Supplemental Reports to *Accomplishments of the Particulate Matter (PM) Centers (1999-2005)*

The following pages contain three integrated reports written by work groups composed of researchers from all five original PM Centers on PM Health Effects Research, Mechanisms of PM Effects, and PM Exposure Research.

PM Health Effects Research: Supplement to *Accomplishments of the Particulate Matter (PM) Centers (1999-2005)*

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This EPA Website document summarizes the progress made by the EPA PM Centers in identifying and understanding the health effects of PM air pollution since the mid-course report prepared in 2002 (Lippmann et al., 2003).

When the PM Centers were designated in 1999, the primary body of evidence for health effects of PM consisted of epidemiologic studies of associations of short-term PM concentrations with daily mortality and long-term average PM with long-term mortality. In the first six years of the PM Centers program there was substantial work to understand and assess potential flaws and weaknesses in this body of evidence, and to develop an understanding of the mechanisms underlying these associations. In the more recent work, the PM Centers research has substantially expanded the range of clinical and preclinical health effects indicators associated with PM exposures. With respect to toxicological studies, both in experimental animals and *in vitro*, increasing efforts were devoted to using real-world particles and exposures (e.g., use of ambient PM concentrators) and mimicking human conditions of compromised organ functions in animals for evaluating PM effects. While interest has continued in measuring the respiratory effects of PM, much of the recent research has focused on identifying and understanding the cardiovascular health effects of $PM_{2.5}$ exposures.

MORTALITY

The Harvard Six Cities and the American Cancer Society Cancer Prevention II (ACS) prospective cohort studies provided some of the most important evidence to support the 1997 and 2006 $PM_{2.5}$ annual average NAAQS. Extended mortality follow-up of the ACS cohort for nine more years more than doubled the number of deaths observed. The extended ACS cohort follow-up verified that cardiopulmonary mortality was significantly associated with $PM_{2.5}$, that excess annual mortality was not associated with larger particles, and that there was a significant association of $PM_{2.5}$ with lung cancer (Pope et al., 2002). The extended follow-up of the Harvard Six-Cities cohort for eight more years approximately doubled the number of deaths observed (Laden et al., 2006). $PM_{2.5}$ concentrations for the extended follow-up years were estimated from PM_{10} and

visibility measures. $PM_{2.5}$-mortality associations were observed for all-cause mortality equivalent to those found in the original analysis. However, $PM_{2.5}$ concentrations were substantially lower in the extended follow-up period than in the original analysis, especially for two of the most polluted cities. Reductions in $PM_{2.5}$ concentrations were associated with reduced mortality risk, and were largest in the cities with the largest declines in $PM_{2.5}$ concentrations. These results suggest that improvements in air quality lead to extended life expectancy. The extended ACS cohort analyses also examined the contributions of each subcategory of excess cardiopulmonary deaths to the total excess, and reported that a very large percentage was due to cardiovascular causes (Pope et al., 2004), stimulating an increase in PM Center focus on cardiovascular effects research. In addition, a recent study, based on 65,893 postmenopausal women in 36 US cities in the Women's Health Initiative (WHI) cohort (Miller et al., 2007) reported that the risk of death from cardiovascular disease was significantly associated with chronic exposure to $PM_{2.5}$.

CARDIOVASCULAR MORTALITY AND MORBIDITY

There is a large body of literature showing associations of cardiovascular mortality with PM. Recent studies have attempted to better understand the pathways of these associations through examination of associations with specific cardiac events, measurements of preclinical markers of cardiac events and risk for such events, and the development and application of technologies for laboratory studies focused on the environmental and host factors that cause or contribute to the expression of adverse cardiovascular events.

Cohort Studies

In the extended follow-up of the Harvard Six Cities cohort, the strongest associations were seen with cardiovascular mortality, specifically a 28% increased risk of cardiovascular mortality associated with each 10 $\mu g/m^3$ increase in average $PM_{2.5}$ (Laden et al., 2006). Moreover, the strongest indication of benefits was also seen with cardiovascular mortality. For each 10 $\mu g/m^3$ reduction in $PM_{2.5}$ in the extended follow-up, there was an estimated 69% lower cardiovascular mortality.

In analysis of cause-of-death data in the ACS study, (Pope et al., 2004) long-term $PM_{2.5}$ exposures were most strongly associated with mortality attributable to cardiovascular causes, specifically ischemic heart disease, dysrhythmias, heart failure, and cardiac arrest. For these cardiovascular causes of death, a 10-$\mu g/m^3$ elevation in $PM_{2.5}$ was associated with 8% to 18% increases in mortality risk. This suggests that $PM_{2.5}$ is a risk factor for cause-specific cardiovascular disease mortality via mechanisms that likely include systemic inflammation, accelerated atherosclerosis, and altered cardiac autonomic function.

In the WHI cohort (Miller et al., 2007), a 10-$\mu g/m^3$ increase in $PM_{2.5}$was associated with a 76% increase in CVD death, as well as a 24% increase in a CVD event (coronary revascularization, myocardial infarction, and stroke, as well as death).

Time-Series Studies

While the investigation of specific causes of death provides insights into possible pathways, there is a cost in reduced statistical power to detect associations because of smaller numbers of events. One approach to reduce this loss of power is to pool event data across multiple cities. In analysis of daily mortality in 20 US cities all-cause and cardiovascular mortality was positively associated with PM_{10} on the previous two days (Zeka et al., 2005). Hospital emergency visits for myocardial infarction among the elderly in 21 US cities increased by 0.65% associated with each 10 $\mu g/m^3$ increase in ambient PM_{10} (Zanobetti and Schwartz, 2005).

Other studies have also assessed associations with myocardial infarctions. Case-crossover analyses of diary data collected in 691 myocardial infarction survivors indicated that time spent in traffic was associated with an almost three-fold increased risk of a myocardial infarction 1 hour later (odds ratio 2.9) (Peters et al., 2004). Time spent in cars, on public transportation, or on motorcycles or bicycles were each linked with an increase in the risk of myocardial infarction, suggesting a specific link to traffic pollution. On the other hand, case-crossover analysis of almost 6,000 confirmed myocardial infarctions in Seattle (Sullivan et al., 2005a) found no increased risk associated with particle concentrations measured by nephelometry in the previous 24 hours.

If PM is associated with ischemic cardiac events, then we should also expect associations with other ischemias. In Boston, increased ambient particle levels were associated with transiently increased risk of ischemic but not hemorrhagic stroke (Wellenius et al., 2005).

Cardiac readmissions among myocardial infarction survivors in five European cities increased in association with concentrations of PM_{10} particle number, and also with CO, NO_2 and O_3 concentrations (von Klot et al., 2005). PM_{10}, CO, NO_2 and SO_2 were associated with the rate of hospital admissions for congestive heart failure in Pittsburgh (Wellenius et al., 2005). Patients with recent myocardial infarction were at the greatest risk of particle related admissions.

In summary, studies of cause-specific daily mortality, hospital admissions, and emergency department visits show associations of PM with more specific indicators of cardiovascular events, including myocardial infarction, readmission following myocardial infarction, congestive heart failure, and ischemic stroke.

INTERMEDIATE MARKERS OF CARDIOVASCULAR EFFECTS

Given the evidence that the frequency of clinical cardiovascular events is associated with PM, studies of physiological, preclinical markers of cardiovascular disease provide evidence of health effects and insights into potential pathways. Repeated measures studies have analyzed the effects of ambient PM on a wide range of pre-clinical markers of health response among healthy or compromised subjects in the community in both clinical experiments and animal models. These studies have considered a range of potential pathways: including cardiac arrhythmias; autonomic function measured by heart rate (HR) and heart rate variability (HRV); vascular changes, including blood pressure and vascular reactivity; and inflammatory markers.

