pollutant gases [NO_2, O_3 (outdoor only), and CO] and outdoor hourly $PM_{2.5}$.

6.1. Materials and Methods

We decreased the potential for exposure error from the use of central regional data by collecting hourly indoor and outdoor home samples of O_3, NO/NO_2, and CO. CARB staff also collected outdoor hourly $PM_{2.5}$. This required equipment that CARB provided, including equipment in their trailer. We expected variable magnitudes of correlation between indoor and outdoor concentrations depending on the pollutant, air exchange rates and other factors. Our main purpose here is to provide data for the regression analysis of health outcomes rather than to model personal exposures. In other words, indoor and outdoor gas exposures were assessed separately in order to better understand their relevance to health effects of PM.

Methods of sampling for Tasks 3-5 were adapted from the QAPP for the SCPCS, available to view at www.epa.gov/ttn/amtic/files/ambient/super/laqapp.pdf. The FACES study SOPs for the trailer was adapted to SCPCS and UCI SOPs where needed.

Briefly, criteria pollutant gases were be monitored continuously using UV photometry for O_3, gas phase chemiluminesence for NO_2, and non-dispersive infrared spectrophotometry for CO. A zero air/span gas generator was in the trailer for instrument calibration. Standard federal reference methods were used to measure criteria gas pollutants (US EPA 2004). Continuous (1-min) NO and NO_2 measurements were obtained at a location in the immediate environment outside each retirement community and collocated with particle samplers. We deployed two parallel samplers for quality assurance and to assure a complete set of data. We used Thermo Environmental NO_x Analyzers (Model 42, Thermo Environmental instruments Inc, Franklin, MA). Dasibi Carbon Monoxide Analyzers (Model 3008, Dasibi Environmental Corp, Glendale, CA) were implemented to measure continuous (1-min) CO levels. Continuous (1-min) outdoor ozone (O_3) concentrations were also monitored at each community by using API Ozone Analyzers (Model 400A, Teledyne Technologies Inc, Los Angeles, CA).

Continuous $PM_{2.5}$ concentrations were measured with two parallel Beta Attenuation Monitors (BAM, Model 1020, Met One instruments, Inc., OR) at each indoor and outdoor location. BAMs were preceded by a 2.5 µm cutpoint impactor. The PM data was 1-hr averaged. The use of two parallel sampler ensured the availablility of continuous data if one instrument drifted or stopped functioning. This was not the case for particle mass and particle number (described below), or EC-OC measurements (described above).

Additional equipment in the trailer were deployed in the study by ARB staff and included the following:

Outdoor black carbon was measured continuously with two parallel Aethalometers (2 channel) [Model AE-21 (UV + BC), Thermo Andersen, Smyrna, GA], which measures the absorption of single-wavelength light through a filter collecting airborne particles (Hansen, 1984). Carbon concentrations from the outdoor Aethalometer were compared to the Sunset Labs carbon analyzer, and other continuous PM and gas measurements.

A weather station for temperature, RH, wind direction and speed.

A station manager/chart recorder.

The following describes measurements made under NIEHS funding:

Size-fractionated Particle mass:

Integrated (24-h) size segregated outdoor particulate matter (PM) samples were collected at all sites by means of Sioutas™ Personal Cascade Impactors (SKC Inc, Eighty Four, PA) (Misra et al. 2002; Singh et al. 2003) from Monday to Friday. This device, described and validated elsewhere Majestic et al. 2006; 2008; Misra et al. 2002; Singh et al. 2003), employs 2 impaction stages and an after-filter. Coarse mode PM (PM > 2.5 μm, $PM_{2.5\text{-}10}$), accumulation mode PM (PM 0.25-2.5 μm, $PM_{0.25\text{-}2.5}$), and quasi-ultrafine mode PM (PM<0.25 μm, $PM_{0.25}$) were sampled on Zefluor filters (3 μm pore-size, Pall Life Sciences, Ann Arbor MI). Misra et al. (2002) describe the performance of the PCIS in greater detail. Briefly, the PCIS sampler was operated at a flow rate of 9 L/min using a diaphragm pump (DOA-P701-AA, Brenner-Fiedler & Associates, Cerritos CA). The entire impactor sampler is enclosed in a cassette holder, 4 cm in diameter and 6 cm high, made of soft aluminum in order to avoid particle losses due to electrostatic deposition.

Outdoor particle mass concentrations of quasi-ultrafine, accumulation and coarse fractions were determined by weighing the Zefluor substrates collected with the Sioutas Impactor. Gravimetric weights were taken before and after each sample collection using a Mettler 5 Microbalance (Mettler-Toledo, Columbus, OH; weight uncertainty ± 2 μg) under controlled conditions (RH, 40-45% and temperature 22-24 °C) in the facilities of the Aerosol Laboratory at USC. Filters were weighed after a 24-hour equilibration period. Laboratory and field blanks were used for quality assurance.

Total particle number concentration:

A Condensation Particle Counter (CPC Model 3022, TSI Inc, Shoreview, MN), was used for number-based concentrations of particles measured near-continuously (i.e., every 15 minutes) and averaged over one hour. The CPC takes advantage of the principal that supersaturated vapor condenses on small particles. Droplets formed in the instrument pass through a photodetector, which sends digital signal data to the microprocessor. The CPCs were co-located with the other PM samplers described above. CPC and Sioutas samplers were purchased in the NIEHS-funded study and run by USC staff.

6.2. Results and Discussion

We describe here exposure measurements made for Task 5, but also Task 3 (EC-OC) and the additional measurements made under NIEHS funding (PN and size-fractionated particle mass) in order to present a complete set of results for the air pollutants.

We show exposure concentrations in the two phases (seasons) of study in Table 16. More detailed results for every group and phase are shown in Appendix Tables A4-A7. Concentrations were generally similar across the two phases, except for higher concentrations of OC_{pri}, PN and NO_x in phase 2 (colder phase), and higher concentrations of SOC and O_3 in phase 1 (warmer phase) (Table 16). This is important since it points to potential differences in the concentration of pollutant components across seasons (although these periods are not entirely distinct from each other in that in each of two study years the first phase (warmer season) of the second studied community preceded the second phase (cooler season) of the first studied community by about a week) (Table 1).

High outdoor $PM_{0.25}$ relative to $PM_{2.5}$ are likely attributable to a large impact of local traffic in the LA basin as compared with $PM_{2.5}$ in the eastern half of the nation with much larger contributions from accumulation mode sulfate aerosols. It is also important that a potentially substantial part of the $PM_{0.25}$ mass is likely to be from accumulation mode particles since as discussed, is not purely an ultrafine measurement.

The average outdoor BAM $PM_{2.5}$ concentration across communities and seasonal phases was 22.3 ± 12.5 μg/m^3, and for indoor BAM $PM_{2.5}$ it was 13.1 ± 7.7 μg/m^3. This can be compared with an ambient annual air quality standard of 12 μg/m^3 for the State standard and 15 μg/m^3 for the federal standard, and to the federal 24-hour average of 35 μg/m^3. A total of 46 days (24-hr average concentrations) were > 35 μg/m^3 for outdoor $PM_{2.5}$ (13% of 342 monitored days) and only 4 days (1%) for indoor $PM_{2.5}$.

Mean outdoor BAM $PM_{2.5}$ levels were higher than the corresponding indoor concentrations across all sites and phases of the study (Table 16 and Appendix B). This suggests that the overall loss of outdoor particles during penetration through the building envelope was higher than the particle generation from indoor sources, which were likely not substantial in the buildings monitored.

The resulting outdoor $PM_{2.5}$ concentrations from adding the two gravimetric size fractions shown ($PM_{0.25}$ + $PM_{0.25\text{-}2.5}$, Table 16) were similar to BAM $PM_{2.5}$ concentrations during both phases of the study.

The San Gabriel Valley sites (Groups 1-3) were closer to freeways than the Riverside site (Group 4) and were impacted by higher levels of CO, NO_2 and NO_x, which are mainly emitted from primary combustion sources such as motor-vehicle emissions (Appendix B). On the other hand, OC and O_3 levels were generally higher in Riverside (except O_3 in Group 3, phase 1). The Riverside site was approximately 110 Km east (and downwind) of downtown Los Angeles, with prevailing easterly winds blowing from the Pacific Ocean. Those winds carry a plume of pollutants generated in the Los Angeles area that includes several reactive organic species likely to form OC through secondary processes (i.e. SOA formation) as the air mass ages and is transported eastwards. Our data confirm that Riverside is a typical receptor area where the contribution of SOA to total measured OC is substantial. This is evidenced by the higher average OC, O_3 and SOA levels (Appendix B) and by the smaller diurnal SOA variation in Riverside as compared to the afternoon increase in both O_3 and SOA in the San Gabriel Valley (section 4.2.1.). In addition, the vegetation surrounding the Riverside community is a potential source of biogenic gas-phase precursors, which form secondary organic aerosols through photochemical reactions (e.g., photochemical oxidation of terpenes) (Kanakidou et al. 2005).

Table 17 shows Spearman rank correlation coefficients for air pollutants for combined phases. EC, BC, OC_{pri}, NO_x, and CO were strongly correlated with each other, likely because they are mostly products of fossil fuel combustion. These correlations were stronger in phase 2 than in phase 1 (not shown). PN and $PM_{0.25}$ concentrations were moderately correlated with these pollutants, and these correlations were stronger in the three San Gabriel Valley communities than Riverside (not shown). This is consistent with the concentration differences in that compared with Riverside, the three San Gabriel Valley communities were closer to freeways that generate high concentrations of PN and $PM_{0.25}$ as discussed above. The finding of a stronger correlation between $PM_{0.25}$ and $PM_{2.5\text{-}10}$ (coarse particles) than between $PM_{0.25}$ and $PM_{0.25\text{-}2.5}$ is because $PM_{0.25}$ and coarse particles come from primary traffic sources in our study region. While $PM_{0.25}$ is primarily a product of fresh emissions, $PM_{0.25\text{-}2.5}$ is primarily a product of ageing and photochemical reactions.

Table 16. Descriptive statistics of air pollutant measurements.

Exposure (24-hr averages)	Phase 1				Phase 2				
	N (missing)	Mean (SD)	IQR	Min/Max	N (missing)	Mean (SD)	IQR	Min/Max	IQR overall[a]
Outdoor hourly PM									
EC ($\mu g/m^3$)	161 (19)	1.45 (0.52)	0.706	0.53 / 3.01	139 (34)	1.55 (0.71)	1.08	0.24 / 3.94	0.87
OC ($\mu g/m^3$)	164 (16)	7.90 (4.65)	4.43	2.32 / 27.26	141 (32)	9.25 (4.33)	7.62	2.51 / 17.72	7.59
BC ($\mu g/m^3$)	180 (0)	1.59 (0.63)	0.86	0.38 / 3.37	172 (1)	1.76 (0.91)	1.24	0.30 / 5.11	1.03
OC_{pri} ($\mu g/m^3$)	161 (19)	4.36 (2.14)	2.91	1.28 / 10.04	139 (34)	6.03 (3.53)	6.25	0.99 / 13.64	4.04
SOC ($\mu g/m^3$)	161 (19)	3.48 (3.40)	2.03	0.28 / 18.74	139 (34)	3.12 (1.62)	2.50	0.00 / 6.91	2.42
PN (particle no./cm^3)	133 (47)	10242.7 (4438.3)	6525.6	1441.4 / 24302.4	152 (21)	14851.3 (6490.0)	8631.1	3296.8 / 31263.9	7354.3
BAM $PM_{2.5}$ ($\mu g/m^3$)	180 (0)	23.96 (8.36)	12.10	5.41/ 47.40	172 (1)	20.56 (15.58)	17.78	2.46/ 89.33	16.05
Indoor hourly PM									
EC uncharacterized ($\mu g/m^3$)	154 (26)	1.31 (0.44)	0.619	0.33/ 2.77	141 (32)	1.24 (0.49)	0.61	0.19/ 2.89	0.675
EC, outdoor origin ($\mu g/m^3$)	146 (34)	0.97 (0.32)	0.441	0.41/ 1.81	128 (45)	1.12 (0.45)	0.572	0.29/ 2.97	0.504
OC uncharacterized ($\mu g/m^3$)	157 (23)	6.11 (1.84)	2.12	2.37/ 11.23	143 (30)	9.06 (4.36)	7.35	2.34/ 18.10	5.22
OC_{pri}, outdoor origin ($\mu g/m^3$)	150 (30)	2.27 (1.21)	1.37	0.00/ 5.62	137 (36)	4.34 (3.33)	4.97	0.32/ 12.30	2.11
SOC, outdoor origin ($\mu g/m^3$)	150 (30)	2.44 (1.81)	1.46	0.24/ 11.11	137 (36)	2.60 (1.40)	2.04	0.00/ 5.92	1.75
PN uncharacterized (/cm^3)	144 (36)	7559.9 (6107.8)	7128.4	681.8 / 32507.2	157 (16)	10656.7 (7460.8)	9845.4	2763.7/ 43027.0	8348.8
PN outdoor origin (/cm^3)	111 (69)	5371.5 (3143.4)	5390.6	598.0/ 12092.8	140 (33)	7826.8 (4394.8)	7233.0	1419.7/ 17700.4	6406.8
BAM $PM_{2.5}$ ($\mu g/m^3$)	180 (0)	13.57 (5.97)	9.06	3.30/ 28.00	157 (16)	12.49 (9.25)	9.62	2.06/ 55.92	10.14

Table 16. (cont)

Exposure (24-hr averages)	Phase 1				Phase 2				
	N (missing)	Mean (SD)	IQR	Min/Max	N (missing)	Mean (SD)	IQR	Min/Max	IQR overall
Outdoor PM mass									
$PM_{0.25}$ ($\mu g/m^3$)	111 (9)	10.27 (3.69)	4.79	3.16/ 22.82	106 (7)	9.25 (4.48)	5.60	2.46 / 30.05	7.00
$PM_{0.25-2.5}$ ($\mu g/m^3$)	115 (5)	12.23 (6.39)	9.54	1.64/ 27.78	111 (2)	10.47 (11.70)	9.95	0.98 / 66.77	10.58
$PM_{2.5-10}$ ($\mu g/m^3$)	110 (10)	11.45 (4.65)	5.32	1.15/ 23.41	107 (6)	7.25 (4.39)	5.22	0.30 / 24.63	5.46
Outdoor hourly gases									
NO_2 (ppb)	179 (1)	26.41 (11.97)	19.17	4.52/ 59.83	172 (1)	28.34 (11.80)	17.57	3.78 / 55.74	14.3
NO_x (ppb)	179 (1)	37.17 (22.44)	28.13	3.70/ 112.43	172 (1)	53.86 (36.14)	50.9	4.26 / 188.0	41.6
CO (ppm)	173 (7)	0.50 (0.25)	0.361	0.11/ 1.30	162 (11)	0.58 (0.35)	0.517	0.01 / 1.68	0.509
O_3 (ppb)	179 (1)	33.30 (11.40)	15.52	8.04/ 76.35	170 (3)	20.62 (8.04)	10.8	6.17 / 44.9	16.09

[a] This overall interquartile range was used to estimate the expected change in the biomarker (coefficient and 95% CI) from exposure to the air pollutant.

IQR: interquartile range

OC_{pri}: Primary organic carbon

SOC: Secondary organic carbon

Table 17. Outdoor Exposure Correlation Matrix.[a]

	OC	BC	OC_{pri}	SOC	PN	$PM_{0.25}$	$PM_{0.25-2.5}$	$PM_{2.5-10}$	NO_2	NO_x	CO	O_3
EC	0.61	0.89	0.97	-0.03	0.50	0.54	0.31	0.36	0.80	0.82	0.78	-0.39
OC	1.00	0.63	0.65	0.72	0.27	0.41	0.33	0.33	0.55	0.46	0.59	-0.05
BC		1.00	0.88	0.07	0.40	0.52	0.43	0.44	0.88	0.83	0.79	-0.38
OC_{pri}			1.00	0.01	0.47	0.55	0.33	0.36	0.78	0.79	0.75	-0.36
SOC				1.00	-0.08	0.09	0.16	0.15	0.07	-0.09	0.11	0.26
PN					1.00	0.36	-0.12	0.06	0.48	0.63	0.45	-0.38
$PM_{0.25}$						1.00	0.17	0.35	0.56	0.51	0.54	0.01
$PM_{0.25-2.5}$							1.00	0.60	0.19	0.01	0.13	0.08
$PM_{2.5-10}$								1.00	0.32	0.18	0.26	0.06
NO_2									1.00	0.88	0.79	-0.42
NO_x										1.00	0.82	-0.53
CO											1.00	-0.29

EC elemental carbon; OC: organic carbon; BC: black carbon; SOC: secondary organic carbon; PN: particle number; OC_{pri}: primary OC; PM: particulate matter.

[a] all exposures are mean centered by group and phase (see Supplemental Material).

7. TASK 6. Analyze the relationship of cardiovascular outcomes to the production of ROS by PM using *in vitro* bioassays of concentrated particle suspensions collected at indoor and outdoor sites.

7.1. Materials and Methods

Statistical analysis methods and model selection procedures are the same as described below for the main analysis of biomarkers and air pollution. Briefly, linear mixed effects models were used to analyze relationships of biomarkers in 60 subjects to *in vitro* bioassay activity for PM (Verbeke and Molenberghs 2001), adjusted for between-subject group and between-phase exposure effects, and temperature at the same averaging time as the air pollutant. We excluded person-weeks with acute infectious illnesses given their known impact on measured biomarkers. We analyzed biomarkers that were informative in the main analysis described below, namely, IL-6, TNF-RII, Cu,ZnSOD and GPx-1, representing both systemic inflammation and erythrocyte antioxidant activity.

7.2. Results and Discussion

There were almost no significant associations between biomarkers of effect and either DTT or DHBA

measured using fine or ultrafine PM (Table 18). Only one significant inverse association between Cu,Zn-SOD and DHBA activity in year 1 fine PM was found (as with air pollutant exposures described below). However, this is no more than expected by chance.

The null results are most likely due to the limited sampling periods from around 9:00-14:00 hours on the Thursdays and Fridays before the Friday blood draws. As discussed below, there were also no clear associations between biomarkers and the air pollutant exposures measured in the 8 hours preceding the blood draw (similar time frame to Friday VACES samples). This is in contrast to the numerous significant associations found for longer-term multi-day average air pollutant exposures. Other limitations include the restriction of each analysis of fine and ultrafine PM to half of the study population, which limits statistical power. However, we published preliminary results for year 1 alone showing many significant associations between biomarkers and multi-day average air pollutant exposures, but not fine mass (Delfino et al. 2008). In addition, there may have been unknown problems in sample quality or in some assays since there were many samples with 0 activities for DTT. The reasons for this are unknown.

Table 18. Associations of circulating biomarkers with *in vitro* DTT and DHBA activity of concentrated fine and ultrafine PM.[a]

***In vitro* assay**	**IL-6 (pg/mL)**	**sP-selectin (ng/mL)**	**TNF-RII (pg/mL)**	**SOD (U/g Hb)**	**GPx-1 (U/g Hb)**
Fine PM (Yr 1)[b]					
DTT indoor	0.05 (-0.48, 0.58)	1.35 (-1.48, 4.18)	90 (-138, 319)	-221 (-547, 104)	-0.73 (-1.91, 0.44)
DTT outdoor	-0.28 (-0.71, 0.15)	0.90 (-1.42, 3.22)	95 (-98, 289)	-271 (-548, 7)	-0.53 (-1.53, 0.47)
DHBA indoor	-0.23(-1.08, 0.62)	2.85 (-1.75, 7.45)	178 (-231, 589)	-691 (-1286, -96)*	-1.16 (-3.32, 1.00)
DHBA outdoor	-0.34 (-0.98, 0.29)	0.28 (-3.16, 3.73)	145 (-164, 455)	-315 (-743, 113)	-0.77 (-2.36, 0.83)
Ultrafine PM (Yr 2)[b]					
DTT indoor	-0.32 (-0.91, 0.27)	-8.18 (-23.1, 6.76)	-177 (-564, 211)	376 (-351, 1103)	1.36 (-3.70, 6.43)
DTT outdoor	-0.32 (-0.98, 0.33)	-3.27 (-19.2, 12.7)	-206 (-609, 197)	571 (-210, 1352)	-1.53 (-6.63, 3.56)
DHBA indoor	-0.17 (-0.49, 0.15)	-5.62 (-13.7, 2.43)	1 (-206, 208)	260 (-130, 650)	1.95 (-0.84, 4.75)
DHBA outdoor[c]	-0.34 (-1.01. 0.33)	-2.22 (-18.4, 14.0)	-212 (-628, 204)	745 (-56, 1546)	-1.10 (-6.36, 4.16)

* $p < 0.05$

[a] Regression coefficients (95% confidence intervals) are for the expected change in the biomarker associated with a one nmoles/min/µg change in DTT and DHBA activity.