Cardiac Arrhythmias

Population Studies: The observed associations of PM with sudden cardiac death suggest a possible role of cardiac arrhythmias. Cardiac patients in Boston with implanted cardioverter defibrillators followed for an average of three years (Dockery et al., 2005) had higher rates of ventricular arrhythmias associated with air pollution over the previous two calendar days. Significant associations were found only for patients who had a previous arrhythmia within the previous three days. A case-crossover analysis of these data (Rich et al., 2005) found $PM_{2.5}$ concentrations in the prior 24 hours was significantly associated with higher risk of ventricular arrhythmias.

In a panel study of elderly Boston residents (Gold et al., 2005), a decreased ST segment in the ECG (an indicator of possible myocardial ischemia or inflammation) was associated with increased ambient concentrations of particles, specifically of BC.

Laboratory Animal Studies: Animal studies have found similar associations. In rats with a surgically induced myocardial infarction, there were marked increases in post-MI arrhythmias (premature ventricular complexes) following exposure to residual oil fly ash (ROFA) in animals with a history of arrhythmias, compared to those exposed to room air (Wellenius et al., 2002). Aged rats showed a significant increase in the frequency of irregular delayed beats after exposure to CAPs, which was not seen after exposures to ultrafine carbon, to air, or to SO_2 (Nadziejko et al., 2004).

Ambient air pollution is a complex mixture of PM and gaseous co-pollutants, such as CO. Rats with induced myocardial infarction were exposed (1 hour) to either filtered air, CO alone, CAPs alone, or CAPs and CO (Wellenius et al., 2004). CO exposure significantly reduced ventricular premature beat frequency, while CAPs exposure increased VPB frequency during the exposure period, although this effect did not reach statistical significance, and was modified by the number of pre-exposure VPBs. Neither CAPs nor CO had any effect on HR, but CAPs increased HR in specific subgroups.

Autonomic Control of the Heart

Population Studies: Studies in both humans and animals have shown $PM_{2.5}$ to be associated with autonomic control of the heart as measured by HR and HRV, a measure of sympathetic/parasympathetic tone and autonomic function. These complementary findings in human panel and controlled CAPs exposure studies demonstrate that increased levels of $PM_{2.5}$ are able to perturb cardiac autonomic function, which may lead to adverse cardiovascular outcomes.

In a panel of elderly subjects in Boston seen repeatedly over twelve weeks, $PM_{2.5}$ was associated with decreased HRV (Schwartz et al., 2005a). Specific associations were found with BC, an indication of traffic particles, and with secondary particles, an indicator of long-range transport. In a panel of 56 men with ischemic heart disease in Erfurt, Germany, changes in repolarization of the heart were associated with $PM_{2.5}$ and also with OC and EC concentrations in the ambient air (Henneberger et al., 2005). A panel study of eighty-eight older healthy or subjects with lung or heart disease in Seattle (Mar et al., 2005) found associations between decreased HR and $PM_{2.5}$ in healthy subjects. However, a study of elderly subjects with and without cardiovascular conditions in Seattle showed no association between outdoor PM measured by nephelometry and HRV (Sullivan et al., 2005b).

In chamber exposure studies of 12 healthy adult and twelve asthmatic volunteers exposed to filtered air or CAPs (~200 $\mu g/m^3$) for 2 hours, both healthy and asthmatic subjects showed CAPs-related parasympathetic stimulation of HRV (Gong et al., 2003). Acute exposure to elevated concentrations of ambient CTPM also altered the autonomic nervous system of the heart in adult volunteers (Gong et al., 2004).

UFP in ambient air may play a role in cardiovascular effects. In randomized controlled studies (Zareba et al., in press) healthy subjects exposed to filtered air and to EC UFP for two hours with intermittent exercise showed a small, but not clinically significant increase in parasympathetic tone and a shortening of the late-corrected QT interval after exposure. Overall, exposures of healthy subjects to EC UFP at these concentrations did not cause clinically important changes in ECG-derived parameters.

One interpretation of these studies is that the cardiovascular effects of $PM_{2.5}$ are seen only in susceptible subgroups of the population. In a study of approximately 500 older men in Boston, long term average $PM_{2.5}$ was associated with decreased HRV, and these associations were stronger among patients with a history of ischemic heart disease, hypertension, and diabetes (Park et al., 2005). In further analyses of these subjects, Schwartz et al. (2005b) found the association between lower high frequency HRV measurements with high $PM_{2.5}$ was reduced in those subjects without the GSTM1 allele (an indication of genetic susceptibility) and those taking statins. Obese patients and patients with high neutrophil counts had increased response to $PM_{2.5}$. This suggests that the effects of $PM_{2.5}$ might be mediated by ROS.

Laboratory Animal Studies: Adult rats exposed by intratracheal instillation of urban air particles or by inhalation of Boston CAPs were evaluated for cardiac dysfunction (Rhoden et al., 2005). HR increased immediately after PM exposure (by instillation or inhalation) but returned to basal levels within 30 min. HRV was unchanged immediately after exposure, but significantly increased during the recovery phase. Pretreatment of the rats with N-acetylcysteine (NAC), a stimulant of glutathione (a powerful antioxidant) production, prior to PM exposure prevented changes in HR and HRV.

Animal studies in the PM Centers have focused on animal models of chronic disease thought to increase susceptibility to PM effects. In spontaneously hypertensive rats, exposure to CAPs caused a decrease in respiratory rate soon after the start of exposure, and stopped when exposure to CAPs ceased (Nadziejko et al., 2002a). The decrease in respiratory rate was accompanied by a decrease in HR.

In Tuxedo, NY, Hwang et al. (2005) found sub-chronic (five month) exposures in the summer of 2003 to CAPs in mice with genetic predisposition to atherosclerotic disease (lacking apoliproprotein, ApoE-/-) produced decreased HR, body temperature, and physical activity (Hwang et al. 2005). No associations were found in normal (C57BL/6) mice. Chen and Hwang (2005) found that the cardiac autonomic function in these ApoE-/- mice was affected by these subchronic CAPs exposures, involving a perturbation of the homeostatic function (parasympathetic/sympathetic balance) in the cardiovascular system (initial enhancement and later depression of the HRV parameters). In a 6-month follow-up subchronic CAPs inhalation study, the $ApoE^{-/-}$ mice were exposed for 6 hours/day to CAPs at an average concentration of 85 $\mu g/m^3$. The av. Ni concentration was 43 ng/m^3 but, on 14 days, there were Ni peaks at ~175 ng/m^3, and unusually low FPM and V. Back-trajectory analyses identified a remote Ni point source.

ECG measurements showed Ni was the only component that was significantly associated with acute changes in HR and HRV (Lippmann et al., 2006).

In Seattle, aged ApoE-/- mice exposed intranasally to saline, local $PM_{2.5}$ or silica (Min-u-Sil 5) showed an initial increase in both HR and activity in all groups, but delayed bradycardia, with no change in activity of the animals in the PM and silica exposed groups. In addition, with PM and silica exposure, there was a decrease in HRV parameters suggesting a decrease in parasympathetic tone, which may lead to cardiac arhythmia (Corey et al., 2005).

In Boston, after exposure to CAPs or filtered air, dogs underwent reversible 5-min coronary artery occlusion (Wellenius et al., 2003). ST-segment elevation during occlusion, a measure of myocardial ischemia, was significantly enhanced following CAPs exposure, compared to filtered air exposures. ST-segment elevation was significantly correlated with crustal elements, possibly associated with urban street dust, but not with CAPs mass or number concentrations. HR was not affected by the CAPs exposures.

Vascular Changes

The observed cardiovascular effects of PM air pollution also may be mediated through vascular changes. Delfino et al. (2005) suggested that UFP may be specifically associated with vascular changes based on PN and surface area.

Population Studies: In cardiac patients undergoing rehabilitation therapy in Boston, ambient $PM_{2.5}$ was associated with increased blood pressure (Zanobetti et al., 2004). A panel study of 88 older healthy or subjects with lung or heart disease in Seattle (Mar et al., 2005) found a small increase in systolic blood pressure associated with $PM_{2.5}$ concentrations in the healthy subjects. In the chamber exposure studies of Gong CAPs exposure among healthy adults, but decreased among asthmatic subjects (Gong et al., 2003). to filtered air or CAPs, systolic blood pressure was modestly increased during CAPs exposure among healthy adults, but decreased among asthmatic subjects.