[b] There were 29 subjects in year 1 and 31 subjects in year 2.

[c] Models do not include the extreme DHBA outlier = 0.625 nmoles/m^3/min.

8. TASK 7 AND 8. Task 7 and 8. Analyze the relationship of cardiovascular outcomes to hourly indoor and outdoor EC-OC concentrations (Task 7) and to hourly indoor and outdoor criteria pollutant gases and particulate air pollutants (Task 8)

8.1. Materials and Methods

We present methods for Tasks 7 and 8 together because results will be presented together and methods of analysis are the same or similar.

8.1.1 Population and Design

Subjects were recruited from four retirement communities in the Los Angeles air basin. Eligibility criteria included age ≥65 years old, a history of CAD, nonsmoker, and unexposed to environmental tobacco smoke. We confirmed CAD diagnoses with a review of subject medical records and reports from treating cardiologists. Study cardiologists and nurses clinically evaluated 105 potentially eligible subjects on site in UC Irvine's Mobile Medicine Clinic. Twenty-one subjects were not eligible and 20 dropped out or participated in < 5 of 12 expected weeks. For the analysis of blood biomarkers, four other subjects had insufficient biomarker data mostly due to exclusions for frequent infections, leaving 60 subjects ages 71 years or older with 5-12 weekly blood draws (N = 578) (Table 19). For ABPM analysis, 18 dropped out and two subjects had an insufficient number of ABPM hours (< 28 out of 140 maximum expected hours) leaving 64 subjects with 6,539 total ABPM hours and a subject average of 103 ± 44 hours (Table 20). For the analysis of ST segment depression using Holter data, 35 out of 64 subjects participating in the Holter study had informative data (presence of any ST segment depression) for use in the analysis (Table 21). There were 328 24-hr ambulatory Holter ECG records for the 35 subjects.

Two communities were studied in 2005-2006 and two communities were studied in 2006-2007 (see Table 1 above). We studied subjects in two periods to enhance known contrasts across the LA basin in particle composition and size distribution by season (Sioutas et al. 2005). In each community, we collected six weeks of data during a period of higher temperature (Jul–mid Oct), and thus higher photochemical activity and mixing depths, and six weeks of data during a cooler period (mid Oct–Feb), with more frequent periods of air stagnation and lower mixing heights (when traffic-related primary air pollutants increase at ground level). This was intended to test differences in association potentially due to differences in pollutant concentrations, or particle size distribution and composition. Over a seven month period, each subject was followed weekly in these two 6-week blocks with blood draws for circulating biomarkers of inflammation and antioxidant activity. Subjects were studied with ambulatory monitoring (ABPM and Holter) in two periods of five consecutive days during each of the two seasonal periods. Ambulatory monitoring started Monday morning and ended Friday late afternoon or early evening. Daily home visits by a research assistant took place for downloads of electronic data, including ABPM, Holter, actigraphs, and personal digital assistant (PDA) diaries.

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

Table 19. Characteristics of subjects in biomarker study (N=60).

Characteristic	Mean ±SD or N (%)
Age (years)	84.1 ± 5.60
Gender	34 (56.7%) Males, 26 (43.3%) Females
Cardiovascular History	
Confirmation of CAD:[a]	
-Myocardial infarction	27 (45.0%)
-Coronary artery bypass graft or angioplasty	20 (33.3%)
-Positive angiogram or stress test	10 (16.7%)
-Clinical diagnosis[b]	3 (5.0%)
Current angina pectoris	18 (30.0%)
Congestive heart failure	13 (21.7%)
History of Hypertension	42 (70.0%)
Hypercholesterolemia (by history)	43 (71.7%)
Other Medical History:	
Type II Diabetes	8 (13.3%)
COPD or Asthma	9 (15.0%)
Stroke or transient ischemic attack	8 (13.3%)
Medications:	
ACE inhibitors and Angiotensin II receptor antagonists	24 (40.0%)
HMG-CoA reductase inhibitors (statins)	31 (51.7%)
Clopidogrel bisulfate (Plavix)c	21 (35.0%)

[a] Each category is hierarchical and excludes being in the above diagnostic category.
[b] includes subjects with anginal symptoms relieved with nitrates plus echocardiogram and ECG evidence of past infarct.
[c] Eight were also taking Coumadin.

Table 20. Characteristics of subjects in ABPM study (N=63).

Characteristic	Mean ±SD or N (%)
Age (years)	83.8 ± 5.66
Gender	38 (60.3%) Males, 25 (39.7%) Females
History of Hypertension	44 (69.8%)
Current hypertension status (SBP/DBP)*	
Normal (<120/80)	41 (65.1%)
Prehypertension (120-139/80-89)	11 (17.5%)
Hypertension (≥140/90)	11 17.5(%)
Antihypertensive medications:	54 (85.7%)
Beta-receptor blocking medications,	37 (58.7%)
Antiadrenergic agents	6 (9.5%)
CA channel blockers	20 (31.8%)
ACE inhibitors and Angiotensin II receptor antagonists	32 (50.8%)
HMG-CoA reductase inhibitors (statins)	41 (65.1%)

* From ambulatory average.

Table 21. Characteristics of subjects in Holter study of ST segment depression (N=35).

Characteristic	Mean ±SD or N (%)
Age (years)	84.2 ± 5.6
Gender	16 (45.7%) Males, 19 (54.3%) Females
Cardiovascular History	
Confirmation of CAD:[a]	
-Myocardial infarction	18 (51.4%)
-Coronary artery bypass graft or angioplasty	7 (20 %)
-Positive angiogram or stress test	8 (22.9 %)
-Clinical diagnosis[b]	2 (5.7 %)
Current angina pectoris	9 (25.7%)
Congestive heart failure	10 (28.6%)
History of Hypertension	26 (74.3%)

[a] Each category is hierarchical and excludes being in the above diagnostic category.
[b] includes subjects with anginal symptoms relieved with nitrates plus echocardiogram and ECG evidence of past infarct.

8.1.2 Outcome measurements

Blood biomarkers measurements

We drew venous peripheral blood samples at the same time of day and day of week to control for circadian rhythm and day-of-week effects. We used chilled anti-coagulant Vacutainer tubes [ethylenediaminetetraacetic acid (EDTA) and citrate theophylline adenosine dipyridamole (CTAD) tubes]. Blood was rapidly separated (< 30 min after blood draw) into erythrocytes and plasma by using an on-site mobile field laboratory to minimize *ex vivo* changes in biomarkers. After centrifugation, each fraction was aliquoted, coded, transported frozen on dry ice from the field to our laboratory, and stored at −80°C until tested. In the analysis of the two years of panel data presented here and published elsewhere (Delfino et al. 2009), we focused on biomarkers that were most informative in the preliminary analysis of the first year of data (Delfino et al. 2008). Plasma samples stored at –80°C were thawed and assayed using 96-well immunoassay kits for the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor-α (IL-6 and TNF-α; Quantikine HS, R&D Systems, Minneapolis, MN), soluble TNF-α receptor II (sTNF-RII; Quantikine, R&D Systems), the acute phase protein, C-reactive protein (CRP; Zymutest, Hyphen BioMed, Neuville-sur-Oise, France), and a marker of platelet activation, soluble platelet selectin (sP-selectin) (Jurk and Kehrel 2005). Frozen-thawed erythrocyte lysates were assayed spectrophotometrically for activities of two antioxidant enzymes, glutathione peroxidase-1 (GPx-1) and copper, zinc-superoxide dismutase (Cu,Zn-SOD) (Cayman Chemical, Ann Arbor, MI), normalized to units per gram of hemoglobin (U/g Hb).

Ambulatory Blood Pressure (ABPM) and related measurements

Ambulatory monitoring of SBP and DBP was conducted using the Burdick Ultralite ABPM model 90217 (Burdick Inc., Deerfield, WI). It fulfilled criteria for accuracy and performance protocols of the Association for the Advancement of Medical Instrumentation and the British Hypertension Society (BHS) (Baumgart and Kamp 1998; O'Brien et al 1995). It uses the oscillometric method of measuring BP, obviating the need for precise placement over the brachial artery as with ausculatory methods. The cuff inflates to 30 mm Hg above the previous SBP, but ≤285 mm Hg, and then deflates linearly at 3 mm Hg/sec with a measurement cycle limited to 180 sec. It automatically initiates up to 2 additional measurements if a reading fails to satisfy program criteria. A microprocessor program discriminates between pressure signals, patient movement, and respiratory artifact. SBP, DBP, time, and error codes are digitally stored. The device was programmed to measure BP at the top of every waking hour (up to 14 hours possible per day).

We electronically monitored physical activity continuously with an actigraph, the Actigraph Mini-motionlogger (Ambulatory Monitoring, Inc., Ardsley, NY). We placed the actigraph with the ABPM near the waist to better capture whole body movements. We used the unit's high sensitivity proportional integrating measure mode, which measures movement intensity by summing the absolute value of deviations from zero volts each 0.1 sec during a series of one-minute epochs. We determined average movement intensity for 5-min periods before each ABP measurement. Periods the actigraph was not worn were identified and put to missing where there was a sustained lack of motion during daytime hours. Subjects also answered an hourly electronic diary after a preprogrammed alarm prompt that immediately followed the end of ABPM measurements. The diary asked the question: "During the blood pressure measurements, what was your posture?" Answers included: 1) standing or walking; 2) sitting; 3) reclining or laying down; 4) changed position. Subjects were instructed to sit whenever possible during their BP measurements.

Ambulatory ECG ST Segment Depression

We used the Burdick model 92513 Compact Digital Holter Recorder and Scanner/Software System (Burdick Inc., Deerfield, WI). It is 7-lead 3-channel ambulatory (Holter) ECG that has a data

acquisition speed of 200 Hz and 8-bit analog-to-digital resolution. It uses digital acquisition and data storage with a 64 MB flash card, which was switched daily for easy data transfer to the Noninvasive Laboratory technician at the UCI Cardiology Division (NIH-funded coinvestigator Dr. John Longhurst). Each day, the subject removed leads and bathed before a research assistant arrived at the subject's home. The research assistant downloaded ECG data and setup the ECG for a new run. The research assistant re-attached the 7 leads V1 to V6 locations on the epigastrium and one reference electrode, and reset the unit for a new run. The Holter ECG signals (3 channels) were read and analyzed by Burdick Vision Premier Holter Analysis System, which includes algorithms for QRS labeling, artifact identification and data correction. It also includes identification of rate-related abnormalities and analysis of ST changes.

All technical specifications of ambulatory ECG monitoring followed recommendations of the American Heart Association (Knoebel 1989; Sheffield 1985). To ensure uniformly high quality data collection, a cardiology fellow working with Dr John Longhurst and the UCI Noninvasive Laboratory provided training in ambulatory procedures and equipment maintenance. The Burdick ambulatory ECG monitors we used automatically detected and flagged recording errors in their internal memory. Data was then edited by a Holter technician from the UCI Noninvasive Laboratory using Burdick's Vision Premier Holter Analysis System, which has arrhythmia detection algorithms. He inspected the entire file for artifacts and outliers with the assistance of Burdick's error codes and by following set rules. A quality assurance (QA) report identified the type of problems in recordings. When the computer program-generated reports did not correspond to ECG strips, the technician edited and flagged the segment. One of our cardiologists then over read data where there are indications of abnormalities, arrhythmias, ST segment changes and flagged ECG regions.

For analyses of ECG ST-segment changes, only beats classified as normal and not preceded by ectopic beats or prolonged RR intervals are included in the beat averaging (Nygårds and Hulting 1979). ST segment changes were assessed as follows. Reference lines were calculated by the Burdick software from the isoelectric line and 24-hr median of the ST-trend curve as previously described (Quintana et al. 1995). Software identified ST segment depression of ischemic type to be a planar or down sloping shift of $\geq$ 1.0 mm (0.1 mV), occurring 80 msec after the J point and lasting $\geq$ 30 seconds. We also identified ST segment elevation of $\geq$ 1.0 mm (0.1 mV) measured at the J point (J + 0-5 ms) and lasting $\geq$ 30 seconds. However, this was a rare event and therefore, not used in the analysis due to low power. For ST depression, an interval of 2 min was required to be counted as another discrete episode.

8.1.3 Analysis

Linear mixed effects models were used to analyze relationships of biomarkers and blood pressure (BP) to air pollutant exposures (Verbeke and Molenberghs 2001). Because within-individual repeated measures of outcomes are correlated, random effects were estimated at the subject level, nested within phase and community. The response data are correlated because repeated daily measurements in each subject constitute a cluster of dependent observations. The covariance structure observed from empirical variograms was representative of an autoregressive-1 correlation and models were fit as such. We used Akaike's Information Criteria to assess the fit of various models.

Generalized estimating equations (GEE) were used to analyze the marginal association of counts of the number of daily ST segment depression observations with daily air pollution averages. The GEE approach can model nonnormal correlated response data (Zeger and Liang 1986). In this case, the distribution is Poisson. The GEE models were tested using the log link function in the SAS Version 9 generalized linear model procedure Genmod, which uses a ridge-stabilized Newton-Raphson algorithm to maximize the log likelihood function for the regression parameters. We used deviance statistics for GEE models to assess the fit of various models and examined the dispersion parameter (deviance / d.f.) for evidence of overdispersion (variance is greater than the mean). GEE

models for the time varying predictors (air pollutants) were best fit with an autoregressive-1 working correlation matrix. ST segment depression was seen in only 33 of 63 subjects who carried the Holters. Because those without any event of ST depression are noninformative in the GEE models, the remaining 30 subjects were excluded from the analysis.

We decided *a priori* to exclude person-weeks with acute infectious illnesses given their known impact on measured biomarkers and possible temporal trends that could be correlated with pollutant exposures. We controlled all models for temperature at the same averaging time as the air pollutant. For BP, we decided *a priori* to control for hour of the day as a representative of circadian variation in BP since pollutants have a diurnal variation as well and the circadian nature of BP is thus a source of confounding. We decided *a priori* to control for posture (from the electronic diary) given its know influence on BP and potential to differ by time of day or other exposure conditions (e.g., indoor vs. outdoor location). We decided *a priori* to control for physical activity using the last 5-min average of actigraph activity, given that ambulatory BP needs to be clinically interpreted in reference to the concurrent physical activity level (Leary et al. 2000).

Using mean centered exposures, we adjusted for between-subject group and between-phase exposure effects. Thus, the interpretation of reported estimates is at the subject-level. Group indoor and outdoor home exposures were assigned to each subject in each of their two phases of study. Thus, there are three different exposure-outcome relationships that will affect the interpretation of pollutant associations with a subject's biomarker measurements: the between-group effect; the within-group, between-phase effect; and the within-subject, within-phase effect. The between-group effect of exposure is the overall outcome levels associated with differences in the air pollutants across groups. This is potentially confounded by time-independent group characteristics, such as the cultural practices, diet, or health-related activities in the retirement community that could affect biomarkers. The within-group, between-phase effect of exposures effect is the overall outcome levels associated with differences in the air pollutants across phases for the same group. Because the phases are at different periods, this exposure effect may be confounded by other unmeasured seasonal factors. The within-phase, within-subject effect of exposure is the parameter of interest. This is the association of overall outcome levels with differences in the air pollutants across weekly measurements of biomarkers or hourly to daily measurements of ambulatory outcomes in the same phase for the same subject.

The following mixed model was tested as proposed by Janes et al. (2008): Let the index *i* indicate the retirement community (*i* = 1,2,3,4), *j* indicate season (phase) within year 1 and 2 (*j* = 1,2,3,4) nested within community, *k* indicate subject (*k* = 1,…,60) within community, and *t* indicate the weekly biomarker measurement (*t* = 1,…,12). Then a given biomarker measurement, $Y_{i,j,k,t}$ will be related to the following three different exposure-outcome relationships:

$\overline{X}_{ik}$ is the between-group (*bg*) component, which is the average exposure for group *i* assigned to each subject *k*, and

$\overline{X}_{ijk} - \overline{X}_{ik}$ is the within-group, between-phase (*wgbp*) component for subject *k* in group *i*, or the average exposure in phase *j* minus the overall average exposure.

We're still most interested in associations for within-phase exposures assigned to each subject: $X_{ijkt} - \overline{X}_{ijk}$ is the within-subject, within-phase (*wswp*) component, which is the assigned exposure at biomarker measurement time *t* for subject *k* minus the average exposure for the phase.

The mixed model is then:

$$Y_{i,j,k,t} = a_{i,j,t} + \alpha Z_{i,j,t} + \beta_{bg}\overline{X}_{ik} + \beta_{wgbp}\left(\overline{X}_{ijk} - \overline{X}_{ik}\right) + \beta_{wswp}\left(X_{ijkt} - \overline{X}_{ijk}\right) + \varepsilon_{i,j,k,t}$$

Where $a_{i,j,k}$ is the random subject intercept nested in group and phase, $Z_{i,j,k}$ is a vector of subject characteristics specifying such covariates as medication use, and $\varepsilon_{i,j,k,t}$ denotes random within-person error in the biomarker measurement. An analogous model can be written for the GEE analysis of ST segment depression. For both BP and ST data, the mean centering method is analogous, except that a subject's "group" and "phase" is defined by the 5-day ambulatory monitoring period rather than the whole 6-week study phase as with the biomarkers. Phase is instead referred to as session.

For exposure variables, we assessed more acute vs. cumulative exposure-response relationships by testing the last 4- and 8-hourly moving averages and the last 24-hr averages of air pollutants (lag 0) as well as cumulative exposures up to 9 days before the outcome measurements (or five days before the blood draw for particle mass and circulating biomarkers only). For circulating biomarkers, we chose a set of daily averaging times that skipped over averages by one day to simplify the presentation while still presenting a view of associations across the span of averaging times (lag 0, 3-day, 5-day, 7-day and 9-day averages). Similarly, for hourly blood pressure, we present daily averaging times of lag 0-, 2-, 5-, and 9-days. For ambulatory ECG data we did not include the same day exposure (lag 0 day, 24-hr average) since the ST segment depression could have occurred anytime during that day. Thus, exposure hours included in lag 0 could have been measured somewhat or mostly in the future. We present results of the Poisson regression models of ST segment depression for lag 1 day and multiday averages that include lag 1 day plus additional daily 24-hr lags (e.g., 2-day average is the average of lag 1 day and lag 2 day averages).

We assessed associations for size-fractionated PM in relation to circulating biomarkers (measured at one fixed time point per week at the end of 5 days of gravimetric sampling). This is because the sampling time frame for the ambulatory data (same 5 days as the PCIS) was inadequate to assess lag effects (no weekend samples were collected) and inadequate to match daily fixed sampling times to the real-time nature of both the ambulatory ECG and ambulatory blood pressure data. Therefore, for ambulatory data we only assessed associations with the continuous exposures. For PM, this meant we were restricted to hourly BAM $PM_{2.5}$.

Many associations were modified by a variety of factors such as medications as hypothesized in the NIEHS-funded and EPA-funded projects. These are briefly presented here as they were not proposed, and are more thoroughly discussed in our publications (Delfino et al. 2008; Delfino et al. 2009). We anticipated differences in association by seasonal phase of study, which is why the study was designed as described above. There were also anticipated to be differences in association by region of study defined by transported vs. local traffic sources. All interactions between air pollutants and potential effect modifiers (medications, phase and group) were tested in models with product terms of the pollutant by modifiers. All stratified results come from these product term models, which includes data for all strata.

Residual diagnostics were examined to investigate deviations from standard linear mixed model assumptions (functional form of independent variables and covariance assumptions) and the presence of influential observations. Residuals for CRP exhibited a highly skewed distribution that was primarily due to a cluster of subjects in the upper quartile with high CRP and two subjects with all measurements below the limit of detection (250 ng/mL). Based upon residual diagnostics, secondary subgroup analyses were conducted among subjects in the upper quartile of mean CRP. While clearly data-driven, similar subgroup effects have also been previously reported elsewhere (Dubowsky et al. 2006; Ruckerl et al. 2006). In an exemplary model for 3-day average $PM_{0.25}$ the skewness was 3.3 including all subjects. The distributional properties of residuals improved when analyses of CRP were stratified by subjects with mean biomarker concentrations in the upper quartile (skewness 1.5) and in the lower three quartiles without the two subjects with constant values and with 6 high outliers reset to next lowest value (skewness 1.7).

Residuals for TNF-α exhibited a modestly skewed distribution (2.2) primarily attributable to three outliers >10 pg/mL (>3 SD above the mean), that improved when outliers were reset to 10 pg/mL (skewness 1.7). Like CRP, we also found that associations were restricted to subjects in the upper

distributions of mean TNF-α. Distributional properties of residuals further improved when analyses of TNF-α were stratified by subjects with mean biomarker concentrations in the upper quartile and in the lower three quartiles. Therefore, mixed models analyses for both CRP and TNF-α were stratified as such to show differential risk by chronic inflammation and to express results for both variables in their measured units.

Residuals for IL-6 exhibited a modestly skewed distribution in an exemplary model for 5-day average EC (skewness 2.6) that was largely due to 4 high outliers > 10 pg/mL. We adjusted values for these observations to equal 10 and skewness of residuals improved (1.5). SP-selectin showed one high outlier (221 ng/mL) that was highly influential leading to stronger associations. It was removed to obtain more representative estimates of association.

To identify influential subject clusters, we tested random slopes models as well as individual autoregressive models. Through this exploratory data analysis, we identified subjects who had potentially high influence. Five subjects formed a highly influential cluster with positive associations between air pollutants and erythrocyte Cu,Zn-SOD, and three subjects formed a highly influential cluster with positive associations between air pollutants and erythrocyte GPx-1. Elsewhere, we present results for the combined group (60 subjects) as well models with the 5 subjects for Cu,Zn-SOD and models with the 3 subjects for GPx-1. In the primary analysis presented here we exclude these highly influential subjects. As these interesting results stem from a sensitivity analysis, the reported results should be interpreted conservatively and viewed as exploratory analyses requiring confirmation in similarly designed studies or experiments.

8.2. Results and Discussion

We present results for tasks 7 and 8 together because they represent a more comprehensive and integrated view of potentially important pollutant components. We add here analysis of the other exposures collected under funding from NIH, NIEHS again to present a more comprehensive and integrated view. Because effects of outdoor air pollutants were of interest in Tasks 7-8, not indoor source pollutants, we focus on outdoor home concentrations of the gases and other particle measurements because we have not estimated indoor exposure of outdoor origin for these exposures. The indoor data we did use is that data where we were able to estimate indoor concentrations of outdoor origin (EC, OC_{pri}, SOC, and PN) from our recent work (Polidori et al. 2007). In general, associations with all indoor PM and gas exposures were weaker than assocations with outdoor exposures to the same pollutants. These results are present in Delfino et al. (2009).

8.2.1 Biomarkers

Descriptive data for biomarker measurements are shown in Table 22.

Table 22. Biomarker concentrations (578 measurements).

Biomarker[a]	Mean±SD	Median (min/max)
IL-6 (pg/mL)	2.42 ± 1.85	1.92 (0.25/10.0)
TNF-α (pg/mL)[b]	1.92 ± 1.64	1.41 (0.5/14.3)
sTNF-RII (pg/mL)	3610 ± 1489	3235 (657/11584)
sP-selectin (ng/mL)	45.0 ± 16.6	42.5 (6.0/119.9)
CRP (ng/mL)[c]	2434 ± 3181	1403 (250/26799)
Cu,Zn-SOD (U/g Hb)	4459 ± 1688	4288 (669/13138)
GPx-1 (U/g Hb)	19.2 ± 7.6	17.8 (5.5/50.7)

[a] Excludes observations for weeks when there was a reported infection.

[b] Values of TNF-α < 0.5 could not be quantified and were set to 0.5
[c] Values of CRP < 250 could not be quantified and were set to 250.

Overview: Many positive associations were found for IL-6, sP-selectin, sTNF RII, TNF-α, and CRP, with markers of traffic-related air pollution, including EC, OC_{pri}, BC, CO, and NO_x, as well as PN. Associations for NO_x were stronger and with tighter confidence intervals than NO_2, therefore, results are presented only for NO_x. All biomarkers of systemic inflammation except sP-selectin and TNF-α were more strongly and significantly associated with $PM_{0.25}$ than larger size fractions. We also found inverse associations of Cu,Zn-SOD and GPx-1 with the same markers of traffic-related air pollution. Associations of Cu,Zn-SOD and GPx-1 with $PM_{0.25}$ were somewhat stronger than larger size fractions. However, this was found only in the restricted subset of 55 (Cu,Zn-SOD) and 57 subjects (GPx-1) described below, whereas models including all 60 subjects were mostly nonsignificant (Delfino et al, 2009).

Several factors suggested strong and highly informative effect modification as exemplified by selected models in several figures described as follows. We present pollutant averaging times in the figures that best represent associations across time rather than the full set of selected averaging times (lag 0, 3-day, 5-day, 7-day and 9-day averages). Selected representative models are shown in the various figures. Models were more strongly positive in the San Gabriel Valley (44 subjects) than in Riverside (16 subjects) for sTNF-RII and sP-selectin (Figure 22). Figure 22B shows an unexpected inverse association of sP-selectin with OC_{pri} and with $PM_{0.25}$ in the Riverside community, whereas there were positive associations in San Gabriel Valley communities. The potential importance of traffic-related particles is supported by these regional differences in associations because the San Gabriel Valley communities are closer to traffic sources than the Riverside community is. To simplify model presentation, we show results of remaining models for sTNF-RII and sP-selectin for subjects in the three San Gabriel Valley communities.

We demonstrated for the first time that associations of IL-6, sP-selectin, and Cu,Zn-SOD with PM markers of primary combustion (EC, BC, OC_{pri}), particle number, and $PM_{0.25}$, were stronger in a cooler 6-week period (phase 2) than a warmer 6-week period (phase 1) (Figure 23). The panels were marginally different across phases since subjects were followed in two phases with few exceptions (5 out of 60 subjects). In models restricted to 55 subjects with data in both phases, phase differences were nearly unchanged (not shown). It is relevant that OC_{pri} and particle number concentrations were higher in cooler months (typically characterized by air stagnation and lower secondary particle formation). Interestingly, concentrations of other pollutants also more strongly associated with biomarkers in the cooler phase (EC, BC, and $PM_{0.25}$) were <u>not</u> higher, suggesting differences in particle composition or size distribution were important, perhaps as better reflected by OC_{pri} and particle number, respectively. This is an important finding because particle mass alone does not provide sufficient information about composition or sources. It is conceivable, for example, that our findings for $PM_{0.25}$ and particle number are in part attributable to nanoparticles. It has been shown that particles 6-12 nm were much higher in the winter than summer near a Los Angeles freeway, but larger particles 50-100 nm showed the opposite trend (Zhu et al. 2004). Semi-volatile organic components associated with particles may have also been important given that biomarker associations were similarly robust for the correlated gases CO and NOx. This included generally stronger associations of biomarkers with gases in phase 2 than in phase 1 when NOx concentrations were lower. These gases were unlikely causal at the observed low concentrations, but instead served as markers for other traffic emission components.

Furthermore, associations were stronger among subjects not taking statins for sTNF-RII, and stronger among subjects not taking clopidogrel for sP-selectin (Figure 24). Findings for statins, which have anti-inflammatory properties, support the hypothesis that effects of air pollution on cardiovascular health are secondary to pro-inflammatory properties of redox active and other pollutant components (Delfino et al. 2005). The new finding for sP-selectin is consistent with a panel study showing an association of ambient particle number concentration with another platelet activation

marker (soluble CD40 ligand) in people with CAD (Ruckerl et al. 2007). Our study is the first to show a protective effect of clopidogrel. This finding supports the plausibility of a pollutant effect on platelet activation because this medication blocks platelet aggregation and is associated with decreased sP-selectin (Xiao and Théroux 2004). Our findings are relevant to the potential for air pollution to affect CAD because sP-selectin activates both leukocytes and endothelial cells, and induces adhesion of leukocytes to platelets and to endothelial cells (Jurk and Kehrel 2005). Therefore, if air pollutants acutely activate platelets as suggested by our finding, this could increase the risk of a potentially fatal thrombotic event in the coronary arteries. Platelet selectin is also critical to the development of neointimal formation after arterial injury (Wang et al. 2005). Potentially relevant findings by two epidemiologic studies are evidence of increased risks of atherosclerosis development with exposure near the home to traffic-related air pollution (Hoffmann et al. 2008; Künzli et al. 2005).

We found that similar to its receptor, TNF-α was positively associated with markers of traffic-related air pollution, but only for subjects with mean TNF-α in the upper quartile of its distribution (Figure 25A). Similarly, among subjects with mean CRP in the upper quartile of its distribution we found positive associations of CRP with $PM_{0.25}$ and markers of traffic-related air pollution (Figure 25B). Consistent with findings of a protective effect of statins for TNR-RII, there were fewer subjects taking statins in the upper quartile of CRP (33%) than in the lower 75th percentile strata (73%, OR 0.18, 95% CI: 0.05, 0.64), but there was little difference in statin use between TNF-α strata. These findings are consistent with reports of smaller associations between air pollutants and CRP among statin users in two other panel studies of susceptible elderly subjects (Dubowsky et al. 2006, Ruckerl et al. 2006). The pro-inflammatory effects of air pollutants may increase cardiovascular risk among those people susceptible due to chronic inflammatory states. This may be reflected by our finding of associations of air pollutants with CRP and TNF-α only among subjects in the upper quartile of biomarker means. Previous panel studies reported similar findings (Dubowsky et al. 2006, Ruckerl et al. 2006).

Across all biomarkers, we found consistently stronger associations for OC_{pri} than for SOC, and this was generally reflected by confidence intervals for total OC that usually crossed zero (Figure 26). We did not find any positive associations with SOC, with most regression coefficients being negative and nonsignificant at $p<0.05$. The present study is the first to estimate epidemiologic associations of cardiovascular outcomes with OC divided into two characteristics representing primary and secondary sources. This early finding is important because little is currently known about the pollutant components behind associations of cardiovascular hospitalization and mortality with ambient PM mass concentrations (Pope and Dockery 2006). Local primary combustion sources of air pollution are primarily from mobile sources in the Los Angeles area, and they generate high concentrations of redox-active organic components and metals (Cho et al. 2005; Ning et al. 2007; Ntziachristos et al. 2007).

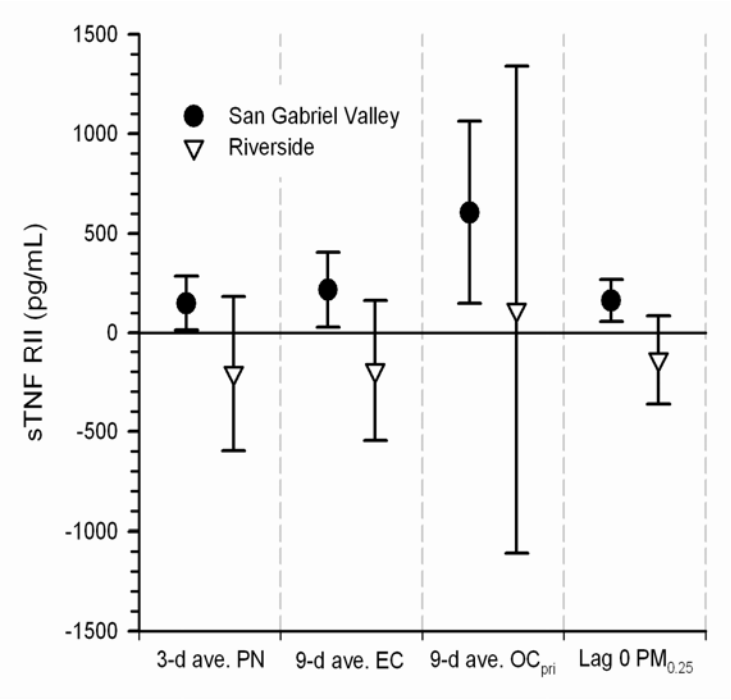
Generally, the strongest and most robust associations for particle mass were for “quasi-ultrafine” $PM_{0.25}$ (Figure 27). For IL-6, sTNF-RII, and CRP, associations were clearly stronger with $PM_{0.25}$ across averaging times (Figures 27A-27B), whereas this was less clear for sP-selectin (Figure 27C). Inverse associations were found for erythrocyte Cu,Zn-SOD with $PM_{0.25}$, $PM_{0.25-2.5}$ and $PM_{2.5-10}$, although associations were stronger with $PM_{0.25}$ (Figure 27D). Inverse associations were similarly stronger for GPx-1 with both $PM_{0.25}$ and $PM_{2.5-10}$ as compared with $PM_{0.25-2.5}$ (Figure 27E). Overall, we detected stronger associations with $PM_{0.25}$ than $PM_{0.25-2.5}$. This finding may be attributable to the higher deposition fraction of the unmeasured UFP fraction ($PM_{0.1}$) of $PM_{0.25}$ than accumulation mode particles and the ability of UFP to translocate systemically to the potentially induce oxidative stress and inflammation (Elder and Oberdörster 2006; Li et al. 2003). Differences in biomarker associations for the two particle size fractions of regulated fine PM ($PM_{2.5}$) have not been as clearly demonstrated in most previous panel studies that have relied on central site data. However, a study by Ruckerl et al. (2007) did show that another marker of platelet activation (plasma sCD40L) was associated with lag day 0 central site accumulation mode particle counts (0.1–1.0 μm) to the same magnitude as number concentration of ultrafine particles from 0.01 to 0.1 μm. They found no association with $PM_{2.5}$ mass. We also showed associations of sP-selectin with total particle number and mass concentration of both 5-day average $PM_{0.25}$ and 3-day average accumulation mode $PM_{0.25-2.5}$ (Figure 27C).

Figures 26-27 show that in many but not all cases associations were strongest for longer-term averages out to the last 5, and in some cases, 9 days. We found no significant association for exposures in the 8 hours preceding the blood draw (not shown). This suggests that cumulative exposures sustained over several days are important in the detection of air pollutant-related increases in systemic inflammation. This finding does not rule out the occurrence of more acute effects throughout each of the monitored weeks (we only drew one blood sample per week).

For many, but not all models, indoor EC and PN of outdoor origin were more strongly associated with biomarkers than the uncharacterized indoor exposure as exemplified by selected models in Figure 28. Effect sizes for indoor EC and PN of outdoor origin were similar or slightly weaker compared with outdoor models for the same pollutants. This is consistent with findings in Figure 26 for indoor OC_{pri} and SOC of outdoor origin vs. outdoor measurements. These findings suggest that indoor monitoring may be unnecessary where the research interest is in outdoor source air pollutants because associations for indoor PM components of outdoor origin (EC, PN, OC_{pri}) were consistent with associations for outdoor home PM components (Figures 26 and 28). This supports the importance of outdoor particles in associations even though subjects spend a majority of their time indoors. This can be attributed to generally high infiltration found in the studied communities (Polidori et al. 2007). These results may not be applicable to other studies where the interest is in indoor source exposures because the communities did not have many of common sources of indoor generated PM such as wood smoke or environmental tobacco smoke, which was by design.

Figure 22. Associations of biomarkers with outdoor air pollutants: differences by study region. (A) TNF-RII (pg/mL). (B) sP-selectin (ng/mL). Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16). There were 44 subjects in the three San Gabriel Valley communities and 16 in the Riverside community.

A



B

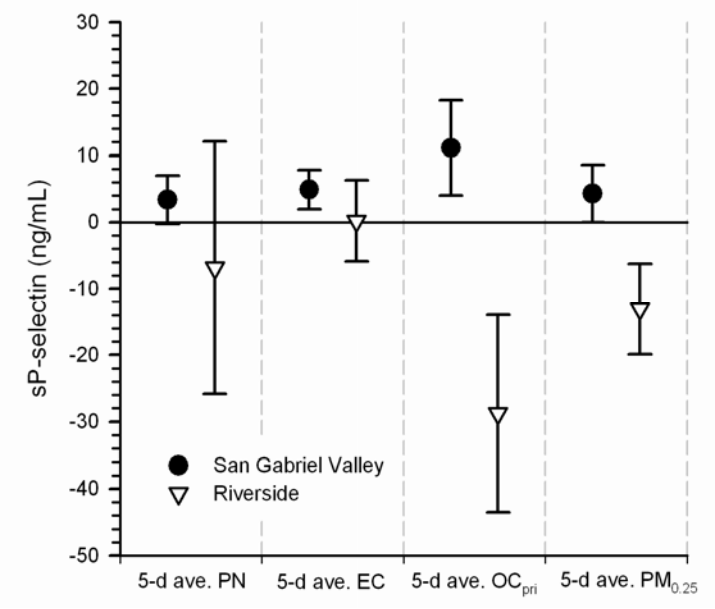


Figure 23. Associations of biomarkers with outdoor air pollutants: differences by phase of study. (A) IL-6 (pg/mL). (B) sP-selectin (ng/mL). (C) Cu,Zn-SOD (U/g Hb). Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16). Phase 1 is a warmer period of greater photochemical activity, and phase 2 is a cooler period of greater air stagnation. Results for sP-selectin are for 44 subjects living San Gabriel Valley.