O'Neill et al. (2005) reported that air pollution in the six days prior to an examination was associated with decreased vascular re-activity, and brachial artery diameter responses among diabetic patients, but not among non-diabetic patients in Boston. Studies in rats (Batalha et al., 2002) and mice (Lemos et al., 2006) showed morphometric measures of vasoconstriction in the lung and heart.

Animal Studies: Exposure of aged male Fischer-344 rats in a mobile laboratory for 6 hours to the traffic aerosol on the New York State Thruway between Rochester and Buffalo resulted in significant increases in plasma endothelin-2, a vasoconstricting protein (Elder et al., 2004). Changes in fibrinogen as an acute phase protein were also observed.

Systemic Inflammation

Population Studies: $PM_{2.5}$ air pollution may also act through increased systemic inflammation. Repeated blood samples of 57 male patients with coronary heart disease from Germany (Ruckerl et al., 2006), found increased C reactive protein, an indicator of acute systemic inflammation, that was associated with $PM_{2.5}$.

In the chamber studies of Gong et al. (2003), systemic effects were assessed in healthy adult and asthmatic volunteers exposed for 2 hours to filtered air or CAPs. Both

groups showed CAPs-related increases in certain blood mediators of inflammation, i.e., soluble intercellular adhesion molecule (ICAM-1) and interleukin-6 (IL-6). However, healthy and asthmatic subjects exposed to EC UFP (~25 nm) for 2 hours with intermittent exercise showed no significant effects on measures of systemic inflammation, including serum amyloid A (SAA), IL-6, and soluble ICAM-1 (Pietropaoli et al., 2004).

Laboratory Animal Studies: Clarke et al. (2000) exposed dogs to CAPs from the Boston area. There was no effect of total PM mass on indicators of systemic inflammation, but factor analysis suggested that specific $PM_{2.5}$ components were associated with inflammatory cell responses (peripheral white blood cell, circulating neutrophils, and circulating lymphocytes) in the blood.

At the end of the third subchronic CAPs inhalation study in Tuxedo, NY in $ApoE^{-/-}$ mice, there were: enhanced vasoconstrictor responses to phenylephrine and serotonin challenge in the thoracic aorta; attenuated relaxation to the endothelium dependent agonist acetylcholine; and marked increases in macrophage infiltration (CD68 Staining), inducible isoform of nitric oxide synthase (iNOS), increased generation of reactive oxygen species, and greater immunostaining for the protein nitration product 3-nitrotyrosine (Sun et al., 2005).

Atherosclerosis

Atherosclerosis is a progressive irreversible condition and an underlying cause of many cardiovascular diseases.

Population Studies: In epidemiologic studies, long-term exposure to $PM_{2.5}$ has been reported to be associated with atherosclerosis measured by carotid intimal media thickness (Kunzli et al., 2005).

Laboratory Animal Studies: Chen and Nadziejko (2005) found that sub-chronic exposure of mice prone to the development of atherosclerotic lesions exposed for five months to CAPs had significantly increased percentage of aortic intimal surface covered by atherosclerotic lesions, and increased plaque cellularity, pointing to PM associated atherosclerosis as a potentially important pathway for the observed cardiovascular effects of long-term $PM_{2.5}$ exposure. In a follow-up study lasting six months, Sun et al. (2005) reported enhanced atherogenesis with accompanied increases in lipid content (ORO staining).

Myocardial Infarction

An association between exposure to vehicular traffic in urban areas and the exacerbation of CVD was suggested in a case-crossover study that was designed to assess whether exposure to traffic effluents can trigger myocardial infarction. Cases of myocardial infarction were identified with the use of data from the Cooperative Health Research in the Region of Augsburg Myocardial Infarction Registry in Augsburg, in southern Germany. (Peters et al., 2004) Subjects that were included had survived for at least 24 hours after the event, completed the registry's standardized interview, and provided information on factors that may have triggered the myocardial infarction. An association was found between exposure to traffic and the onset of a myocardial infarction within one hour afterward (odds ratio, 2.92; 95 percent confidence interval, 2.22 to 3.83; $p<0.001$). The time the subjects spent in cars, on public transportation, or on motorcycles or bicycles was consistently linked with an increase in the risk of myocardial

infarction. Adjusting for the level of exercise on a bicycle or for getting up in the morning changed the estimated effect of exposure to traffic only slightly (odds ratio for myocardial infarction, 2.73; 95 percent confidence interval, 2.06 to 3.61; $p<0.001$). The subject's use of a car was the most common source of exposure to traffic; nevertheless, there was also an association between time spent on public transportation and the onset of a myocardial infarction one hour later. Thus, transient exposure to traffic may increase the risk of myocardial infarction in susceptible persons.

Coagulation Studies

Adverse effects of inhaled PM may be the indirect result of a PM-induced increase in blood coagulability.

Population Studies: Healthy and asthmatic subjects inhaling EC UFP showed no effects of inhalation on measures of coagulation (platelet count, serum fibrinogen, factor VII, or Von Willibrand's factor antigen) in venous blood (Pietropaoli et al., 2004). Thus there is no evidence from the PM Centers for PM acting through this pathway.

Laboratory Animal Studies: Blood samples were collected from healthy rats before, and 0, 6, and 12 hours after 6 hours of exposure to filtered air or CAPs. Prothrombotic changes in blood coagulation parameters (platelet count, fibrinogen level, factor VII activity, thrombin-antithrombin complex [TAT] level, tissue plasminogen activator [tPA] activity, and plasminogen activator inhibitor [PAI] activity) were not associated with CAPS exposures (Nadziejko et al., 2002b).

RESPIRATORY EFFECTS

Since the lungs represent the portal of entry and the first line of defense, early studies focused on the respiratory effects of PM air pollution. Investigations continue into the respiratory health effects of PM, particularly among children with developing lungs and among adults and children with chronic respiratory disease.

Respiratory Mortality and Morbidity

In cause-of-death data in the ACS cohort study (Pope et al., 2004), long-term $PM_{2.5}$ exposures were only weakly associated with respiratory causes of death. Similarly, in the Harvard Six Cities cohort study (Laden et al., 2006), the number of respiratory deaths remained small in the extended follow-up, and their associations with $PM_{2.5}$ were weak, and not statistically significant.

Respiratory mortality was positively associated with PM_{10} exposure on the same and previous two days in an analysis of daily mortality in twenty US cities (Zeka et al., 2005). Respiratory emergency room visits in Spokane were associated with $PM_{2.5}$ but not with the coarse fraction (Slaughter et al., 2005). No association was found with cardiac or respiratory hospital admissions.

Asthma

PM exposures have been associated with acute and chronic respiratory health effects. Recent studies have focused on the effects of PM on the developing respiratory

system of children, and potential associations with the development of asthma. Children living in Southern California exposed to higher levels of PM $PM_{2.5}$ and PM_{10} had reduced rates of growth and reduced attained level of FEV_1 by age 18 (Gauderman et al., 2004). In a study of 208 children in Southern California with NO_2 measured outside their homes, the rates of respiratory illness or asthma were associated with outdoor NO_2 levels and proximity to freeways, suggesting asthma is associated with fresh traffic related air pollutants (Gauderman et al., 2005). This was supported in a survey of 5,341 kindergarten and 1st grade school children in Southern California, which showed residence within 75 m of a major road was associated with increased risk of asthma and wheeze (McConnell et al., 2006).

Air pollution may exacerbate disease and/or decrease lung function in patients with chronic respiratory disease. In a panel of asthmatic children in Southern California, FEV_1 decreased with increasing $PM_{2.5}$ exposures in the prior 24 hours (Delfino et al., 2004). In a panel study of asthmatic adults and children in Spokane, WA, sputum production, runny nose and cough was associated with various measures of PM in the children, but not in adults (Mar et al., 2004). These studies suggest that both coarse and fine particles may aggravate asthma symptoms.