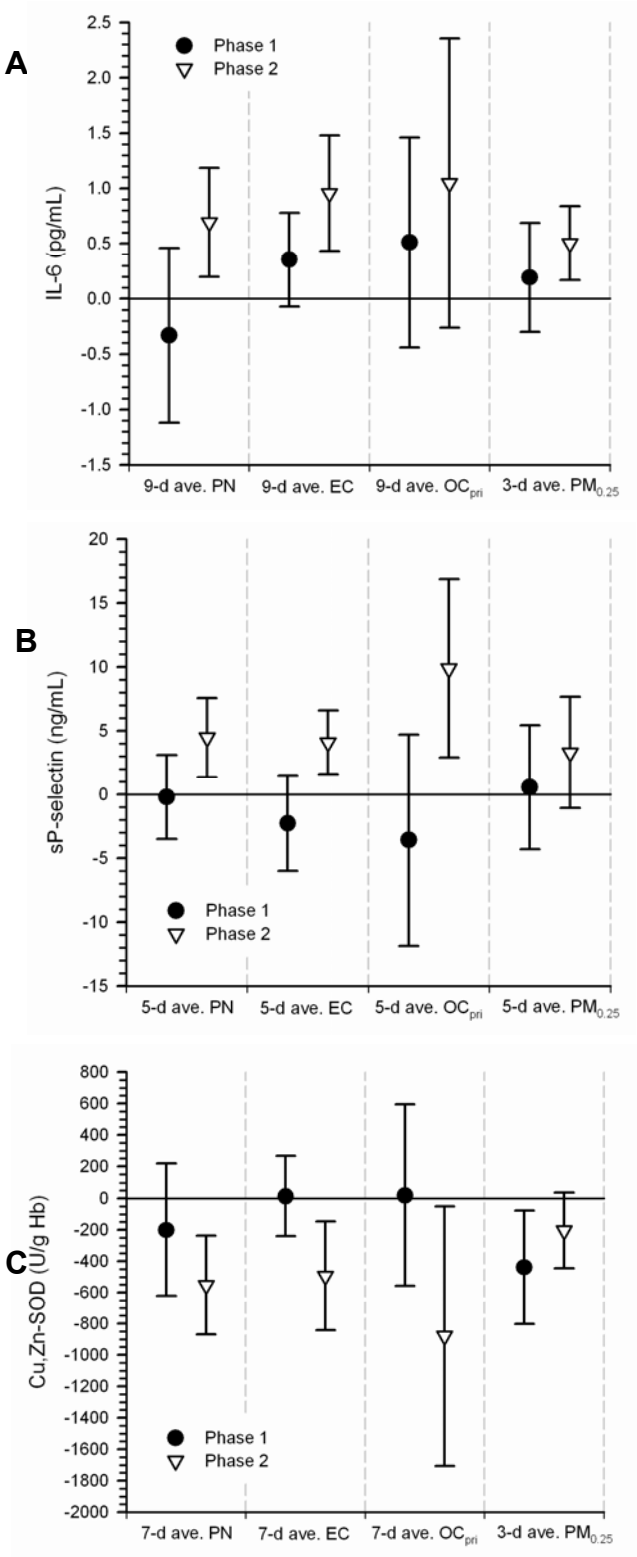


Figure 24. Associations of biomarkers with outdoor air pollutants: differences by medication use among 44 subjects living in the San Gabriel Valley. (A) TNF-RII (pg/mL) and statin use. (B) sP-selectin (ng/mL) and clopidogrel use. Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16).

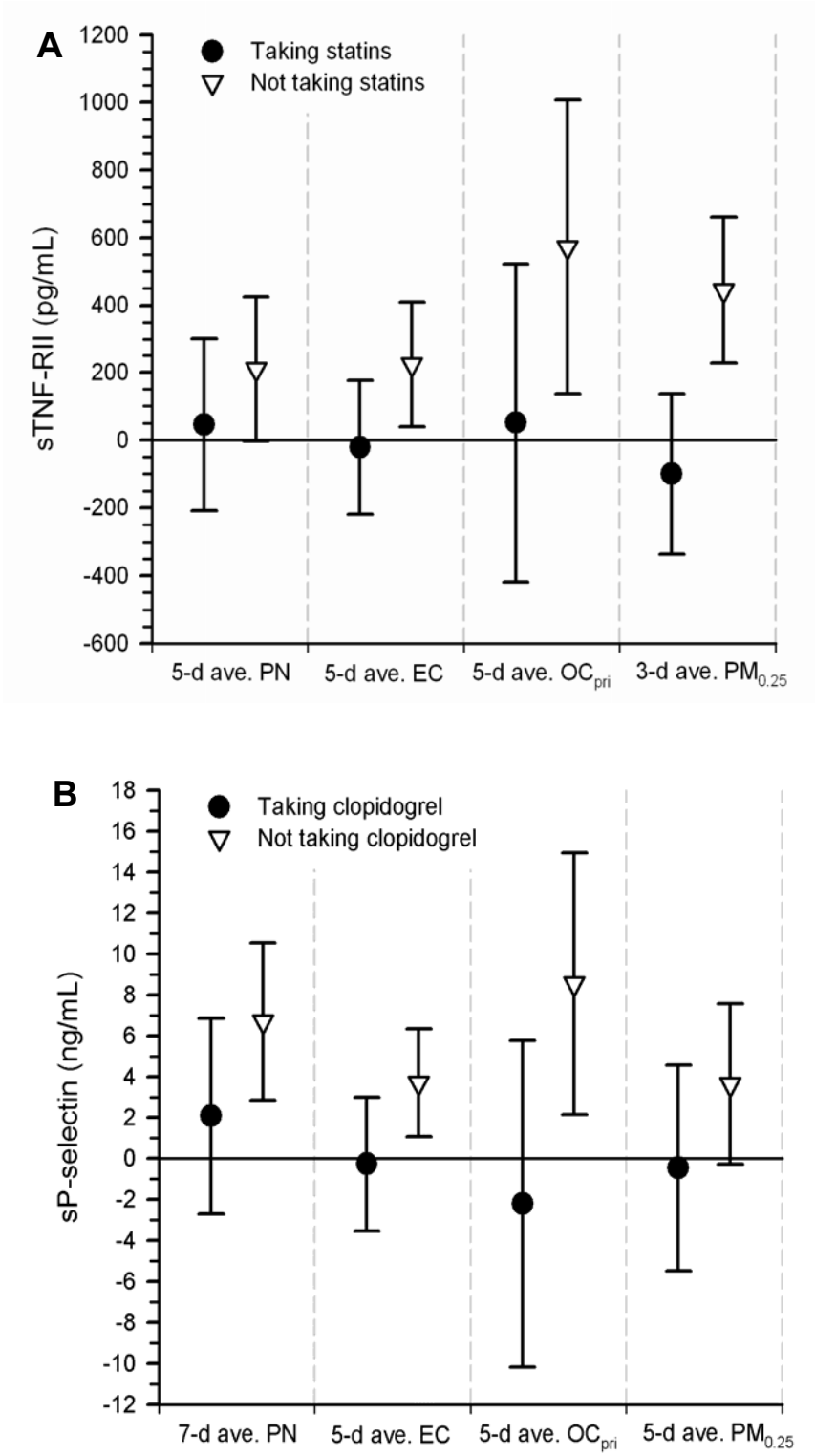
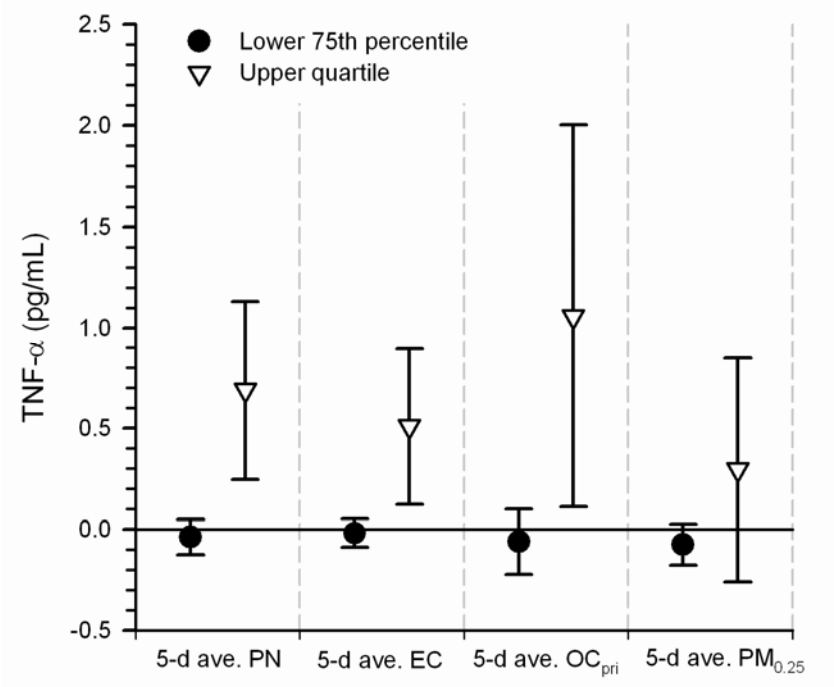


Figure 25. Associations of TNF-α and CRP with outdoor air pollution: differences between subjects with mean biomarkers in the lower 75th percentile vs. upper quartile. (A) TNF-α (pg/mL). (B) CRP (ng/mL). Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16).

A



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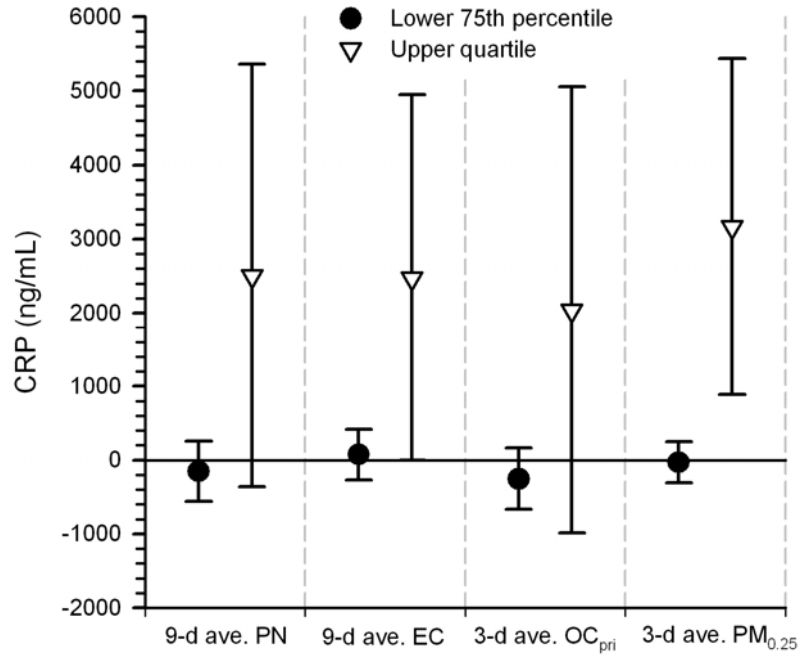


Figure 26. Associations of biomarkers with indoor and outdoor organic carbon (OC): differences by primary OC (OC_{pri}) and secondary OC (SOC) fractions. (A) IL-6 (pg/mL). (B) TNF-RII (pg/mL). (C) sP-selectin (ng/mL). (D) Cu,Zn-SOD (U/g Hb). (E) GPx-1 (U/g Hb). Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16). Models for TNF-RII and sP-selectin are restricted to 44 subjects living in the San Gabriel Valley. Indoor OC_{pri} and SOC are estimated concentrations of outdoor origin (Polidori et al., 2007).

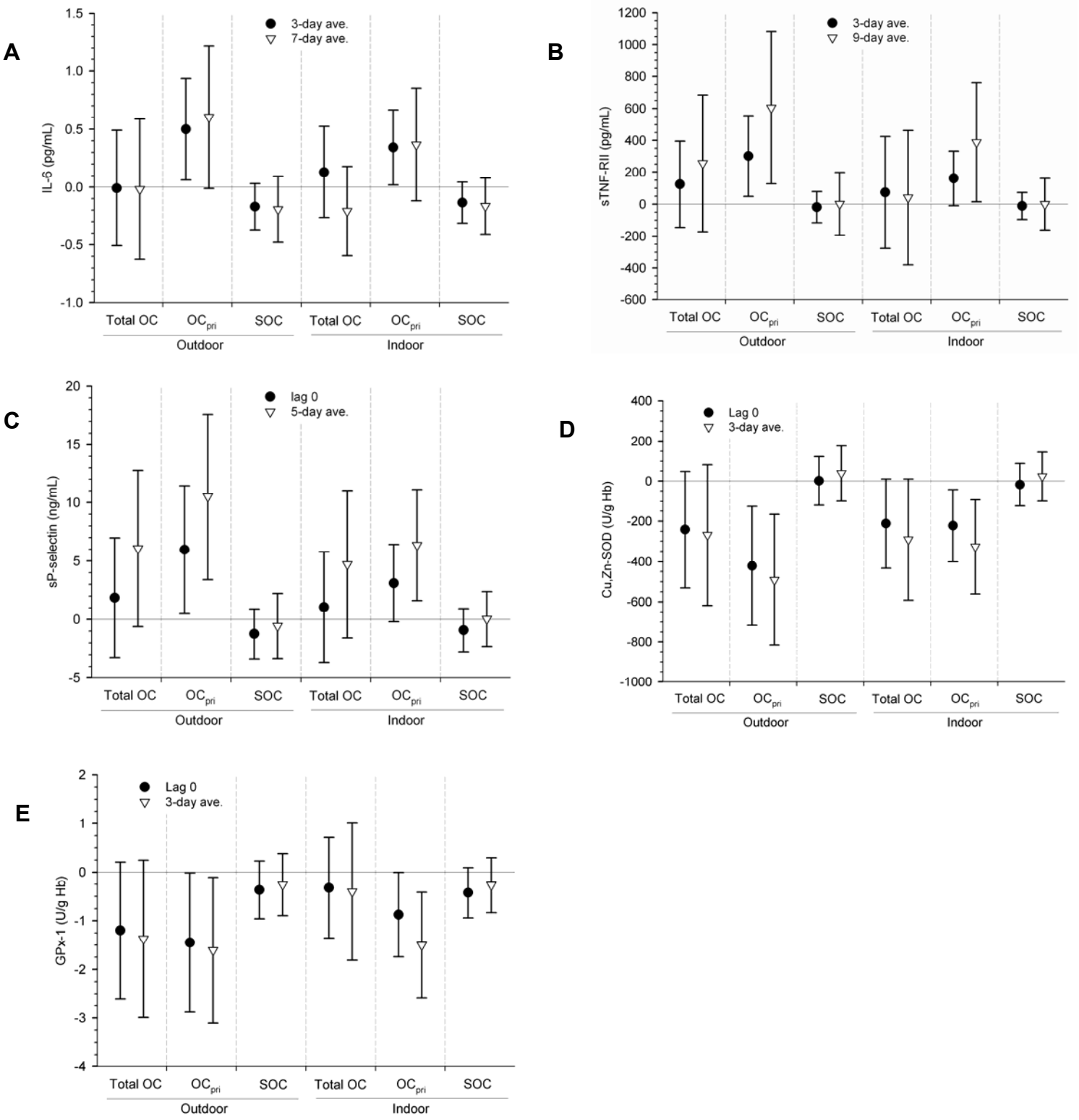


Figure 27. Associations of biomarkers with outdoor size-fractionated particle mass. (A) IL-6 (pg/mL). (B) TNF-RII (pg/mL). (C) sP-selectin (ng/mL). (D) Cu,Zn-SOD (U/g Hb). (E) GPx-1 (U/g Hb). Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16). Models for TNF-RII and sP-selectin are restricted to 44 subjects living in the San Gabriel Valley.

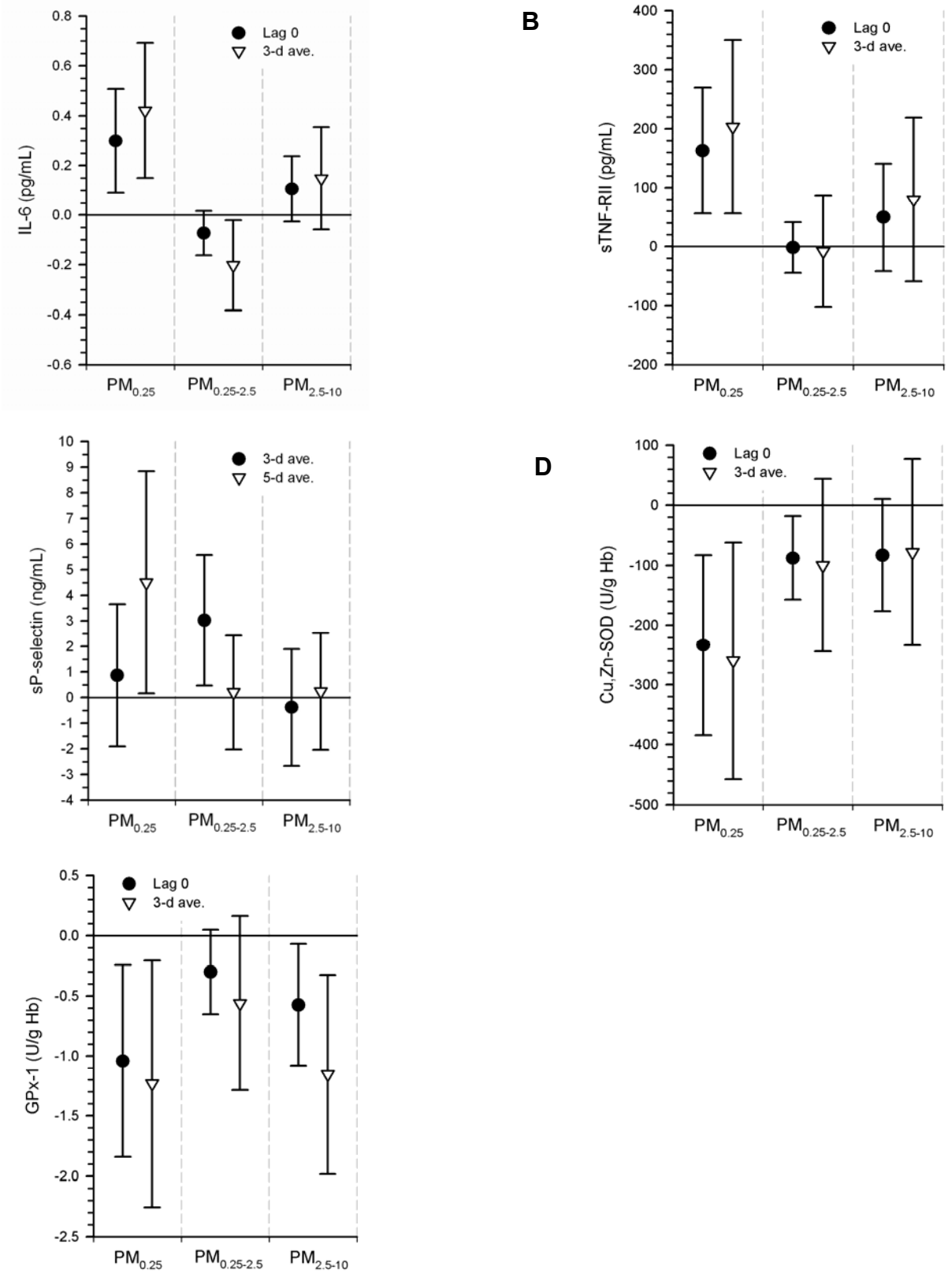
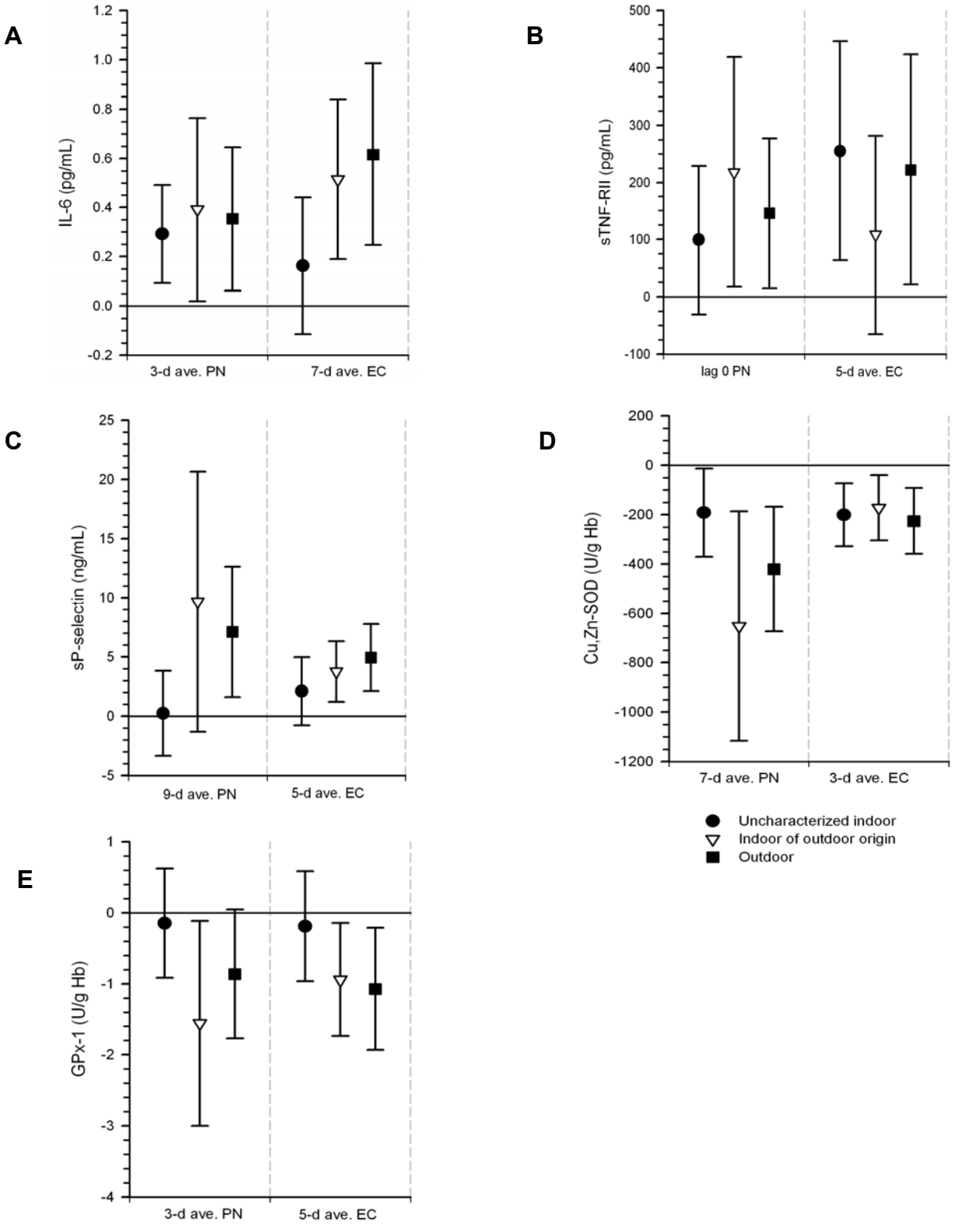


Figure 28. Associations of biomarkers with uncharacterized indoor air pollution as compared with indoor air pollution of outdoor origin and with outdoor air pollution. *(A)* IL-6 (pg/mL). (B) TNF-RII (pg/mL). (C) sP-selectin (ng/mL) (D) Cu,Zn-SOD (U/g Hb). (E) GPx-1 (U/g Hb). Expected change in the biomarker (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16). Models for TNF-RII and sP-selectin are restricted to 44 subjects living in the San Gabriel Valley. PN, particle number; EC, elemental carbon.