In an analysis of a registry of cystic fibrosis patients across the US, higher average PM air pollution concentrations in their communities was associated with increased risk of pulmonary exacerbations and lower lung function in the cystic fibrosis patients (Goss et al., 2004).

Airway Inflammation

Recent epidemiologic studies and animal and human inhalation studies have shown that short-term ambient $PM_{2.5}$ may have inflammatory effects in the lungs.

In panels of elderly subjects, ambient $PM_{2.5}$ was associated with increased exhaled NO (Adamkiewicz et al., 2004), an indicator of inflammation in the lungs. Koenig et al. (2005) found exhaled NO was associated with personal exposures to ambient outdoor PM, but not to indoor generated PM.

In the human clinical studies, Ghio et al. (2000) found modest increases in inflammatory cells in bronchoalveolar lavage fluid from healthy subjects after inhalation of CAPs from the Chapel Hill, NC area. In contrast, Gong et al. (2004) found no increases in inflammatory cells in induced sputum or changes in pulmonary function following $PM_{2.5}$ CAPs inhalation, in young healthy or asthmatic subjects or in healthy elderly or subjects with COPD. Similarly, no inflammatory effects were observed with exposures to CTPM (Gong et al., 2004).

Pietropaoli et al. (2004) exposed subjects to 10 to 25 $\mu g/m^3$ EC UFP for 2 hours, with intermittent exercise. They found no effects on sputum inflammatory cells, pulmonary function, or exhaled NO. Additional studies at 50 $\mu g/m^3$ also showed no changes in pulmonary function or exhaled NO.

Clarke et al. (2000) exposed dogs to CAPs from the Boston area. There was no effect of total PM mass on indicators of airway inflammation, but factor analysis suggested that specific PM components were associated with specific inflammatory cell responses in the lung.

Pulmonary Vascular Effects

PM exposure may cause pulmonary vasoconstriction. In randomized, double-blind studies, healthy and asthmatic subjects exposed to EC UFP (50 $\mu g/m^3$) showed decreased pulmonary diffusing capacity for CO at 21 hours after exposure, a finding consistent with decreased pulmonary capillary blood volume in response to UFP (Frampton et al., 2006).

NERVOUS SYSTEM EFFECTS

Effects of ambient air PM on the nervous system were produced in $ApoE^{-/-}$ mice undergoing six months of 30 hr/wk subchronic CAPs inhalation exposures in Tuxedo, NY at an av. concentration of 110 $\mu g/m^3$. This strain of mice has elevated oxidative stress in the brain. At the end of the exposure sequence, the neurons from the substantia nigral nucleus compacta were significantly reduced (29%) as compared to the sham-exposed mice, and there was a significant increase in immunocytochemically stained astrocytes (Veronesi et al., 2005). Gene expression analyses on these mice (Gunnison and Chen, 2005) showed exposure-related changes indicative of alteration in circadian rhythm that could be related to the changes in the brain. These unanticipated changes associated with subchronic CAPs inhalation exposures at environmentally relevant concentrations indicate a need for further research on the effects of $PM_{2.5}$ on the central nervous system.

Inhalation by rats of EC UFP using ^{13}C for six hours showed significant and persistent increases of ^{13}C in the olfactory bulbs over 1 to 7 days post-exposure, with other brain regions showing no or less and inconsistent increases (Oberdörster et al., 2004). The authors attributed this to a direct access of the inhaled UFP to the CNS from deposits in the nose *via* the olfactory nerve.

CONCLUSIONS

The research conducted at the EPA PM Centers on health effects has provided a substantial body of support for the recent associations between ambient $PM_{2.5}$ and cardiovascular disease exacerbation. The potential pathophysiological changes identified include an induction of systemic inflammation and the exacerbation of atherosclerosis, as well as alteration in the autonomous nervous system control and induction of cardiac arrhythmia. These changes are consistent with the increased risk of hospitalization due to myocardial infarctions, congestive heart failure and ischemic stroke. However, considerable heterogeneity was observed comparing $PM_{2.5}$ at different locations as well as study different population subgroups, indicating that the underlying biological mechanisms have not been completely unraveled. Concurrently, PM Center research has helped to clarify: 1) the role of chronic $PM_{2.5}$ exposure on respiratory disease-related mortality and lung function growth in children; and 2) the effects of short-term peak exposures to $PM_{2.5}$ on airway inflammation. It has also indicated that repeated $PM_{2.5}$ exposure can result in the loss of cells in the brain and that inhaled UFP can translocate *via* sensory neurons to the brain.

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Research on the Mechanisms of PM Effects: Supplement to *Accomplishments of the Particulate Matter (PM) Centers (1999-2005)*

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This document summarizes additional mechanistic information obtained in the first 6 years of the PM Centers' research since their establishment in 1999. Research performed as part of the EPA-funded Particle Centers has increased the understanding of the mechanisms of PM health effects. Progress has been made in understanding the dosimetry and distribution of particles throughout the body, pulmonary inflammation and vascular effects, asthma and allergen responses, cardiac effects, vascular and systemic inflammatory effects, and possible impact on the central nervous system. These findings have increased our understanding of the mechanisms of injury at the organ, tissue, cellular, and subcellular levels, and have begun to shed light on systemic interactions in the whole organism that contribute to adverse health outcomes.

1. **Introduction**

A number of plausible mechanisms have been proposed to explain the adverse health impact of ambient air particulate matter (PM) (Table 1). Various aspects of the inflammatory response cascade are postulated to play key roles in PM effects. Data from a number of EPA Centers studies have shown that inhalation of PM can elicit pro-inflammatory effects in tissue culture cells, the lung, and the cardiovascular system (1-13). However, it remains unclear whether the systemic and cardiovascular responses are primarily driven by local (pulmonary) inflammation with production of pro-inflammatory cytokines and chemokines in the affected airways, or by translocation of particles into the pulmonary and systemic circulation, with more direct effects on the endothelium and other organs. In either case, evidence is building that reactive oxygen species (ROS) may play an important role as a mechanism of injury.

The major emphasis on the mechanistic report will be on the role of oxidative stress and inflammation in mediating the adverse effects of PM. This does not exclude the contribution of other mechanisms of injury (Table 1).

2. The Role of ROS and Inflammation in PM-induced Adverse Health Effects

A potential mechanistic link between PM exposures and inflammation involves the generation of ROS and oxidative stress (1, 6, 10, 12, 14-17). Several centers have demonstrated that ambient PM and DEP induce ROS production in target cells such as macrophages, bronchial epithelial and endothelial cells (2, 11, 12, 16-18). More direct evidence that ROS is involved in PM effects in vivo has been provided by chemiluminescence studies in rats, in parallel with a demonstration that antioxidants suppress these oxidative stress effects (17). In vivo chemiluminescence originating from H_2O_2 production in the lungs or heart, is a sensitive measure of inhaled CAPs effects,

including their impact on the lung (17). N-acetylcysteine (NAC) suppressed the chemiluminescence response (10) and was effective in suppressing ovalbumin (OVA) sensitization in mice co-challenged with aerosolized DEP, daily for 10 days (6, 10). NAC also interfered in the generation of carbonyl proteins in the lung of these animals; carbonylation is an oxidative modification of protein structure (6).

In order to explain the link between ROS production and tissue injury, a series of in vitro and in vivo experiments explored the link between oxidative stress and inflammation (6, 10-12, 16-18). This led to the characterization of a hierarchical oxidative stress model (Fig. 1), which posits that at the lower levels of oxidative stress (Tier 1), there is an induction of phase II enzymes by a genetic response pathway that involves the transcription factor, Nrf2 (2, 15, 19, 20). Diesel exhaust particles (DEP) and ambient ultrafine particles (UFP) increase the accumulation of Nrf2 in the nucleus, including their ability to activate the antioxidant response element (ARE) (21). Interestingly, the buildup of Nrf2 in the nucleus is dependent on a prolongation of protein half-life by interference in proteosomal degradation (21). Nrf2 drives the ARE in the promoter of phase II genes, leading to the expression of antioxidant and detoxification enzymes (21). Treatment with DEP, organic DEP extracts and ambient ultrafine particles induced the expression of heme oxygenase 1 (HO-1), glutathione-S- transferase (GST), NADPH quinone oxidoreductase, catalase, superoxide dismutase (SOD), glutathione peroxidase and glucoronosyltransferase (20, 21). It has also been demonstrated that source constituents of ambient particles and NIST ambient particulate can inactivate some of these protective enzymes. (22). The induction of phase II enzymes protect against oxidative stress injury (Tiers 2 and 3), and a reduced Tier 1 defense may promote PM susceptibility. In humans, this can result from phase II enzyme polymorphisms, e.g., the GST M1 null genotype which predisposes to the development of asthma and enhanced sensitization to common environmental allergens during nasal DEP challenge (23). Conversely, induction of a phase II response may be a key factor in adaptation to a polluted environment, and may explain why persistent inflammatory changes in the lung were not observed after repeated exposure to low CAPs levels in one study (24). Some phase II enzymes, such as HO-1, exert anti-inflammatory effects based on their antioxidant abilities.

If Tier 1 protection fails, a further increase in oxidative stress could lead to the generation of pro-inflammatory responses (Tier 2) or cytotoxic effects (Tier 3) (Fig. 1). Tier 2 responses are linked to the activation of intracellular signaling pathways which impact cytokine and chemokine production (1, 2, 25). An example is activation of the MAP kinase and NF-κB cascades (2, 25). This cascade is responsible for the expression and activation of AP-1 transcription factors (e.g., c-Jun and c-Fos), which in turn is responsible for the expression of pro-inflammatory genes, including those encoding for cytokines, chemokines and adhesion molecules. European investigators have recently confirmed that the Jun kinase and NF-κB cascades are activated in lung epithelial cells obtained from human subjects exposed to DEP inhalation (26). Tier 3 responses involve mitochondrial perturbation by pro-oxidative chemicals (15, 16, 27, 28). Although the *in vivo* significance of the mitochondrial pathway is uncertain, PM-induced interference in one electron transfers in the mitochondrial inner membrane and perturbation of the mitochondrial permeability transition (PT) pore can contribute to superoxide generation and the induction of cellular apoptosis (16, 28). These effects can be mimicked by organic extracts made from DEP as well as redox cycling quinones and functionalized aromatic hydrocarbons present in these particles (16, 27, 28). Each tier of oxidative stress is sensitive to the effects of NAC (16, 28).

The link between oxidative stress and inflammation has been confirmed by microarray and real-time PCR analyses (3, 21). Total lung RNA was collected to study the pulmonary response to CAPs in rats (3). This showed increases in pro-inflammatory mediators such as C-C chemokines,

IL-1, IL-6 and TNFα, in parallel with an overall decrease in immune enhancers such as IL-2 and interferon. (3). There is also evidence in microarray studies for increases in ROS activity, as well as evidence for activation of organic chemical metabolism and detoxification mechanisms (3). In another study in which exhaust aerosol and LPS were used, the expression of TNFα and the TNFα receptor were found to be consistently increased in the lung, heart, and olfactory bulb, although to different degrees in each tissue (29). Combining these stimuli, led to an additive response (29). A comparison of microarray results from the lung and heart showed that more genetic changes occur in the lung compared to the heart (30). There was a significant response at all dose levels in mice exposed to PM versus saline. The responding genes could be grouped into distinct functional entities such as inflammation, immune responsivity, oxidant responses, apoptosis, growth factors, transcription factors, kinase activity, protease activity, transport and cellular adhesion (30).

While there is still considerable debate about which particle components are responsible for the pro-oxidative and pro-inflammatory effects, there is accumulating evidence that transition metals, such as copper, vanadium, chromium, nickel, cobalt and iron, as well as aromatic and polar organic substances play a role in ROS production (1, 2). The particle backbone could play an important role in acting as a template for single electron transfers reactions, including electron transfer to molecular dioxygen (11, 14, 16, 20). This could involve redox cycling reactions, as demonstrated by the ability of the ambient PM collections to generate superoxide in the presence of dithiothreitol (DTT) (two, 14, 21). In fact, the DTT oxidation event can be assessed by a colorometric reaction to assay for the content of redox cycling chemicals in urban PM samples (14, 63). Biologically catalyzed oxidation-reduction reactions in the cell, as well as interference in one-electron transfers in the mitochondrial inner membrane, contribute to ROS generation. In addition to the ability of ROS to damage cellular proteins, DNA and cell membranes, electrophilic PM chemicals such as the quinones can modify cellular proteins by Michael acceptor reactions (2, 14, 64). It is likely that this type of reaction leads to Nrf2 release to the nucleus by the covalent modification of its cytosolic chaperone, Keap-I (21). The covalent modification of intracellular and tissue proteins was also confirmed by studying their tyrosylation and carbonylation (6). Much remains to be discovered about the mechanisms by which PM lead to ROS generation.

In order to characterize the redox cycling chemicals present in PM, silica gel chromatography was used to fractionate organic DEP extracts (11, 20, 21, 28). Increasing polar solvents eluted aliphatic, aromatic and polar chemical fractions. This demonstrated that the quinone-enriched polar material was more active than the polycyclic aromatic hydrocarbon (PAH)-enriched aromatic fraction in the DTT assay, which, in turn, correlates with glutathione depletion in epithelial cells and macrophages (14, 20, 21). The aliphatic fraction was inactive in these assays.

The relationship between the organic chemical composition and the redox cycling potential of ambient PM was confirmed in a study where UFP were compared to coarse and fine particles collected in the Los Angeles basin (14). Ambient UFP were more active than coarse and fine particles in the DTT assay, and also more prone to generate oxidative stress in macrophages and epithelial cells (14). Both the in vitro and cellular responses showed an excellent correlation with the PAH content of UFP (14). Another important observation in this study was the ability of UFP to lodge in and disrupt the mitochondrial architecture (14). This involves cellular apoptosis and apo-necrosis by a pathway that requires opening of the mitochondrial permeability transition pore (PTP) (14, 16, 28). Functional effects on the PTP and inability to sustain one electron transductions in the mitochondrial inner membrane was confirmed in isolated mitochondrial preparations through the use of calcium-dependent swelling, calcium retention capacity and dissipation of the mitochondrial membrane potential (28). Moreover, UFP effects could be reproduced by polar and aromatic

chemicals fractionated from DEP, while commercial polystyrene nanoparticles were inactive (28). These data show differential particle toxicity based on size, composition, and subcellular localization.

Old age also predisposes to PM susceptibility (12, 29). This susceptibility could also be premised on the increased propensity of aged tissue to generate ROS and oxidative stress. Particle-induced cytokine gene expression is enhanced in macrophages from aged animals (12). Geriatric rats exposed to vehicular traffic on a freeway also showed changes in HRV, suggesting a shift in parasympathetic and sympathetic influences (29).

In summary, ROS generation by the particles themselves as well as their chemical constituents appear to be a major pathway of injury and could lead to oxidative stress and pro-inflammatory effects on the lung, heart and possibly also the CNS. Figure 2 summarizes the events that lead to ROS generation and the biological consequences of oxidative stress. Other important mechanisms of injury include endotoxin-mediated cellular and tissue responses, covalent modification of key regulatory proteins in the cell, vasoconstriction and altered blood coagulability.

3. Mechanisms of Cardiovascular Health Effects

3.1. Introduction to cardiovascular mechanisms

Ambient PM initiates adverse cardiovascular health effects in humans and animals. Evidence that ambient PM can affect cardiovascular health comes from studies that show: (i) associations between daily changes in PM concentration and cardiovascular deaths and hospitalization admissions, as well as (ii) increased adult cardiac and pulmonary mortality associated with spatial differences in PM concentrations. Pathways that have been suggested as potential mechanisms to explain these associations are shown schematically in Figure 3. The first pathway involves altered cardiac autonomic function resulting from particle inhalation. Studies have observed that changes in cardiac autonomic function, as measured by heart rate variability (HRV), are predictors of cardiovascular disease and mortality. Environmental epidemiological studies also report associations between the same HRV predictors and air pollution. The second mechanistic pathway involves lung and systemic inflammation leading to vascular dysfunction. Inhaled PM may provoke a low-grade inflammatory response in the lung, release potentially harmful cytokines into the blood, induce changes in platelets and blood coagulation, increase vascular reactivity, and trigger acute cardiovascular events specifically associated with ischemic heart disease.