As previously reported and discussed in our publication of year 1 data (Delfino et al 2008), but not presented here, regression coefficients for ozone had opposite signs compared with other pollutants and were completely confounded by markers of primary combustion (EC, BC, OC_{pri}, CO, NOx) with which O_3 was inversely correlated.

Our most novel findings are the inverse associations between air pollutants and two antioxidant enzymes (GPx-1 and Cu,Zn-SOD). This at first glance appears to be inconsistent with experimental results show urban UFP can induce a positive antioxidant response represented by hemoxygenase-1 in epithelial and macrophage cell cultures (Li et al. 2003). In fact, in the proposed research for the EPA SCPC renewal, we hypothesized positive associations, in part based on these *in vitro* findings in other cells. However, erythrocytes do not have nuclei and thus have a relatively fixed amount of antioxidant enzymes after maturation from reticulocytes. The findings for GPx-1 and Cu,Zn-SOD in most of the elderly subjects studied suggest enzyme inactivation within erythrocytes by pollutant components or $PM_{0.25}$. This speculation is supported by experimental evidence (Hatzis et al. 2006; Pigeolet et al. 1990) including the present work discussed above showing *in vitro* inactivation of GAPDH by concentrated PM (Shinyashiki et al. 2008). Furthermore, quasi-ultrafine particles $\leq$0.2 μm in diameter and nanoparticles, but not larger particles, freely enter the erythrocyte (Rothen-Rutishauser et al. 2006).

Erythrocytes are critical in protecting the body against oxidative stress (Tsantes et al. 2006). We hypothesize that erythrocyte antioxidant enzyme inactivation is in part responsible for pollutant-related increase in biomarkers of inflammation and thrombosis. This is supported by the following findings. We tested mixed effects models of the relation between the antioxidant enzymes and biomarkers of inflammation. We found within-subject inverse associations of IL-6 with GPx-1, and sP-selectin with Cu,Zn-SOD. For an interquartile range decrease in GPx-1 of 10.4 U/g Hb, IL-6 increased 0.25 pg/mL (95% CI: −0.03, 0.53) or 10% of mean IL-6. Similarly, for an interquartile range decrease in SOD of 2026 U/g Hb, sP-selectin increased 5.8 ng/mL (95% CI: 3.3, 8.3), or 13% of mean sP-selectin. In general, biomarkers of inflammation were positively associated with each other, and GPx-1 was positively associated with Cu,Zn-SOD (not shown).

8.2.2 Ambulatory Blood Pressure

In the multiple regression analysis, we found positive associations between air pollution and both DBP and SBP (Table 23). Significant associations were found for the particulate air pollutant measurements BC, EC, OC, OC_{pri}, SOA, and $PM_{2.5}$, but not for particle number. OC showed stronger associations than BC, EC and $PM_{2.5}$. Of the two OC fractions, OC_{pri} showed generally stronger associations than SOC. NO_x and CO were also associated with DBP, but these associations were mostly nonsignificant. Ozone was not associated with either SBP or DBP. Associations were more significant for DBP than SBP, possibly due to higher variation in SBP. Also, the longer the averaging time, the stronger and more significant the association, especially for 5-day moving averages. For some pollutants, associations were stronger still for 9-day averages (BC, EC, NO_x and CO). Figure 29 illustrates these findings for selected models of 2-day and 5-day average particle mass measurements. Only BC and $PM_{2.5}$ were significantly or borderline significantly associated with DBP (but not SBP) for shorter averaging times of 8-hr and 1-day (Figure 30).

A previous repeated measures study most similar to our own followed 62 subjects with preexisting cardiac disease attending a cardiac rehabilitation program involving exercise therapy (Zanobetti et al. 2004). They showed a significant in-clinic increase in resting SBP (2.8 mm Hg) and DBP (2.7 mm Hg) associated with 10.5 $\mu g/m^3$ 5-day average ambient $PM_{2.5}$, which was stronger than shorter averaging times. This compares well with our finding for 5-day $PM_{2.5}$ scaled to the same concentration change and SBP (2.2 mm Hg) but is larger than our finding for DBP (1.4 mm Hg). They also found no associations for $\leq$ 24-hr averages of $PM_{2.5}$, but we did for 8-hr and 24-hr

averages, perhaps because we analyzed hourly ABPM with hourly air pollutant data, and thus may have had enhanced ability to assess acute exposure-response relationships.

The present results provide clues to possible causal pollutant components not otherwise explained by US EPA-regulated particle mass concentrations typically analyzed in similar studies (Delfino et al. 2005). Our strongest overall associations were for organic carbon (Figure 29), especially 2-day average OC_{pri}, which is the OC fraction that is a marker of primary combustion products of fossil fuel (Polidori et al 2007). This is concordant with our findings that blood biomarkers of inflammation such as IL-6, which were also more strongly associated with this OC fraction. The strongest associations overall were for 9-day total OC, showing a large increase of 6.93 mm Hg in diastolic BP (95% CI: 1.90, 11.96) and 5-day total OC, showing a large increase of 6.20 mm Hg in systolic BP (95% CI: 1.18, 11.22).

We showed similar magnitudes of association for both hourly BC and $PM_{2.5}$ from 8-hr to 5-day averages, but associations of BP with BC were somewhat stronger than $PM_{2.5}$ at 9-day averages (Table 23, Figure 30).

We also showed that the strongest and most significant associations were for multiday moving averages of air pollutants including 5- and 9-day averages as compared with 8-24 hour averages, consistent with our findings for biomarkers. We speculate that cumulative effects on systemic oxidative stress and inflammation from sustained elevations of air pollution over several days are linked with increased blood pressure, perhaps related to greater endothelial damage and hence diminished vasodilation.

In other NIEHS-funded research we also tested confounding and interaction for variables from the electronic diary including stress and anxiety. Intense, strong, or moderate stress or anxiety, adjusted for posture and hour of day, was positively associated with SBP (2.21 mm Hg, 95% CI: 0.01, 4.41) and DBP (2.12 mm Hg, 95% CI: 0.78, 3.45) compared with little to no stress or anxiety. These responses representing emotional states did not confound or interact with associations of air pollutants and BP and were therefore were not included in adjusted models. We assume that stress reports come from a variety of factors, including occasional high noise levels. However, we did not measure noise in any of the communities monitored, but it can be assumed that levels were relatively low since these are residential facilities for elderly retired individuals.

Table 23. Associations of diastolic and systolic blood pressure with outdoor air pollutants.

	Diastolic BP		Systolic BP	
Exposure / averaging time	**Coeff. (95% CI)[a]**	***p*-value**	**Coeff. (95% CI)**	***p*-value**
Particle number				
8-hr	0.01 (-0.54, 0.57)	0.9643	0.38 (-0.60, 1.36)	0.4512
24-hr	-0.26 (-1.10, 0.58)	0.5463	0.11 (-1.40, 1.61)	0.8910
2-day	-0.65 (-1.92, 0.63)	0.3203	-1.86 (-4.10, 0.38)	0.1028
5-day	-0.84 (-3.21, 1.53)	0.4863	-2.05 (-6.30, 2.20)	0.3451
9-day	-1.19 (-5.73, 3.34)	0.6065	-3.00 (-11.17, 5.18)	0.4725
Black carbon				
8-hr	0.29 (-0.06, 0.65)	0.1064	0.23 (-0.41, 0.88)	0.4807
24-hr	0.37 (-0.10, 0.84)	0.1235	0.25 (-0.63, 1.12)	0.5796
2-day	0.82 (0.23, 1.41)	0.0064	1.15 (0.06, 2.25)	0.0389
5-day	2.37 (0.98, 3.75)	0.0008	3.07 (0.50, 5.64)	0.0192
9-day	4.19 (1.81, 6.56)	0.0006	5.34 (0.93, 9.75)	0.0176
Elemental carbon				
8-hr	0.09 (-0.30, 0.47)	0.6579	-0.12 (-0.83, 0.60)	0.7480
24-hr	0.13 (-0.39, 0.65)	0.6225	-0.26 (-1.23, 0.70)	0.5934
2-day	0.57 (-0.12, 1.26)	0.1058	0.71 (-0.57, 2.00)	0.2779
5-day	1.60 (0.12, 3.09)	0.0340	2.00 (-0.73, 4.73)	0.1506
9-day	3.18 (0.38, 5.99)	0.0263	2.74 (-2.50, 7.98)	0.3061
Organic Carbon (OC)				
8-hr	0.34 (-0.73, 1.41)	0.5351	0.62 (-1.37, 2.61)	0.5392
24-hr	0.54 (-0.65, 1.73)	0.3705	0.05 (-2.17, 2.27)	0.9644
2-day	1.43 (-0.09, 2.95)	0.0651	2.17 (-0.65, 4.99)	0.1319
5-day	4.68 (2.02, 7.34)	0.0006	6.20 (1.18, 11.22)	0.0154
9-day	6.93 (1.90, 11.96)	0.0069	3.71 (-5.76, 13.17)	0.4429
Primary OC				
8-hr	0.33 (-0.82, 1.47)	0.5751	1.31 (-0.80, 3.42)	0.2239
24-hr	0.33 (-1.30, 1.97)	0.6901	0.83 (-2.21, 3.86)	0.5936
2-day	2.34 (0.08, 4.59)	0.0422	4.39 (0.27, 8.52)	0.0368
5-day	3.69 (-0.43, 7.80)	0.0792	5.22 (-2.29, 12.73)	0.1728
9-day	2.04 (-6.82, 10.90)	0.6517	-6.36 (-22.48, 9.75)	0.4387

Table 23 (cont)	Diastolic BP		Systolic BP	
Exposure / averaging time	**Coeff. (95% CI)[a]**	***p*-value**	**Coeff. (95% CI)**	***p*-value**
Secondary OC				
8-hr	0.44 (-0.23, 1.11)	0.1984	0.94 (-0.29, 2.17)	0.1353
24-hr	0.65 (-0.15, 1.44)	0.1113	1.24 (-0.23, 2.72)	0.0987
2-day	0.83 (-0.21, 1.87)	0.1184	1.66 (-0.25, 3.57)	0.0877
5-day	2.33 (0.87, 3.79)	0.0018	3.17 (0.47, 5.86)	0.0214
9-day	2.38 (-0.91, 5.67)	0.1568	1.00 (-5.10, 7.11)	0.7468
$PM_{2.5}$				
8-hr	0.42 (-0.02, 0.86)	0.0606	0.25 (-0.55, 1.06)	0.5368
24-hr	0.59 (0.08, 1.10)	0.0227	0.53 (-0.41, 1.48)	0.2699
2-day	0.99 (0.38, 1.59)	0.0015	1.54 (0.41, 2.67)	0.0074
5-day	2.10 (0.90, 3.30)	0.0006	3.43 (1.20, 5.65)	0.0025
9-day	3.38 (1.37, 5.38)	0.0010	3.40 (-0.34, 7.13)	0.0748
NO_x				
8-hr	0.20 (-0.27, 0.67)	0.3982	0.18 (-0.67, 1.03)	0.6795
24-hr	0.21 (-0.42, 0.85)	0.5073	0.17 (-1.00, 1.33)	0.7768
2-day	0.54 (-0.27, 1.36)	0.1888	0.61 (-0.89, 2.12)	0.4254
5-day	1.73 (-0.27, 3.72)	0.0893	0.71 (-3.00, 4.42)	0.7079
9-day	2.76 (-0.78, 6.30)	0.1260	4.02 (-2.52, 10.57)	0.2282
CO				
8-hr	0.26 (-0.29, 0.81)	0.3583	-0.03 (-1.03, 0.97)	0.9511
24-hr	0.29 (-0.44, 1.02)	0.4307	-0.32 (-1.65, 1.02)	0.6416
2-day	0.67 (-0.29, 1.63)	0.1720	0.35 (-1.42, 2.11)	0.7001
5-day	1.48 (-0.40, 3.36)	0.1221	-0.49 (-3.95, 2.96)	0.7795
9-day	4.28 (0.37, 8.19)	0.0319	1.44 (-5.73, 8.62)	0.6931
O_3				
8-hr	-0.19 (-0.67, 0.29)	0.4402	0.29 (-0.57, 1.14)	0.5127
24-hr	-0.23 (-1.10, 0.64)	0.6076	0.52 (-1.07, 2.11)	0.5228
2-day	-0.51 (-1.52, 0.50)	0.3206	0.30 (-1.55, 2.16)	0.7478
5-day	-1.53 (-4.16, 1.10)	0.2548	1.34 (-3.53, 6.21)	0.5901
9-day	-1.99 (-5.74, 1.76)	0.2973	-3.18 (-10.13, 3.77)	0.3695

[a] Regression coefficients and 95% confidence intervals are for the expected change in blood pressure in 63 subjects associated with an interquartile range change in air pollutants (see Table 16).

Figure 29. Associations of blood pressure with outdoor home air pollutants. (A) systolic blood pressure (SBP); (B) diastolic blood pressure (DBP). Expected change in blood pressure (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16).

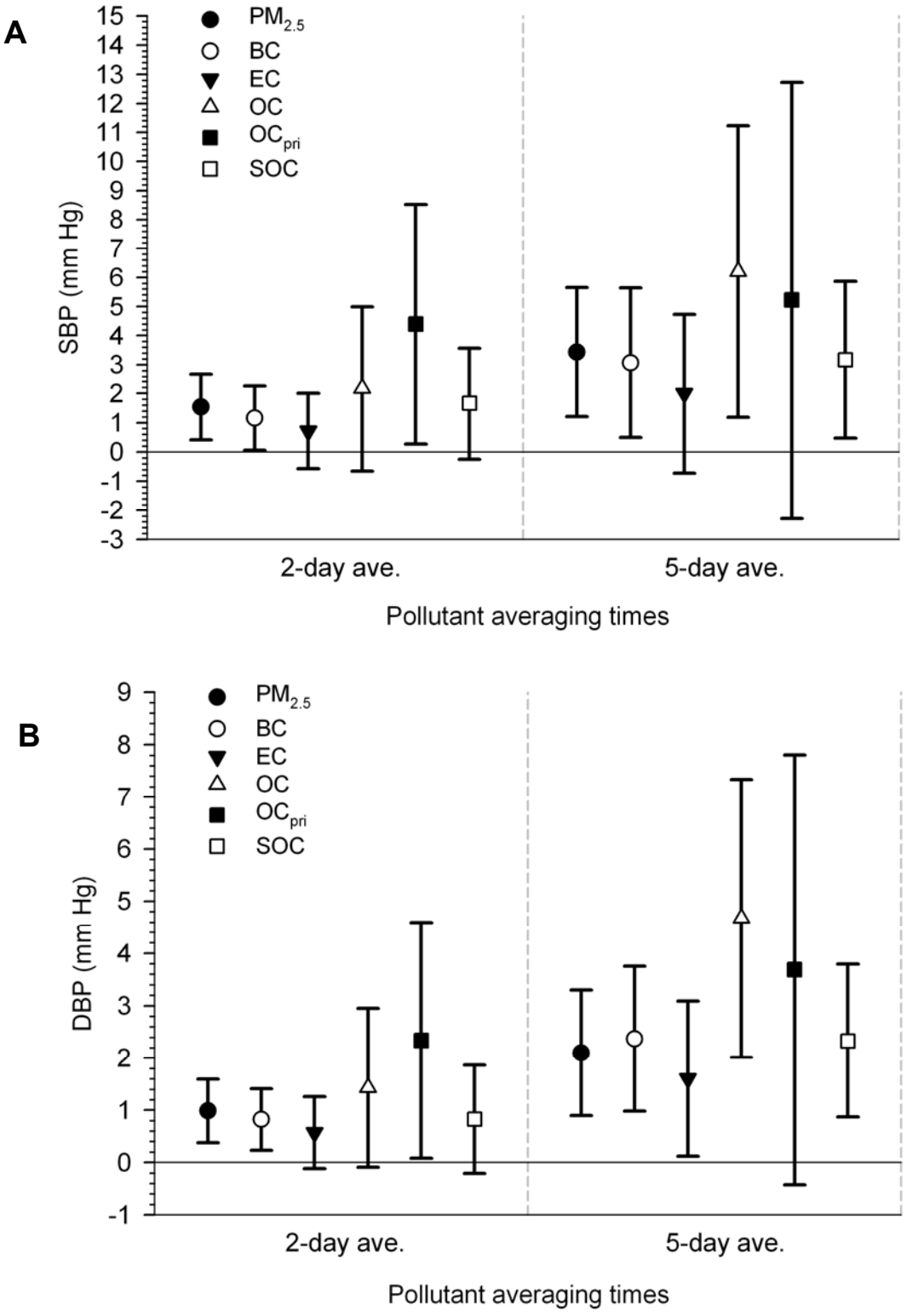
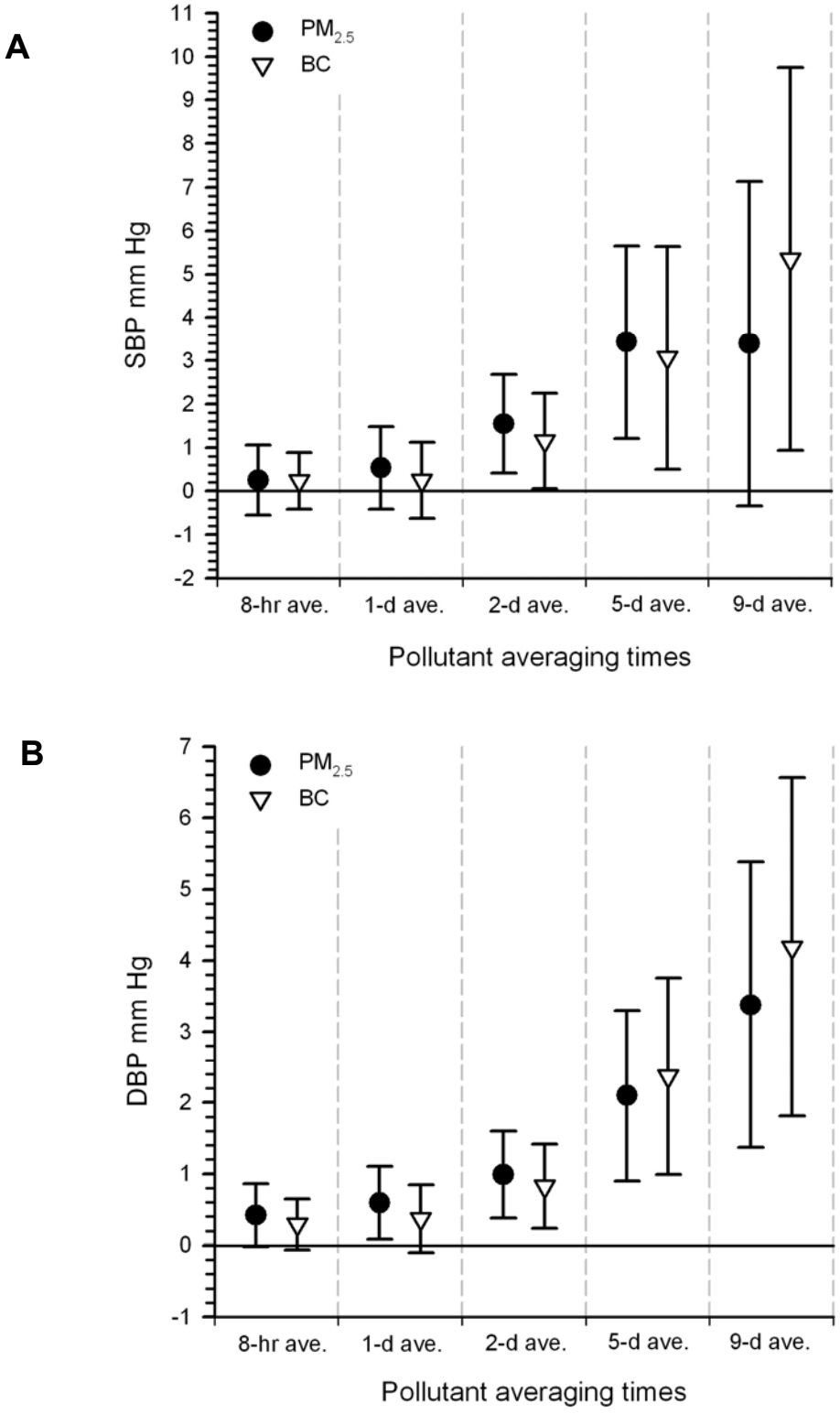


Figure 30. Associations of blood pressure with outdoor home PM2.5 and black carbon (BC). A) systolic blood pressure (SBP); (B) diastolic blood pressure (DBP). Expected change in blood pressure (adjusted coefficient and 95% CI) corresponds to an interquartile range change in the air pollutant over all measurements (Table 16).



8.2.3 Ambulatory ECG ST Segment Depression

Preliminary Poisson regression results from GEE models are presented in Table 24 out to the 6-day average. Associations of air pollutant variables with risk of ST segment depression were seen out to the 6-day average, which includes lag 1 day through lag 7 day (see Methods). Magnitudes of association diminished with longer averaging times (not shown). There were no associations of ST segment depression with particle number as was seen for blood pressure models. For both BC and EC, significant associations with ST segment depression were observed out to the 3-day average, while associations for OC were larger and significant out to the 6-day average. Compared with SOC, the OC_{pri} fraction was more strongly and significantly associated with ST segment depression. However, in contrast to the biomarkers, but similar to diastolic blood pressure, SOC was significantly associated with longer-term averages of 5-6 days. There were no significant associations with BAM $PM_{2.5}$, although several models from lag 1 day to 6-day average were of borderline significance. The relative rates for $PM_{2.5}$ were similar in magnitude to EC and BC but around half that of total OC and OC_{pri} as illustrated in Figure 31 for a standardized interquartile range (IQR) change in the air pollutant (IQRs are shown in Table 16). Consistent estimates were found for NO_x and CO but were only significant for NO_x lag 1 day and 2-day average. Inverse associations was observed for O_3 out to the 3-day average (significant at the 2-day average), but similar to biomarkers, this was confounded by markers of primary combustion (not shown).

Our findings are consistent with findings for $PM_{2.5}$ in other panel studies (Gold et al. 2005; Lanki et al. 2008; Pekkanen et al. 2002), including the only other panel studies that employed ambulatory ECG (Chuang et al. 2008). In the present study total PN (combined ultrafine and accumulation mode) was not associated with ST segment depression. However, Pekkanen et al. (2002) found equivalent and largely independent associations of lag 2 day ambient ultrafine PN data (10 to 100 nm), accumulation mode PN (100 to 1000 nm), and $PM_{2.5}$ mass with ST segment depression measured in clinics using exercise challenge tests in 45 subjects with CAD (342 submaximal exercise challenges) living in Helsinki, Finland. Using the same panel data, Lanki et al. (2006) tested a multipollutant model that included indicator elements for ambient $PM_{2.5}$ sources (Si, S, Ni, Cl and BC) in relation to ST segment depression and found only BC was significantly associated with ST segment depressions. Lanki et al (2008) found associations of ST segment depression with recent hourly ambient BAM $PM_{2.5}$ mass but not number concentrations of ultrafine particles in 41 exercise-challenged subjects with CAD (179 visits). The panel study in Helsinki, Finland (Lanki et al. 2006; Pekkanen et al. 2002) relied on central site data and so results are variably accurate with respect to personal exposures or exposures near the home as measured in our study of retirement communities. Only Lanki et al. 2008 used personal $PM_{2.5}$ exposure during the 24 hours prior to in-clinic ECG measurements. They found similar magnitudes of positive association with ST segment depression during submaximal exercise testing for personal and ambient $PM_{2.5}$ exposures. Authors inferred from this finding that ambient and not indoor sources were important.

In another panel study of 48 subjects with CAD (128 24-hr Holters), Chuang et al (2008) found an interquartile increase in the previous 24-hour mean ambient BC (0.47 $\mu g/m^3$) was associated with a 1.50-fold increased risk of ambulatory ECG-measured ST-segment depression $\geq$0.1 mm (95% CI, 1.19, 1.89), and smaller and nearly significant associations were observed for $PM_{2.5}$ (6.9 $\mu g/m^3$, RR 1.22, 95% CI: 0.99, 1.50). Our results are not directly comparable in that they used a continuous scale for ST changes. Our ECG software data only read out a continuous depression scale at or greater than the more clinically relevant (with regard to ischemia) level of -1.0 mm. Chuang et al (2008) did not report on risk of "cardiac ischemia" at this level of ST segment depression typically used to define potential cardiac ischemia. The present study involved more data (328 24-hr ambulatory Holters in 35 subjects with any ST segment depression $\geq$1 mm), which may have allowed us to observe associations for ischemic ST segment depression.

Table 24. Relative rate of ST segment depression in relation to outdoor air pollutants.

Exposure / averaging time[a]	RR (95% CI)[b]	*p*-value
Particle number		
24-hr lag 1 day	1.19 (0.90, 1.56)	0.2213
2-day	1.30 (0.88, 1.92)	0.1924
3-day	1.00 (0.53, 1.87)	0.9909
4-day	0.55 (0.25, 1.25)	0.1549
5-day	0.91 (0.36, 2.30)	0.8346
6-day	0.65 (0.21, 1.97	0.4436
Black carbon		
24-hr lag 1 day	1.36 (1.03, 1.79)	0.0290
2-day	1.59 (1.16, 2.19)	0.0043
3-day	1.80 (1.16, 2.81)	0.0092
4-day	1.37 (0.69, 2.71)	0.3674
5-day	1.35 (0.82, 2.21)	0.2363
6-day	1.59 (0.85, 2.95)	0.1440
Elemental carbon		
24-hr lag 1 day	1.20 (0.95, 1.53)	0.1276
2-day	1.52 (1.12, 2.06)	0.0069
3-day	1.59 (1.16, 2.19)	0.0043
4-day	1.34 (0.78, 2.32)	0.2925
5-day	1.47 (0.94, 2.30)	0.0915
6-day	1.60 (0.82, 3.15)	0.1694
Organic Carbon (OC)		
24-hr lag 1 day	1.53 (0.91, 2.56)	0.1074
2-day	2.16 (0.80, 5.83)	0.1295
3-day	2.11 (1.09, 4.08)	0.0272
4-day	2.04 (1.03, 4.03)	0.0398
5-day	4.53 (2.33, 8.81)	0.0000
6-day	3.47 (1.03, 11.66)	0.0443
Primary OC		
24-hr lag 1 day	1.69 (1.04, 2.74)	0.0339
2-day	3.24 (1.97, 5.35)	0.0000
3-day	3.39 (1.91, 6.02)	0.0000
4-day	2.52 (1.18, 5.37)	0.0165
5-day	3.26 (1.29, 8.23)	0.0126
6-day	3.58 (0.79, 16.27)	0.0986

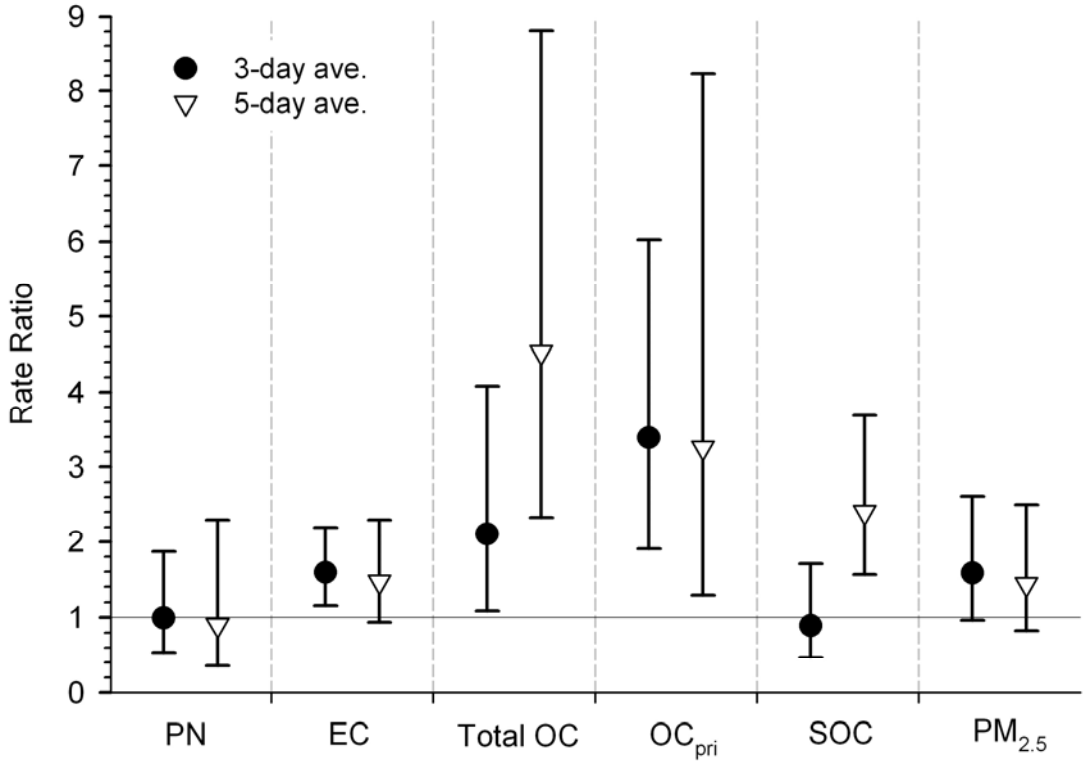
Table 24 (cont)

Exposure / averaging time[a]	RR (95% CI)[b]	*p*-value
Secondary OC		
24-hr lag 1 day	1.00 (0.68, 1.48)	0.9799
2-day	0.73 (0.45, 1.19)	0.2070
3-day	0.90 (0.47, 1.71)	0.7377
4-day	1.38 (0.70, 2.72)	0.3516
5-day	2.40 (1.56, 3.69)	0.0001
6-day	2.35 (1.04, 5.33)	0.0401
$PM_{2.5}$		
24-hr lag 1 day	1.38 (0.97, 1.96)	0.0706
2-day	1.40 (0.90, 2.20)	0.1368
3-day	1.59 (0.96, 2.61)	0.0700
4-day	1.57 (0.88, 2.82)	0.1295
5-day	1.44 (0.82, 2.50)	0.2012
6-day	1.67 (0.91, 3.06)	0.1008
NO_x		
24-hr lag 1 day	1.32 (1.04, 1.67)	0.0206
2-day	1.48 (1.01, 2.17)	0.0452
3-day	1.56 (0.86, 2.83)	0.1462
4-day	0.98 (0.44, 2.18)	0.9635
5-day	0.98 (0.46, 2.08)	0.9488
6-day	1.00 (0.43, 2.32)	0.9972
CO		
24-hr lag 1 day	1.20 (0.91, 1.58)	0.2026
2-day	1.20 (0.74, 1.93)	0.4612
3-day	1.30 (0.64, 2.62)	0.4667
4-day	0.94 (0.43, 2.03)	0.8675
5-day	1.09 (0.54, 2.20)	0.8025
6-day	1.00 (0.48, 2.08)	0.9969
O_3		
24-hr lag 1 day	0.65 (0.38, 1.10)	0.1104
2-day	0.42 (0.25, 0.72)	0.0014
3-day	0.59 (0.31, 1.16)	0.1250
4-day	0.97 (0.41, 2.32)	0.9458
5-day	1.37 (0.62, 3.02)	0.4366
6-day	1.67 (0.65, 4.32)	0.2885

[a] The multiday averages include lag 1 day 24-hr average plus averages of additional daily 24-hr lags (e.g., 2-day average is the average of lag 1 day and lag 2 day averages).

[b] Rate ratios and 95% confidence intervals are for the expected change in ST segment depression among 33 subjects associated with an interquartile range change in the air pollutant over all measurements (Table 16).

Figure 31. Relative rate of ST segment depression in relation to outdoor particulate air pollutants.



8.3 Limitations

Limitations include a lack of hourly personal exposures. Subjects may have been exposed outside of their communities, leading to some exposure error from using outdoor home exposure data. However, the participating retirement community residents stayed at home 88.4% of time (from diary data). We also did not have hourly particle size distribution data, which could have given us better idea about the overall inhaled dose. The fraction of PM depositing in the respiratory system is highly dependent on particle size, and in particular PM reaching the alveolar region can conceivably translocate into the circulation. Although we made measurements of daily size-fractionated gravimetric particle mass, the time frames did not match the hourly ambulatory BP and ECG data.

Although we believe that the present exposure data represents key sources and components, we cannot link exposure to specific sources nor can we identify specific component classes such as polycyclic aromatic hydrocarbons as being responsible for associations. Nevertheless, the major source of fossil fuel emissions in the LA basin is motor vehicle exhaust, and because EC, BC, OC_{pri}, CO, and NOx are linked to these emissions and strongly correlated with each other, our data suggests that motor vehicle exhaust may be responsible for the reported associations.

9. SUMMARY AND CONCLUSIONS

9.1. Exposure Data Characterization (Tasks 3 and 6)

Our major findings from the exposure assessment perspective of the study can be summarized as follows:

Measured indoor and outdoor concentrations of $PM_{2.5}$, OC, EC, PN, O_3, CO, and NO_X were generally comparable. The study average percentage contribution of outdoor SOA to outdoor particulate OC (representative for the San Gabriel Valley) was 40% and varied between 40–45% in the summer (during G1P1) and 32-40% in the winter (during G2P2). Quantifying the SOA contribution to measured OC is important for the following reasons: (1) to test evolving predictive SOA models; (2) to link the organic PM concentration to its emissions and precursors; and (3) to develop effective control strategies for PM. The low air exchange rates (AERs 0.25 to 0.33 hr^{-1}) calculated for G1 and G2 are consistent with the structural characteristics of the sampling sites, the low number of open windows and doors, and the presence of central air conditioners. Infiltration factor (F_{inf}) estimates were highest for EC (a nonvolatile species mostly found in the 0.1-0.4 μm range and also for OC. Lower F_{inf} values were obtained for $PM_{2.5}$ and PN, because these measurements are composed of both volatile and nonvolatile inorganic and organic components.

We found that only 13-17% (G2P2) to 16-26% (G1P1) of measured indoor OC was emitted or formed indoors. These results are consistent with low indoor activity levels at both retirement communities and with the prevailing use of central air conditioning.

We also found that outdoor $PM_{0.25}$ and, to a lesser extent, accumulation mode particles were the two PM fractions that best correlated with outdoor concentrations of CO, NO_2, and NO_x. This is consistent with their common mobile sources.

Perhaps our most significant finding is that primary sources of OC showed the highest contribution among the apportioned sources of OC for both indoor and outdoor particles at all sites. The contribution of mobile sources to indoor levels illustrates the significance of these sources on indoor PM concentrations. A major implication of these findings is that, even if people (particularly the elderly retired population of our study) generally spend most of their time indoors, a major portion of PM to which they are exposed comes from outdoor mobile sources. The significance of this conclusion is supported by the fact that indoor infiltrated particles from mobile sources were more

strongly associated with the adverse health effects observed in the elderly subjects living in the studied retirement communities compared to uncharacterized indoor particles. A caveat is that the elderly population with CAD studied and their exposures, which were restricted to four residential communities, may not represent the general population with respect to the health effects observed or the range of exposures encountered in indoor and outdoor environments.

9.2. In Vitro Redox Activity of Concentrated PM and Relevance to Health Effect (Tasks 1-2, 4 and 6)

We did not find evidence that biomarkers were associated with *in vitro* redox activity of concentrated PM as hypothesized. The null results are most likely due to the limited sampling periods, although other unmeasured factors influencing activity could have affected results. We believe the wide variations in redox activity between samples are evidence of this unmeasured interference and likely limited their use in testing associations.

An important new finding is the results using GAPDH in an *in vitro* redox assay to assess the electrophilic properties of PM. This may be relevant to our suggestive finding that air pollutants may inactivate antioxidant enzymes. Because electrophiles lead to irreversible inactivation of the protein GAPDH, then it is conceivable that this is the mechanism behind out findings for Cu,Zn-SOD and GPx-1 proteins.

9.3. Epidemiologic Analysis of the Cohort Panel (Tasks 7-8)

Our results for circulating biomarkers of effect suggest that pollutant components linked to emission sources of primary $PM_{2.5}$ OC, quasi-UFP ($PM_{0.25}$) and particle number concentrations are associated with increased systemic inflammation, platelet activation, and decreased circulating erythrocyte antioxidant enzyme activity in elderly people with coronary artery disease. The strongest overall associations were for the OC fraction that is a marker of primary combustion products of fossil fuel (OC_{pri}) (Polidori et al 2007). The importance of outdoor particles in associations even though subjects spend a majority of their time indoors is supported by associations for indoor PM components of outdoor origin (EC, PN, OC_{pri}) that were consistent with associations for outdoor home PM components.