3.2 Studies using CAPS exposure during or after coronary occlusion

The effect of inhalation exposure to CAPs on myocardial ischemia was assessed in a canine model of coronary artery occlusion (31). Exposure to CAPs significantly ($p = 0.007$) enhanced occlusion-induced peak ST-segment elevation in precordial leads. ST-segment elevation was significantly correlated with the Si concentration ($p = 0.003$) of the particles and other crustal elements. No associations were found with CAPs mass or number concentrations. Heart rate was not affected by CAPs exposures. These results suggest that exacerbation of myocardial ischemia during coronary artery events may be an important mechanism of environmentally related acute cardiac mortality. The suggested mechanism for these observations involves vasoconstriction of cardiac arteries as a result of PM exposure. Micro-array studies provide evidence of an increase in endothelin gene expression in the lung and a substantial decrease in endothelial nitric oxide synthase (eNOS) expression with CAPs exposure (3). Other studies in rats and mice (32, 33), showing morphometric

measures of vasoconstriction in the lung and heart, indicate that blood vessels are an important target of ambient PM health effects. Another recent study showed that diabetics have greater brachial artery diameter responses from increased exposure to ambient particles (34).

3.3 Chemiluminescence and pharmacological approaches to assess ROS production in the heart

To evaluate the ability of particulate air pollution to promote oxidative stress and tissue damage *in vivo,* a rat model of short-term CAPs exposure was used (17). CAPs exposure in the range of 300 $\mu g/m^3$ induced significant oxidative stress, determined as *in situ* chemiluminescence in the lungs and in the heart, but not in the liver. Increases in oxidant levels were also triggered by residual oil fly ash, but not by particle-free air nor by carbon black aerosols. Increases in chemiluminescence in the lung showed strong associations with the CAPs content of iron, manganese, copper, and zinc. Increases in cardiac chemiluminescence were associated with Fe, aluminum, silicon, and titanium. CAPs inhalation also led to tissue-specific increases in the activities of the antioxidant enzymes, SOD and catalase.

Pharmacological strategies were used to determine whether oxidants are implicated in PM-dependent cardiac dysfunction and whether a PM-induced increase in autonomic stimulation on the heart mediates cardiac oxidative stress and toxicity (35). In rats, intratracheal instillation of urban air particles (UAP 750 μg) or inhalation of CAPs (700 +/-180 $\mu g/m^3$) for 5 hr led to significant increases in heart oxidants measured as organ chemiluminescence or thiobarbituric acid reactive substances. Heart rate increased immediately after exposure and returned to basal levels over the next 30 min. HRV was unchanged immediately after exposure, but significantly increased during the recovery phase. To determine the role of ROS in the development of cardiac malfunction, rats were treated with 50 mg/kg N-acetylcysteine (NAC) 1 hr prior to UAP instillation or CAPs inhalation (10). NAC prevented changes in HRV in UAP-exposed rats. To investigate the role of the autonomic nervous system in PM-induced oxidative stress, rats were given 5 mg/kg atenolol (beta-1 receptor antagonist), 0.30 mg/kg glycopyrrolate (muscarinic receptor antagonist) or saline immediately before exposure to CAPs aerosols. Both atenolol and glycopyrrolate effectively prevented CAPs-induced cardiac oxidative stress (10). These data indicate that PM exposure increases cardiac oxidants via autonomic signals.

3.4 Studies on HRV and arrhythmias

If PM does cause serious cardiovascular effects shortly after exposure, one would expect to see some physiological change during exposure. In one study, spontaneously hypertensive rats with surgically implanted blood pressure transmitters were exposed to concentrated ambient PM (CAPs) for 4 hr to determine whether CAPs inhalation causes immediate effects (36). At other times, the rats were exposed to sulfuric acid aerosols because acid is one of the components of PM that could activate irritant receptors that cause effects. Exposure to CAPs or sulfuric acid aerosols caused a decrease in respiratory rate and heart rate that was apparent soon after the start of exposure and stopped when exposures ceased. The similarity between the effects of fine acid aerosol and CAPs suggests that CAPs activate airway-irritant receptors during exposure.

Pulmonary and systemic effects were assessed in 12 healthy human adult and 12 asthmatic volunteers exposed once for 2 hr in a whole-body chamber (37). The exposure dose was approximately 200 $\mu g/m^3$ CAPs in the fine size range. Each subject was also exposed once to

filtered air. Neither healthy nor asthmatic subjects showed significant changes in symptoms, spirometry, or routine hematologic measurements attributable to CAPs exposure compared with filtered air. Both groups showed CAPs-related (i) decreases of columnar cells in induced sputum after exposure, (ii) increases in certain blood mediators of inflammation (i.e., soluble intercellular ICAM-1 and IL-6), and (iii) parasympathetic stimulation of HRV. In the asthmatic group, systolic blood pressure modestly increased during filtered air exposure and decreased during CAPs exposure, whereas the pattern was reversed in the healthy group. Observed changes in some mediators of inflammation in blood and changes in HRV were consistent with PM-related effects reported from epidemiologic studies suggesting that exposure to concentrated $PM_{2.5}$ tends to elicit more systemic than pulmonary effects. (38).

UFP in ambient air may play a role in cardiovascular effects because of the possibility of penetrating the alveolar epithelium and entering the vascular space. The effects of inhalation of elemental carbon UFP were examined in healthy subjects using continuous ECG monitoring and measurement of HRV, markers of myocardial repolarization, ischemia, and arrhythmias (39). In two separate randomized, double-blinded, controlled studies, subjects were exposed to filtered air and to elemental carbon UFP at mass concentrations of 10 and 25 $\mu g/m^3$. The particle counts in these studies were approximately 2 and 7 x 10^6 particles/cm^3 respectively; particle median diameter was 25 nm. Exposures were for two hours with intermittent exercise. The observed changes were generally small and not clinically significant, although there were trends indicating an increase in parasympathetic tone. One of the studies indicated a dose-related shortening of the late-corrected QT interval with UFP exposure, compared with air. Overall, exposures of healthy subjects to carbon UFP at these concentrations did not cause clinically important changes in ECG-derived parameters.

An ECG telemetry study was done on aged (1-year) $ApoE^{-/-}$ mice (40) exposed intranasally to 50 µg of saline or 50 µg each of $PM_{2.5}$ or silica (Min-u-Sil 5). The mice were monitored for a one-day baseline prior to and for 4 days following exposure. After an initial increase in both heart rate and activity in all groups, there was delayed bradycardia with no change in activity of the animals in the PM and silica exposed groups. In addition, with PM and silica exposure, there was a decrease in HRV parameters suggesting a decrease in parasympathetic tone which may lead to cardiac arrhythmia (40).

Long-term exposure to $PM_{2.5}$ significantly decreased heart rate, body temperature, and physical activity for mice lacking apoliproprotein ($ApoE^{-/-}$) over 5 mo of exposure, with smaller and nonsignificant changes for C57BL/6 mice (41). The effect of subchronic CAPs exposure on HRV parameters that are sensitive to cardiac sympathetic and parasympathetic nerve activity was also studied (42). HRV in the late afternoon and overnight for the $ApoE^{-/-}$ mice showed a gradual increase for the first 6 wk, a decline for about 12 more wk, and a slight turn upward at the end of the study period. For C57BL/6 mice, there were no chronic changes in HRV in the late afternoon, and a slight increase after 6 wk for the overnight period. The response patterns of $ApoE^{-/-}$ mice implies a perturbation of the homeostatic function in the cardiovascular system (initial enhancement and later depression of the HRV parameters). These results complement the findings in human panel and controlled CAPs exposure studies in demonstrating that increased levels of particle pollution are able to perturb cardiac autonomic function, which may lead to adverse cardiovascular outcomes.

Ambient air pollution is a complex mixture of PM and gaseous pollutants such as CO. The effect of exposure to CO, alone or in combination with ambient PM, on arrhythmia incidence has been difficult to sort out in epidemiological studies. To evaluate these effects, left-ventricular myocardial infarction was induced in Sprague-Dawley rats by thermocoagulation, and 12-18 hr later the rats

were exposed (1 hr) to either filtered air (n = 40), CO (35 ppm), CAPs (median concentration = 350.5 $\mu g/m^3$), or CAPs and CO (CAPs median concentration = 318.2 $\mu g/m^3$) (43). CO exposure reduced ventricular premature beat (VPB) frequency by 60.4% ($p = 0.012$) during the exposure period compared to controls. CAPs exposure alone increased VPB frequency during the exposure period, and the response to CAPs plus CO was a decrease in arrhythmia similar to CO alone. No significant interactions were observed between the effects of CO and CAPs. Thus, in this animal model, the responses to CO and CAPs are distinctly different. Other studies have shown marked increases in post-MI arrhythmia in rats using PM surrogates (43, 44).

3.3 3.5 Systemic inflammation, acute phase reactions and effects on coagulation

Adverse effects of inhaled PM may be the indirect result of a PM-induced increase in blood coagulability. This explanation is biologically plausible because prospective studies have shown that increases in blood coagulation parameters are significantly associated with risk of adverse cardiovascular events. The hypothesis that acute exposure to elevated levels of PM causes prothrombotic changes in blood coagulation parameters was examined (45). Rats with indwelling jugular vein catheters were exposed for 6 hr to filtered air or CAPs in New York City air. Blood samples were taken at four time points: before and immediately after exposure and at 12 and 24 hr after the start of exposure. At each time point, six coagulation parameters (platelet count, fibrinogen level, factor VII activity, thrombin-antithrombin complex level, tissue plasminogen activator activity, and plasminogen activator inhibitor activity) were measured as well as all standard blood count parameters. Five concentrated-PM exposure experiments were performed over a period of 8 weeks. PM exposure concentrations ranged from 95 to 341 $\mu g/m^3$. There were no consistent exposure-related effects on any of the endpoints across the five experiments and no indication of any dose-dependent effects. The results do not indicate that exposure to ambient CAPs causes adverse effects on blood coagulation in healthy rats.

Systemic release of cytokines from the lung and vasculature may impact the production of clotting factors and anticoagulant enzymes in the liver. Laboratory-generated 30 nm elemental carbon UFP were intravenously administered to rats (46). It was shown that doses as low as 1 mg per rat were able to induce thrombus formation in the ear vein model (46). While the role of particle-adsorbed chemicals in these adverse cardiovascular events is uncertain, UFP gain access to the systemic circulation by alveolar penetration. This is illustrated by ear vein thrombus formation upon intratracheal instillation of UFP (46); even a low dose (0.2 μg per rat) was able to produce a significant effect.

Human studies failed to show an effect of UAP inhalation on coagulation and systemic inflammatory responses (47). Healthy and asthmatic subjects inhaled 10 to 50 $\mu g/m^3$ elemental carbon UFP (~25 nm) for 2 hr with intermittent exercise. There were no exposure-related increases in platelet count, serum fibrinogen, factor VII, or Von Willibrand's factor antigen in venous blood. There were no significant effects on measures of systemic inflammation, including serum amyloid A (SAA), IL-6, and soluble ICAM-1.

3.6 Accelerated atherosclerosis models (ApoE and variations)

Studies were conducted to determine whether PM can exacerbate atherosclerosis, which is a chronic inflammatory disease of the vessel wall. In one study, C57BL/6, $ApoE^{-/-}$ and $ApoE^{-/-}$/LDL-receptor (DK)-deficient mice were exposed to CAPs for 6 hr/5 days/wk, for up to 5 months (48). The overall mean exposure concentration for these groups of animals was 110 $\mu g/m^3$. The cross-sectional area

of the aorta root of DK mice was examined morphologically using confocal microscopy for the severity of lesion, extent of cellularity, and lipid contents. Aortas from the arch to the iliac bifurcations were also sectioned longitudinally and lesion areas were stained with Sudan IV. All DK mice, regardless of exposure, had developed extensive lesions in the aortic sinus regions, with lesion areas that covered > 79% of the total area. In male DK mice, the lesion areas in the aortic sinus regions appeared to be enhanced by CAPs, with changes approaching statistical significance ($p = 0.06$). In addition, plaque cellularity was increased by 28% ($p = 0.014$, combined) whereas there were no CAPs-associated changes in the lipid content in these mice. When examining the entire aorta opened longitudinally, both the $ApoE^{-/-}$ and DK mice had prominent areas of atherosclerosis covering 40% or more of the luminal surface. Visual examination of all images suggested that plaques tend to form in clusters concentrating near the aortic arch and the iliac bifurcations. Quantitative measurements showed that CAPs exposure increased the percentage of aortic intimal surface covered by grossly discernible atherosclerotic lesion by 57% in the $ApoE^{-/-}$ mice ($p = 0.03$). This study demonstrated in susceptible animals had a significant impact on the size, severity, and composition of aortic atherosclerotic plaques.

3.7 Implications of the cardiovascular mechanistic studies

Ischemic heart disease, arrhythmias, hypertension, and atherosclerosis are important outcomes that have been linked to ambient air pollution via epidemiological and experimental studies. The specific mechanisms involved are complex. It is clear that the autonomic nervous system, the responses of the endothelium, and ROS play important roles.

4. Mechanisms of Respiratory Effects of PM

4.1 Introduction to mechanisms of respiratory health effects

The respiratory epithelium is the first tissue to encounter, and respond to inhaled PM. Local responses include injury, inflammation, release of inflammatory mediators, alteration of allergen responsiveness, and activation of neuronal pathways. The respiratory tract is also the portal for distribution of PM to other organs. There are three potential pathways for PM effects: (i) local airway and respiratory effects: (ii) local mediator responses causing effects beyond the respiratory system, and (iii) effects from PM translocated beyond the respiratory tract. This section will focus on respiratory effects, including new findings on particle dosimetry, airway inflammation, asthma and allergen responsiveness, and the pulmonary vasculature.

4.2 Dosimetry and Particle distribution

The health effects of PM exposure are proportional to the locally deposited particle dose and this dose is influenced by the proportion of inhaled particles that are retained in the lung with each breath. Fractional particle deposition and distribution within compartments of the respiratory tract are markedly influenced by particle size. Some ultrafine particles have a high rate of deposition in the alveolar region of the lung because they deposit by diffusional mechanisms.

Human clinical studies with inhalation of ultrafine carbon particles were undertaken with a specially developed inhalation facility (49). In healthy subjects exposed at rest to 10 or 25 $\mu g/m^3$ UFP (~23 nm) for 2 hours, the number deposition fraction exceeded 0.6, and increased further during exercise (8). In subjects with mild asthma, UFP deposition was further enhanced, both at rest and with exercise (8). Remarkably, the number deposition fraction in asthmatics during exercise was 0.86

±0.04. Not only does exercise increase particle dose but the fraction of inhaled particles that deposits is increased as well.

4.3 Airway Inflammation

Airway inflammation can be measured (i) directly by histological examination or recovery of inflammatory cells from airway sampling such as bronchoalveolar lavage fluid or sputum, and (ii) indirectly via changes in inflammatory cytokine gene or protein expression, decrements in pulmonary function secondary to airway narrowing, increases in non-specific airways responsiveness, or increases in systemic markers of inflammation.