Our findings support the possibility that inactivation of antioxidant enzymes may be one mechanism of air pollutant-related increases in systemic inflammation. These effects may be partly behind reported morbidity and mortality associations with ambient $PM_{2.5}$ mass concentrations (Pope and Dockery 2006). Stronger associations during the cooler phase of study despite similar $PM_{0.25}$ mass concentrations in cooler and warmer phases, further support the view that the greatest impacts on systemic responses may be attributable to nanoparticles not adequately represented by the present particle mass concentrations, as well as to unmeasured toxic air pollutants that increase near ground level in the winter. Our related experimental work utilizing particles collected in the Los Angeles air basin at the Southern California Particle Center suggests that this might include redox active and electrophilic organic components of traffic exhaust particles in the ultrafine range (Araujo et al. 2008; Gong et al. 2007; Li et al. 2003; Ntziachristos et al. 2007; Shinyashiki et al. 2008).

We also found exposure to outdoor home air pollutants is associated with increased blood pressure in this panel cohort of elderly subjects with a history of coronary artery disease. This physiologic response may additionally contribute to associations of cardiovascular morbidity and mortality with regional ambient air pollution observed in many epidemiologic studies (Pope and Dockery 2006). This was the first study using ABPM over multiple days that could test the time course of pollutant exposure-response relationships. We found 4-hr and 8-hr exposure averages in various models were significantly associated with increased BP, suggesting a very acute effect. However, associations were stronger for longer averaging times out to five or nine days, and this was

similarly found for biomarkers of systemic inflammation. This suggests that cumulative effects on systemic oxidative stress and inflammation from sustained elevations of air pollution over several days may be linked with increased blood pressure and cardiac ischemia, perhaps related to greater endothelial damage and hence diminished vasodilation and/or disturbances in autonomic function.

We found positive associations between particulate air pollutants (BC, EC, OC, OC_{pri}, SOA, and $PM_{2.5}$) and both DBP and SBP, but not for particle number. The strongest association overall was for OC_{pri}. Findings with ST segment depression are similar to the BP findings, including stronger assocaitons with OC_{pri}. This is concordant with our findings that blood biomarkers of inflammation such as IL-6, which were also more strongly associated with this OC fraction. However. while we did not find that biomarkers were associated with SOC or O_3, BP was associated with SOC (5-day average) and ST segment depression was associated with SOC (5-6 day averages) and O_3 (2 day average). It is conceivable that airway inflammation or other pathways induced by oxidant gases and aerosols influence cardiac function.

Overall, we found significant associations of biomarkers of inflammation, blood pressure, and ischemic ST segment depression with PM markers of traffic-related air pollution, including EC, OC_{pri}, and BC. In many models for biomarkers and blood pressure, significant associations were also found for criteria pollutant gases CO and NO_x that similarly track traffic-related air pollution in Los Angeles. Furthermore, health outcomes were generally more strongly (or in the case of biomarkers, only associated) with the primary OC as compared with the secondary OC fraction of total OC. Because all of these pollutants are highly correlated, the information about potential causal components of air pollution cannot be easily inferred.

With regard to size fraction, $PM_{0.25}$ was only moderately correlated with the surrogate measures of traffic-related air pollution (EC, OC_{pri}, BC, CO and NO_x), and therefore, stronger associations of biomarkers with this size fraction compared with larger size fractions must be interpreted to indicate another characteristic of air pollution that is potentially causal. Differences in associations for the two particle size fractions of regulated fine PM ($PM_{2.5}$) have not been as clearly demonstrated in previous panel studies that have relied on central site data. Several factors may explain this difference. First, the unmeasured UFP fraction ($PM_{0.1}$) of our $PM_{0.25}$ has a higher lung deposition fraction than accumulation mode particles (Elder and Oberdörster 2006). Second, the potential ability of UFP to translocate systemically may lead to an induction of oxidative stress and inflammation (Elder and Oberdörster 2006; Li et al. 2003).

In this regard, measurements across seasons helped us further characterize differences in associations due to particle size distribution and indirectly, chemical composition. This approach enabled us to detect stronger associations with $PM_{0.25}$ than $PM_{0.25\text{-}2.5}$, and to demonstrate for the first time that associations of IL-6, sP-selectin, and SOD with PM markers of primary combustion (EC, BC, OC_{pri}), particle number, and $PM_{0.25}$, were stronger in a cooler 6-week period than a warmer 6-week period. The seasonal findings suggest differences in particle composition or size distribution were important, perhaps as better reflected by OC_{pri} and particle number as these were found in higher concentrations in the cooler season, while $PM_{0.25}$ was not, even though associations were stronger for $PM_{0.25}$ in the cooler season. This may indicate that particle mass does not provide sufficient information about composition or sources. Our findings for $PM_{0.25}$ and particle number may in part attributable to nanoparticles, which have little mass. It has been shown that particles 6-12 nm were much higher in the winter than summer near a Los Angeles freeway, but larger particles 50-100 nm showed the opposite trend (Zhu et al. 2004).

The potential importance of traffic-related particles is supported by stronger positive biomarker associations (TNF-RII and sP-selectin) in the San Gabriel Valley communities closer to traffic sources than the Riverside community. As discussed, associations for all outcomes were also generally strongest for OC_{pri}. OC_{pri} and the directly related EC are in highest concentration in ultrafine particles compared with larger size fractions (Li et al. 2003). Because $PM_{0.25}$ includes some accumulation mode particles, it likely represents both fresh and aged traffic-related particles. Based on these results, both components and $PM_{0.25}$ or smaller size fractions appear to be important in the

associations we observed. This is pertinent to ambient air quantity standards in that there are no ambient air quality standards for the PM size fraction that includes ultrafine PM and the general class of organic components from traffic that appear to be driving many of the associations in this study.

10. RECOMMENDATIONS

With respect to the exposure assessment, given the considerable effort expended collecting indoor air samples, we were able to assess effects of indoor PM of outdoor origin. Our main interest in the present study was in the effects of outdoor air pollution. The epidemiologic findings strongly suggest that indoor monitoring may be unnecessary in future studies because associations for indoor PM components of outdoor origin (EC, PN, OC_{pri}) were consistent with associations for outdoor home PM components. Nevertheless, this recommendation may not apply to other outcomes in other groups potentially susceptible to health effects of air pollutant exposure. This includes people with asthma who may experience exacerbations of asthma due to indoor source particles, including aeroallergens, or volatile organic pollutants.

Although stronger associations between weekly biomarkers and $PM_{0.25}$ compared with larger size fractions was an important finding, future studies, especially those using more frequently measured outcomes such as blood pressure of ECG outcomes, should employ hourly particle size distribution and surface area data, which we did not have.

With respect to the assessment of the redox potential of ambient PM, we recommend the following for future work in cohort panels or other epidemiologic designs that aim to assess associations of health outcomes with *in vitro* redox activity of concentrated PM. Most importantly, we need to understand the determinants of variability in the assay that may be due to sample handling or experimental conditions. Sampling periods should be over longer durations of time than the several hours employed in the present study, especially given results for air pollutant exposures showing the strongest associations were for multi-day averages. The timing of sampling should be cumulative and comprehensive prior to the health measurement, regardless of selected duration. None of these design characteristics was part of the present study, in part due to limitations in current technology for the collection and assay of concentrated PM.

Additional studies to confirm the many novel findings are needed, including mechanistic studies as well as additional epidemiologic research using similar highly sensitive methods. Our robust findings are likely attributable to the use of repeated blood draws for biomarkers immediately processed and frozen on site prior to assay, real-time monitoring of ambulatory blood pressure, ECG, and physical activity, measurements of air pollution near subject residences, and detailed exposure measurements including global markers of particle source and composition linked to fossil fuel combustion. Future panel studies should not rely on cruder methods used in previous studies, including the use of central site fine particle mass not fractionated by particle size and without markers of composition or source. The novel finding of possible inactivation of erhythrocyte antioxidant enzymes is potentially important and should be retested in similarly designed panel studies and experimental studies. Our analysis methods to detect the GPx-1 and Cu,Zn-SOD subgroups is exploratory as stated, but should be employed in future repeated measures studies to better identify potentially susceptible subgroups, which in this case involved most of the subjects. One limitation of the present study in this regard that should be addressed is the need to assess relationships between health outcomes and exposure to PM from specific sources and exposure to specific component classes such as polycyclic aromatic hydrocarbons. This will require monitoring semivolatile organic components.

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Manuscripts in Preparation

Delfino RJ, Tjoa T, Gillen D, Staimer N, Polidori A, Arhami M, Jamner L, Sioutas C, Longhurst J. Traffic-related air pollution enhances the blood pressure response to physical activity in elderly subjects with coronary heart disease.

Glossary of terms, Abbreviations and Symbols

ACE: angiotensin-converting enzyme
ABPM: ambulatory blood pressure monitoring
BC: black carbon
BP: blood pressure
DBP: diastolic blood pressure
CAD: coronary artery disease
CI: confidence interval
CO: carbon moxide
CRP: C reactive protein
Cu,Zn-SOD: Copper-zinc superoxide dismutase
EC: elemental carbon
F_{inf}: infiltration factors
GPx-1: glutathione peroxidase-1
Hb: hemoglobin
HMG CoA: 3-hydroxy-3-methylglutaryl coenzyme A
IL-6: interleukin-6
IQR: interquartile range
NO_2: nitrogen dioxide
NO_x: nitrogen oxides
O_3: ozone
OC: organic carbon
OC_{pri}: primary OC
PM: particulate matter
$PM_{0.25}$: 0-0.25 μm in diameter
$PM_{0.25-2.5}$: 0.25-2.5 μm in diameter
$PM_{2.5-10}$: 2.5-10 μm in diameter
PM_{10}: particulate matter < 10 μm in aerodynamic diameter
$PM_{2.5}$: particulate matter < 2.5 μm in aerodynamic diameter
PN: particle number
SBP: systolic blood pressure
SOC: secondary organic carbon
sP-selectin: soluble platelet selectin
sTNF-RII: soluble receptor II
TNF-α: tumor necrosis factor-α
UFP: ultrafine particles

Appendices

Appendix A. Concentrated PM and *in vitro* redox assay data.

Table A1. Descriptive results for collection of concentrated PM

Sampling Dates	Sample Type	Concentrated ($\mu g/m^3$)	Estimated Ambient ($\mu g/m^3$)	Total PM Mass Collected (μg)
Group 1, phase 1				
July 21-22, 2005	Fine-indoor	584	29.2	3152
	Fine-outdoor	348	17.4	1880
July 28-29, 2005	Fine-indoor	704	35.2	3800
	Fine-outdoor	345	17.3	1864
August 4-5, 2005	Fine-indoor	208	10.4	1000
	Fine-outdoor	590	29.5	3364
August 11-12, 2005	Fine-indoor	346	17.3	1972
	Fine-outdoor	575	28.7	3276
August 18-19, 2005	Fine-indoor	652	32.6	3716
	Fine-outdoor	966	48.3	5504
Group 2, phase 1				
September 1-2, 2005	Fine-indoor	461	23.0	2212
	Fine-outdoor	182	9.1	876
September 15-16, 2005	Fine-indoor	341	17.1	1844
	Fine-outdoor	362	18.1	1956
September 22-23,2005	Fine-indoor	161	8.0	916
	Fine-outdoor	597	29.8	3580
September 29-30, 2005	Fine-indoor	172	8.6	928
	Fine-outdoor	187	9.3	1008
October 06-07, 2005	Fine-indoor	176	8.8	952
	Fine-outdoor	370	18.5	1996
October 13-14, 2005	Fine-indoor	83	4.2	448
	Fine-outdoor	485	24.3	2620
Group 1, phase 2				
October 27-28, 2005	Fine-indoor	338	16.9	1824
	Fine-outdoor	424	21.2	2292
November 03-04, 2005	Fine-indoor	181	9.1	980
	Fine-outdoor	301	15.1	1628
November 10-11, 2005	Fine-indoor	124	6.2	668
	Fine-outdoor	250	12.5	1348
November 17-18, 2005	Fine-indoor	477	23.9	2576
	Fine-outdoor	169	8.5	912
December 01-02, 2005	Fine-indoor	813	40.7	4392
	Fine-outdoor	810	40.5	4376
December 08-09, 2005	Fine-indoor	250	12.5	1352
	Fine-outdoor	160	8.0	864

Appendix Table A1 (cont.)

Sampling Dates	Sample Type	Concentrated (µg/m3)	Estimated Ambient (µg/m3)	Total PM Mass Collected (µg)
Group 2, phase 2				
January 12-13, 2006	Fine-indoor	456	22.8	2464
	Fine-outdoor	319	15.9	1720
January 19-20, 2006	Fine-indoor	91	4.6	492
	Fine-outdoor	147	7.3	792
January 26-27, 2006	Fine-indoor	213	10.6	1148
	Fine-outdoor	154	7.7	832
February 02-03, 2006	Fine-indoor	451	22.6	2436
	Fine-outdoor	733	36.6	3956
February 09-10, 2006	Fine-indoor	219	10.9	1180
	Fine-outdoor	213	10.6	1148
February 16-17, 2006	Fine-indoor	128	6.4	692
	Fine-outdoor	235	11.7	1268
Group 3, phase 1				
July 06-07, 2006	Ultrafine-indoor	79	4	427
	Ultrafine-outdoor	197	10	1066
July 13-14, 2006	Ultrafine-indoor	117	6	634
	Ultrafine-outdoor	168	8	909
July 20-21, 2006	Ultrafine-indoor	100	5	538
	Ultrafine-outdoor	231	12	1249
July 27-28, 2006	Ultrafine-indoor	133	7	720
	Ultrafine-outdoor	172	9	931
August 03-04, 2006	Ultrafine-indoor	155	8	837
	Ultrafine-outdoor	221	11	1192
August 10-11, 2006	Ultrafine-indoor	156	8	845
	Ultrafine-outdoor	235	12	1268
August 17-18, 2006	Ultrafine-indoor	107	5	580
	Ultrafine-outdoor	219	11	1184
Group 4, phase 1				
August 31-September 01, 2006	Ultrafine-indoor	177	9	954
	Ultrafine-outdoor	200	10	1082
September 14-15, 2006	Ultrafine-indoor	110	5	592
	Ultrafine-outdoor	128	6	693
September 21-22, 2006	Ultrafine-indoor	104	5	562
	Ultrafine-outdoor	157	8	849
September 28-29, 2006	Ultrafine-indoor	92	5	498
	Ultrafine-outdoor	178	9	962
October 05-06, 2006	Ultrafine-indoor	90	5	487
	Ultrafine-outdoor	186	9	1003
October 12-13, 2006	Ultrafine-indoor	109	5	589
	Ultrafine-outdoor	124	6	670

Appendix Table A1 (cont.)

Sampling Dates	Sample Type	Concentrated (µg/m3)	Estimated Ambient (µg/m3)	Total PM Mass Collected (µg)
Group 3, phase 2				
October 26-27, 2006	Ultrafine-indoor	143	7	774
	Ultrafine-outdoor	259	13	1530
November 02-03, 2006	Ultrafine-indoor	164	8	820
	Ultrafine-outdoor	251	13	1281
November 07-08, 2006	Ultrafine-indoor	109	5	662
	Ultrafine-outdoor	113	6	733
November 16-17, 2006	Ultrafine-indoor	124	6	668
	Ultrafine-outdoor	208	10	1124
November 23-24, 2006	Ultrafine-indoor	134	7	672
	Ultrafine-outdoor	149	7	744
Group 4, phase 2				
January 11-12, 2007	Ultrafine-indoor	74	4	312
	Ultrafine-outdoor	100	5	420
January 18-19, 2007	Ultrafine-indoor	64	3	268
	Ultrafine-outdoor	87	4	364
January 25-26, 2007	Ultrafine-indoor	72	4	324
	Ultrafine-outdoor	151	8	680
February 01-02, 2007	Ultrafine-indoor	106	5	572
	Ultrafine-outdoor	108	5	584
February 08-09, 2007	Ultrafine-indoor	77	4	439
	Ultrafine-outdoor	145	7	781
February 15-16, 2007	Ultrafine-indoor	107	5	512
	Ultrafine-outdoor	112	6	637

Table A2. Indoor and outdoor DTT and DHBA activity per PM mass (nmoles/min/µg): Year 1 concentrated $PM_{2.5}$ data.

Sample date	DHBA, indoor	DHBA, outdoor	DTT, indoor	DTT outdoor
East San Gabriel Valley				
Phase 1				
7/15/2005	N/A	N/A	N/A	N/A
7/22/2005	0.011	0.023	0.018	0.028
7/29/2005	0.012	0.011	0.017	0.037
8/05/2005	0.055	0.015	0.034	0.032
8/12/2005	N/A	N/A	0.028	0.019
8/19/2005	N/A	N/A	0.022	0.02
Phase 2				
10/28/2005	0.016	0.013	0.033	0.027
11/04/2005	0.011	0.012	0.033	0.022
11/11/2005	0.037	0.014	0.118	0.049
11/18/2005	0.008	0.013	0.021	0.026
12/02/2005	0.016	0.018	0.025	0.035
12/09/2005	0.026	0.043	0.016	0.068
West San Gabriel Valley				
Phase 1				
9/02/2005	0.008	0.101	0.025	0.184
9/16/2005	0.014	0.039	0.029	0.069
9/23/2005	0.038	0.02	0.15	0.045
9/30/2005	0.03	0.044	0.046	0.077
10/07/2005	0.017	0.016	0.034	0.044
10/13/2005	0.009	0.003	0.076	0.021
Phase 2				
1/13/2006	0.009	0.027	0.051	0.049
1/20/2006	0.013	0.009	0.016	0.021
1/27/2006	0.02	0.036	0.022	0.039
2/3/2006	0.025	0.022	0.018	0.034
2/10/2006	0.017	0.027	0.025	0.061
2/17/2006	0.035	0.023	0.046	0.03

Table A3. Indoor versus outdoor DTT and DHBA activity on a per PM mass (nmoles/min/µg): Year 2 concentrated $PM_{0.15}$ data.

Sample date	DHBA, indoor	DHBA, outdoor	DTT, indoor	DTT outdoor
East San Gabriel Valley				
Phase 1				
07/14/2006	0.004	0.004	0.000	0.000
07/21/2006	0.015	0.007	0.004	0.023
07/28/2006	0.003	0.003	0.000	0.031
08/04/2006	0.007	0.001	0.038	0.000
08/11/2006	0.005	0.003	0.000	0.000
08/18/2006	0.008	0.003	0.034	0.018
Phase 2				
10/27/2006	0.009	0.002	0.033	0.029
11/03/2006	0.011	0.005	0.036	0.041
11/10/2006	0.008	0.012	0.045	0.046
11/17/2006	0.015	0.008	0.030	0.050
12/01/2006	N/A	N/A	N/A	N/A
12/08/2006	N/A	N/A	N/A	N/A
Riverside				
Phase 1				
00/01/2006	0.002	0.002	0.016	0.023
09/15/2006	0.003	0.004	0.030	0.036
09/22/2006	0.005	0.002	0.031	0.022
09/29/2006	0.004	0.002	0.023	0.015
10/06/2006	0.004	0.003	0.030	0.026
10/13/2006	0.004	0.003	0.029	0.034
Phase 2				
01/12/2007	0.024	0.004	0.026	0.014
01/19/2007	0.024	0.144	0.026	0.051
01/26/2007	0.099	0.014	0.046	0.023
02/02/2007	0.229	0.013	0.119	0.017
02/09/2007	0.016	0.009	0.027	0.037
02/16/2007	0.012	0.012	0.024	0.033

Samples for G3P2 week of Dec 1 and Dec 8 were not collected.

Appendix B. Descriptive statistics of air pollutant measurements by retirement community group and seasonal phase.

Table B1. San Gabriel Valley Group 1 descriptive statistics of air pollutant measurements.

Exposure (24-hr averages)	Phase 1				Phase 2				
	N (missing)	Mean (SD)	IQR	Min/Max	N (missing)	Mean (SD)	IQR	Min/Max	IQR overall[a]
Outdoor hourly PM									
EC ($\mu g/m^3$)	42 (2)	1.42 (0.41)	0.6	0.53 / 2.26	40 (6)	1.67 (0.71)	0.95	0.35 / 3.94	0.56
OC ($\mu g/m^3$)	42 (2)	5.00 (1.15)	1.44	3.28 / 8.03	40 (6)	5.22 (1.94)	2.56	2.51 / 12.15	2.07
BC ($\mu g/m^3$)	44 (0)	1.75 (0.51)	0.57	0.76 / 2.92	46 (0)	2.16 (0.85)	1.15	0.90 / 5.11	0.83
OC_{pri} ($\mu g/m^3$)	42 (2)	2.94 (0.73)	0.82	1.28 / 4.74	40 (6)	3.13 (1.21)	1.63	1.04 / 7.08	1.22
SOC ($\mu g/m^3$)	42 (2)	2.02 (0.97)	1.14	0.78 / 5.56	40 (6)	1.97 (1.17)	1.12	0.03 / 4.73	1.02
PN (particle no./cm^3)	42 (2)	13058 (1842)	1697	6960 / 15930	39 (7)	13007 (3419)	3424	6982 / 20737	1915
BAM $PM_{2.5}$	44 (0)	29.83 (5.81)	9.73	20.13 / 39.87	46 (0)	26.76 (20.66)	22.76	6.46/ 89.33	15.04
Indoor hourly PM									
EC uncharacterized ($\mu g/m^3$)	37 (7)	1.43 (0.51)	0.59	0.55 / 2.77	28 (18)	1.44 (0.57)	0.59	0.62 / 2.89	0.60
EC, outdoor origin ($\mu g/m^3$)	42 (2)	0.99 (0.28)	0.31	0.41 / 1.61	38 (8)	1.29 (0.49)	0.66	0.63 / 2.97	0.46
OC uncharacterized ($\mu g/m^3$)	37 (7)	5.72 (0.69)	0.98	4.63 / 7.36	28 (18)	5.29 (1.51)	2.22	3.16 / 8.03	1.41
OC_{pri}, outdoor origin ($\mu g/m^3$)	42 (2)	2.41 (0.60)	0.80	1.07 / 3.86	40 (6)	2.32 (0.91)	1.12	0.76 / 5.21	0.91
SOC, outdoor origin ($\mu g/m^3$)	42 (2)	1.73 (0.83)	0.97	0.68 / 4.79	40 (6)	1.68 (1.01)	0.97	0.03 / 4.07	0.87
PN uncharacterized (/cm^3)	40 (4)	10600 (1796)	2150	6084 / 14655	36 (10)	13277 (3478)	4955.5	6960 / 20778	2940
PN outdoor origin (/cm^3)	33 (11)	8698 (798)	788	6890 / 10341	32 (14)	10146 (2145)	1817	6676 / 15205	1518
BAM $PM_{2.5}$	44 (0)	19.55 (4.06)	6.24	13.00 / 28.00	45 (1)	20.15 (11.49)	11.60	6.59 / 55.92	9.04

Table B1. (cont)	Phase 1				Phase 2				
Exposure (24-hr averages)	**N (missing)**	**Mean (SD)**	**IQR**	**Min/Max**	**N (missing)**	**Mean (SD)**	**IQR**	**Min/Max**	**IQR overall**
Outdoor PM mass									
$PM_{0.25}$ ($\mu g/m^3$)	28 (1)	9.90 (2.68)	3.88	4.99 / 16.85	25 (5)	8.52 (2.62)	3.16	3.31 / 13.43	3.51
$PM_{0.25-2.5}$ ($\mu g/m^3$)	28 (1)	14.80 (4.04)	4.92	7.97 / 21.94	29 (1)	15.71 (17.33)	11.03	2.83 / 66.77	8.57
$PM_{2.5-10}$ ($\mu g/m^3$)	28 (1)	12.08 (2.90)	4.25	8.50 / 19.14	27 (3)	7.99 (3.77)	4.99	2.63 / 16.92	5.45
Outdoor hourly gases									
NO_2 (ppb)	44 (0)	30.46 (8.17)	10.33	11.22 / 46.91	46 (0)	30.43 (8.21)	10.48	17.17 / 50.87	10.26
NO_x (ppb)	44 (0)	40.46 (13.48)	16.76	11.74 / 70.48	46 (0)	56.76 (25.76)	41.87	17.30 / 124.57	27.13
CO (ppm)	38 (6)	0.69 (0.25)	0.27	0.20 / 1.30	46 (0)	0.68 (0.26)	0.37	0.19 / 1.31	0.33
O_3 (ppb)	44 (0)	30.15 (5.67)	6.32	22.71 / 51.48	44 (2)	14.58 (5.10)	5.33	6.17 / 28.91	15.04

[a] This overall interquartile range was used to estimate the expected change in the biomarker (coefficient and 95% CI) from exposure to the air pollutant.

IQR: interquartile range

OC_{pri}: Primary organic carbon

SOC: Secondary organic carbon

Table B2. San Gabriel Valley Group 2 descriptive statistics of air pollutant measurements.

Exposure (24-hr averages)	Phase 1				Phase 2				
	N (missing)	Mean (SD)	IQR	Min/Max	N (missing)	Mean (SD)	IQR	Min/Max	IQR overall[a]
Outdoor hourly PM									
EC ($\mu g/m^3$)	44 (2)	1.66 (0.63)	1.02	0.61 / 3.01	40 (4)	1.69 (0.69)	1.05	0.24 / 3.42	1.05
OC ($\mu g/m^3$)	44 (2)	6.67 (2.43)	3.30	2.85 / 13.60	40 (4)	6.85 (2.06)	2.52	3.55 / 12.48	2.86
BC ($\mu g/m^3$)	46 (0)	1.98 (.72)	1.14	0.57 / 3.37	43 (1)	2.12(.89)	1.15	0.62 / 4.45	1.24
OC_{pri} ($\mu g/m^3$)	44 (2)	3.83 (1.28)	1.94	1.77 / 7.01	40 (4)	3.55 (1.34)	1.95	0.99 / 7.11	1.90
SOC ($\mu g/m^3$)	44 (2)	2.80 (1.75)	1.82	0.62 / 8.10	40 (4)	3.16 (1.64)	1.58	0.57 / 6.91	1.88
PN (particle no./cm^3)	28 (18)	14139 (4935)	5999	6838 / 24302	43 (1)	22952 (5126)	8588	10316 / 31264	10629
BAM $PM_{2.5}$	46 (0)	23.76 (10.06)	16.46	5.42 / 47.40	43 (1)	21.03 (13.77)	18.73	3.31 / 67.42	16.31
Indoor hourly PM									
EC uncharacterized ($\mu g/m^3$)	46 (0)	1.24 (0.47)	0.79	0.33 / 2.17	37 (7)	1.18 (0.51)	0.73	0.19 / 2.31	0.77
EC, outdoor origin ($\mu g/m^3$)	43 (3)	1.07 (0.37)	0.65	0.46 / 1.81	35 (9)	1.11 (0.32)	0.50	0.63 / 2.03	0.58
OC uncharacterized ($\mu g/m^3$)	46 (0)	6.47 (1.40)	1.89	4.17 / 10.75	37 (7)	4.97 (1.81)	2.02	2.34 / 10.79	2.44
OC_{pri}, outdoor origin ($\mu g/m^3$)	44 (2)	2.42 (0.78)	1.19	1.21 / 4.04	40 (4)	1.55 (0.65)	1.00	0.32 / 3.51	0.99
SOC, outdoor origin ($\mu g/m^3$)	44 (2)	2.40 (1.48)	1.57	0.54 / 6.87	40 (4)	2.51 (1.40)	1.53	0.49 / 5.92	1.54
PN uncharacterized (/cm^3)	27 (19)	14292 (9289)	18752	1016 / 32507	41 (3)	19498 (7201)	7666	10797 / 43028	10640
PN outdoor origin (/cm^3)	20 (26)	7042 (3440)	5921	1016 / 12093	39 (5)	12844 (2589)	3445	6085 / 17700	5674
BAM $PM_{2.5}$	46 (0)	16.56 (5.30)	7.97	5.97 / 27.58	36 (8)	10.95 (6.30)	8.37	3.77 / 31.50	10.44

Table B2. (cont)	Phase 1				Phase 2				
Exposure (24-hr averages)	**N (missing)**	**Mean (SD)**	**IQR**	**Min/Max**	**N (missing)**	**Mean (SD)**	**IQR**	**Min/Max**	**IQR overall**
Outdoor PM mass									
$PM_{0.25}$ ($\mu g/m^3$)	25 (5)	9.38 (2.56)	3.48	4.67 / 14.66	28 (2)	9.95 (3.73)	5.72	4.99 / 18.75	4.78
$PM_{0.25-2.5}$ ($\mu g/m^3$)	29 (1)	13.39 (8.21)	12.27	1.64 / 27.05	29 (1)	10.26 (7.88)	9.88	1.29 / 29.33	12.04
$PM_{2.5-10}$ ($\mu g/m^3$)	24 (6)	11.89 (3.55)	4.11	5.76 / 22.38	27 (3)	8.31 (4.21)	6.94	1.76 / 17.20	6.32
Outdoor hourly gases									
NO_2 (ppb)	45 (1)	36.08 (10.92)	18.83	16.87 / 59.83	43 (1)	35.52 (9.76)	12.17	11.96 / 52.48	14.20
NO_x (ppb)	45 (1)	58.09 (26.43)	50.83	18.09 / 112.43	43 (1)	88.12 (39.28)	55.04	17.04 / 188.00	50.65
CO (ppm)	45 (1)	0.64 (0.24)	0.42	0.24 / 1.02	40 (4)	0.85 (0.36)	0.58	0.14 / 1.68	0.48
O_3 (ppb)	45 (1)	21.95 (6.51)	11.35	8.04 / 34.35	43 (1)	21.91 (6.30)	6.87	15.17 / 44.87	7.61

[a] This overall interquartile range was used to estimate the expected change in the biomarker (coefficient and 95% CI) from exposure to the air pollutant.

IQR: interquartile range

OC_{pri}: Primary organic carbon

SOC: Secondary organic carbon

Table B3. San Gabriel Valley Group 3 descriptive statistics of air pollutant measurements.

Exposure (24-hr averages)	Phase 1				Phase 2				
	N (missing)	Mean (SD)	IQR	Min/Max	N (missing)	Mean (SD)	IQR	Min/Max	IQR overall[a]
Outdoor hourly PM									
EC ($\mu g/m^3$)	39 (5)	1.51 (0.44)	0.54	0.65 / 2.44	33 (6)	1.75 (0.71)	0.99	0.64 / 3.27	0.74
OC ($\mu g/m^3$)	39 (5)	6.11 (1.47)	1.95	2.32 / 9.28	35 (4)	11.90 (1.56)	2.07	8.49 / 13.86	6.29
BC ($\mu g/m^3$)	44 (0)	1.48 (0.49)	0.79	0.46 / 2.26	39 (0)	1.80 (.75)	1.06	0.76 / 3.49	0.83
OC_{pri} ($\mu g/m^3$)	39 (5)	3.26 (0.80)	1.01	1.65 / 4.86	33 (6)	8.24 (1.34)	1.78	6.13 / 11.07	4.64
SOC ($\mu g/m^3$)	39 (5)	2.85 (1.11)	1.32	0.28 / 4.72	33 (6)	3.70 (1.39)	2.00	1.37 / 6.00	1.64
PN (particle no./cm^3)	27 (17)	6281 (2613)	3950	1441 / 10184	33 (6)	12629 (3612)	3683	3296 / 20808	6802
BAM $PM_{2.5}$	44 (0)	20.84 (5.44)	6.36	8.92 / 31.54	39 (0)	21.04 (13.05)	23.38	5.97 / 54.52	11.48
Indoor hourly PM									
EC uncharacterized ($\mu g/m^3$)	43 (1)	1.26 (0.42)	0.62	0.78 / 2.36	36 (3)	1.39 (0.49)	0.67	0.64 / 2.76	0.72
EC, outdoor origin ($\mu g/m^3$)	38 (6)	0.92 (0.28)	0.37	0.44 / 1.57	31 (8)	1.23 (0.47)	0.73	0.48 / 2.15	0.57
OC uncharacterized ($\mu g/m^3$)	43 (1)	4.45 (1.05)	1.51	2.37 / 7.07	38 (1)	10.35 (2.12)	2.66	6.09 / 15.27	5.69
OC_{pri}, outdoor origin ($\mu g/m^3$)	38 (6)	1.39 (0.66)	0.87	0.07 / 2.51	33 (6)	5.97 (1.56)	1.70	1.74 / 8.85	4.49
SOC, outdoor origin ($\mu g/m^3$)	38 (6)	2.32 (0.87)	0.92	0.24 / 3.97	33 (6)	3.17 (1.20)	1.77	1.18 / 5.14	1.46
PN uncharacterized (/cm^3)	40 (4)	2770 (1404)	2184	681 / 6438	37 (2)	5375 (1048)	1059	3237 / 7546	2923
PN outdoor origin (/cm^3)	24 (20)	2096 (1158)	2292	598 / 3831	32 (7)	4532 (1142)	1393	1420 / 7040	2707
BAM $PM_{2.5}$	44 (0)	9.19 (2.59)	3.14	3.65 / 14.02	39 (0)	11.61 (6.12)	8.98	4.17 / 24.62	4.83

Table B3. (cont)	Phase 1				Phase 2				
Exposure (24-hr averages)	**N (missing)**	**Mean (SD)**	**IQR**	**Min/Max**	**N (missing)**	**Mean (SD)**	**IQR**	**Min/Max**	**IQR overall**
Outdoor PM mass									
$PM_{0.25}$ ($\mu g/m^3$)	28 (3)	10.35 (3.86)	5.10	3.16 / 22.02	23 (0)	11.06 (6.09)	9.85	4.69 / 30.05	6.87
$PM_{0.25-2.5}$ ($\mu g/m^3$)	29 (2)	11.20 (5.05)	5.11	3.37 / 22.84	23 (0)	11.19 (11.29)	14.98	0.98 / 42.71	9.97
$PM_{2.5-10}$ ($\mu g/m^3$)	29 (2)	9.93 (3.28)	5.48	4.13 / 15.17	23 (0)	8.56 (5.75)	7.19	0.30 / 24.63	6.78
Outdoor hourly gases									
NO_2 (ppb)	44 (0)	25.73 (9.03)	16.09	9.43 / 39.96	39 (0)	32.68 (9.07)	10.22	17.17 / 55.74	14.17
NO_x (ppb)	44 (0)	34.14 (13.92)	23.80	11.30 / 60.30	39 (0)	52.25 (21.45)	29.35	20.22 / 115.65	28.83
CO (ppm)	44 (0)	0.41 (0.13)	0.23	0.20 / 0.70	39 (0)	0.56 (0.18)	0.21	0.26 / 1.01	0.25
O_3 (ppb)	44 (0)	43.69 (10.65)	10.20	22.13 / 76.35	39 (0)	17.15 (5.80)	8.13	7.04 / 33.70	27.96

[a] This overall interquartile range was used to estimate the expected change in the biomarker (coefficient and 95% CI) from exposure to the air pollutant.

IQR: interquartile range

OC_{pri}: Primary organic carbon

SOC: Secondary organic carbon

Table B4. Riverside (Group 4) descriptive statistics of air pollutant measurements.

Exposure(24-hr averages)	Phase 1				Phase 2				IQR overall[a]
	N(missing)	Mean(SD)	IQR	Min/Max	N(missing)	Mean(SD)	IQR	Min/Max	
Outdoor hourly PM									
EC(μg/m^3)	36(10)	1.15(0.45)	0.59	0.58 / 2.34	26(18)	0.91(0.34)	0.43	0.36 / 1.78	0.51
OC(μg/m^3)	39(7)	14.22(5.21)	9.25	8.44 / 27.26	26(18)	15.59(1.52)	2.30	12.41 / 17.72	5.96
BC(μg/m^3)	46(0)	1.15(.46)	0.61	0.38 / 2.26	44(0)	0.94(.51)	0.67	0.30 / 3.04	650.50
OC$_{pri}$(μg/m^3)	36(10)	7.84(0.96)	1.40	6.66 / 10.04	26(18)	11.48(0.84)	1.09	10.11 / 13.64	3.74
SOC(μg/m^3)	36(10)	6.68(5.70)	10.03	0.42 / 18.74	26(18)	4.12(1.47)	2.35	1.06 / 6.67	4.21
PN(particle no./cm^3)	36(10)	6899(1094)	1144	4113 / 8494	37(7)	9362(2123)	2696	4688 / 13442	2494
BAM $PM_{2.5}$	46 (0)	21.53 (8.16)	12.46	37.33 / 7.74	44 (0)	13.20 (9.28)	11.67	2.46 / 45.40	14.50
Indoor hourly PM									
EC uncharacterized(μg/m^3)	28(18)	1.34(0.25)	0.23	0.71 / 1.92	40(4)	1.03(0.27)	0.36	0.57 / 1.95	0.41
EC, outdoor origin(μg/m^3)	23(23)	0.86(0.29)	0.42	0.42 / 1.46	24(20)	0.72(0.27)	0.33	0.29 / 1.46	0.40
OC uncharacterized(μg/m^3)	31(15)	8.36(1.72)	3.36	5.55 / 11.23	40(4)	14.24(2.23)	3.22	8.52 / 18.10	6.63
OC$_{pri}$, outdoor origin(μg/m^3)	26(20)	3.05(2.14)	4.51	0.01 / 5.62	24(20)	10.13(0.80)	1.08	8.74 / 12.30	6.06
SOC, outdoor origin(μg/m^3)	26(20)	3.83(3.27)	4.87	0.34 / 11.11	24(20)	3.50(1.28)	1.89	0.91 / 5.73	3.05
PN uncharacterized(/cm^3)	37(9)	4539(1053)	1105	2183 / 6690	43(1)	4577(1317)	1418	2764 / 9035	1345
PN outdoor origin(/cm^3)	34(12)	3472(540)	770	2288 / 4206	37(7)	3382(636)	696	1966 / 4976	759
BAM $PM_{2.5}$	46 (0)	9.05 (2.81)	4.14	3.30 / 14.04	37 (7)	5.61 (2.49)	3.51	2.17 / 11.85	5.24

Table B4.(cont)	Phase 1				Phase 2				
Exposure(24-hr averages)	**N(missing)**	**Mean(SD)**	**IQR**	**Min/Max**	**N(missing)**	**Mean(SD)**	**IQR**	**Min/Max**	**IQR overall**
Outdoor PM mass									
$PM_{0.25}$($\mu g/m^3$)	30(0)	11.29(4.89)	7.30	4.84 / 22.82	30(0)	7.81(4.50)	6.64	2.46 / 22.96	6.36
$PM_{0.25-2.5}$($\mu g/m^3$)	29(1)	9.61(6.41)	8.89	3.02 / 27.78	30(0)	5.07(3.81)	4.74	1.05 / 15.43	7.31
$PM_{2.5-10}$($\mu g/m^3$)	29(1)	12.02(7.20)	8.36	1.15 / 23.41	30(0)	4.62(2.55)	3.26	0.46 / 11.15	8.46
Outdoor hourly gases									
NO_2(ppb)	46(0)	13.73(5.83)	7.59	4.52 / 31.78	44(0)	15.30(8.37)	10.13	3.78 / 43.35	8.65
NO_x(ppb)	46(0)	16.48(7.45)	11.48	3.70 / 37.52	44(0)	18.77(11.90)	13.83	4.26 / 65.30	11.78
CO(ppm)	46(0)	0.29(0.13)	0.22	0.11 / 0.54	37(7)	0.17(0.16)	0.17	0.01 / 0.83	0.18
O_3(ppb)	46(0)	37.49(8.48)	10.42	20.22 / 55.70	44(0)	28.48(6.83)	10.50	13.83 / 40.43	10.43

[a] This overall interquartile range was used to estimate the expected change in the biomarker(coefficient and 95% CI) from exposure to the air pollutant.

IQR: interquartile range

OC_{pri}: Primary organic carbon

SOC: Secondary organic carbon