Until recently, there was little evidence that inhalation of ambient PM, even at relatively high concentrations, caused significant inflammation in the respiratory tract. Recent animal and human inhalation studies, using particle concentrators, have shown that short-term exposure to concentrated ambient fine particles may have pro-inflammatory effects. Similarly, exposure of dogs to CAPs in the Boston area showed no effects of total PM mass on indicators of airway or systemic inflammation (50). However, further analysis suggested that specific PM components were associated with specific inflammatory cell responses, both in the lung and the blood.

Pulmonary inflammatory responses to CAPs were assessed by exposing normal rats followed by collection of total lung RNA (3, 51). The RNA was pooled, labeled, and hybridized to multiple Affymetrix rat micro-array chips to explore the range of responses to CAPs exposure. Using the A-chip results, data from the sham-exposed group was subtracted from the CAPs group. Since these chips typically include multiple measurements of the same gene, cluster analyses of the results as well as biologic responder cluster assessments of these micro-array studies strongly support the pro-inflammatory potential of CAPs. Increases in pro-inflammatory mediators such as C-C chemokines, IL-1, IL-6 and TNFα are illustrated with an overall decrease in immune Th1 enhancers such as IL-2 and interferon. In addition to enhanced pro-inflammatory mediators, there is evidence of vascular endothelial responses to inhaled CAPs. There is also evidence in the microarray studies for increases in ROS activity, as well as evidence for activation of chemical metabolism and detoxification pathways.

In a human clinical exposure study, investigators in the Chapel Hill area, NC, found modest increases in inflammatory cells in BAL a fluid from healthy subjects after inhalation of concentrated ambient fine particles (52). In contrast, another group found no increases in inflammatory cells in induced sputum or changes in pulmonary function following concentrated fine PM inhalation in young healthy or asthmatic subjects (53), or in healthy elderly subjects with chronic obstructive pulmonary disease (54). Similarly, no inflammatory effects were observed with exposures to concentrated coarse PM (38). Pietropaoli et al. exposed subjects to 10 to 25 $\mu g/m^3$ elemental carbon ultrafine particles (UFP) for 2 hours, with intermittent exercise (47). They found no effects on sputum inflammatory cells, pulmonary function, or exhaled nitric oxide (NO), a noninvasive measure of airway inflammation. Additional studies at a higher concentration of 50 $\mu g/m^3$ also showed no changes in pulmonary function or exhaled NO, and no changes were seen in systemic markers of inflammation or coagulation (55). However, ambient UFP may have a greater pulmonary inflammatory potential than pure elemental carbon particles.

Mild pulmonary inflammatory responses have been seen in a study of "on-road" exposures to ambient particles in rats (56, 57, 58). In one experiment, a single 6 hr exposure to on-road aerosols was found to increase the total number of cells in BAL fluid 3 days after exposure in comparison to filtered air controls. In a separate experiment, the aerosols were found to induce a decrease in the percentage of circulating PMNs relative to filtered air controls after a single 6 hr exposure.

In summary, there is evidence that inhalation of PM at concentrations near ambient can initiate a mild acute inflammatory response in the lung. However, the absence of inflammatory cells or structural changes in the chronic CAPs inhalation studies suggests that pulmonary inflammation may resolve with chronic exposure in animals or humans without lung disease, perhaps due to upregulation of antioxidant and adaptive responses, as discussed in Section 2. The demonstration of long-term effects on the vascular endothelium and heart in the absence of pulmonary effects suggests the lung is better able to adapt to the effects of PM exposure than is the cardiovascular system. However, the effect of long-term PM exposures on the pulmonary inflammatory response in people with or without underlying lung disease, such as asthma or chronic obstructive lung disease, is unknown.

4.4 Asthma and Allergen Responsiveness

A number of human and animal studies have shown that DEP act as an adjuvant that can enhance the allergic inflammatory response to common environmental allergens. Exposure to aerosolized DEP can enhance antigen (OVA)-specific IgE production in a murine inhalation model (6). This adjuvant effect could be suppressed by NAC, in parallel with a decrease in carbonyl protein content in the lung (6). However, this route of sensitization does not lead to vigorous airway inflammation (2, 6), prompting the development of additional murine models to test the effect of DEP on the enhancement of airway inflammation and AHR (7). Two new protocols were developed (7). In the first, low-grade sensitization is achieved by injecting the antigen in BALB/c without alum, followed by challenge with aerosolized OVA ± DEP. This allows DEP to act as an adjuvant during secondary sensitization, with the ability to induce airway inflammation and AHR. In the second model, delivery of DEP post-OVA challenge in the classical sensitization model leads to a resurgence of airway inflammation and AHR (7).

4.5 Pulmonary Vascular Effects

There is evidence that inhalation of fine and ultrafine PM may alter the function of the vascular endothelium in the lung as well as systemically. In morphometric studies of the pulmonary vasculature in rats (32), exposure to CAPs produced a decrease in the lumen-to-wall ratio, indicating vasoconstriction in the pulmonary vascular bed. Sulfate and silicon were the chemical species most strongly associated with these vascular effects. Microarray studies showed a strong increase in mediators and receptors associated with vasoconstriction and endothelial injury with an inhibition of vasodilator mediator activity (57). The lungs and hearts of rats exposed to 3 days of CAPs, compared with sham exposures, showed morphologic evidence of both pulmonary and cardiac vascular endothelial activation. Similar effects were seen in human studies. Exposure to 50 $\mu g/m^3$ carbon UFP decreased the pulmonary diffusing capacity for carbon monoxide 21 hours after exposure (-1.76 ± 0.66 ml/min/mmHg (UFP) vs. -0.18 ± 0.41 ml/min/mmHg (air), p = 0.040) (55). This finding is consistent with decreased pulmonary capillary blood volume in response to UFP. Taken together, these findings provide evidence of endothelial cell changes with exposure to ambient particles.

In a series of randomized, double-blind studies, healthy and asthmatic subjects were exposed to elemental carbon UFP at concentrations of 10, 25 and 50 $\mu g/m^3$, and blood leukocytes were analyzed by flow cytometry for changes in surface expression of adhesion molecules (13). There were reductions in expression of adhesion molecules on blood monocytes and PMNs compared with control air exposures. For example, monocyte expression of ICAM-1 was decreased following inhalation of 10 and 25 $\mu g/m^3$ carbon UFP, in a concentration-related manner. This decrease could result from produced pulmonary capillary blood flow secondary to vasoconstriction.

In summary, the respiratory effects of exposure to ambient PM are influenced by the inhaled dose and airway distribution of the particles, which is determined both by particle characteristics and the presence or absence of airway obstruction. Inhaled fine and ultrafine particles appear to induce mild, possibly transient inflammatory responses. DEP appear to enhance the allergen response in an animal model of allergic airway inflammation. There is both direct and indirect evidence that inhalation of fine and UFP may affect the pulmonary vascular endothelium, leading to pulmonary vasoconstriction. Alteration of pulmonary vascular function may have effects on cardiac preload, with potential clinical consequences in patients with either pulmonary or cardiovascular disease.

5. Central Nervous System

EPA PM Center investigators have clearly demonstrated that inhalation of PM is associated with extrapulmonary effects. As discussed elsewhere in this report, it is unclear whether circulating mediators, such as cytokines, which originate in the lung, may elicit these extrapulmonary responses or whether particles themselves leave the respiratory tract and produce the toxic effects observed in other organs. While both mechanisms have merit, PM Center investigators have clearly demonstrated that the ultrafine fraction of PM can translocate from the respiratory tract to extrapulmonary tissues. In one study, animals were exposed to ultrafine elemental ^{13}C particles and isotope concentration was determined in lungs, cerebrum, cerebellum, and olfactory bulbs at 1, 3, 5, and 7 days postexposure (59). ^{13}C concentration in the olfactory bulb consistently increased from day 1 through day 7 postexposure, but not in cerebrum and cerebellum. In a second study, rats were exposed to ^{192}Ir-radiolabeled ultrafine iridium particles. Within the first week after exposure, radiolabel was measurable in the liver, spleen, heart, and brain. The radiolabeled particles were cleared from the body via excretion and the tissue concentrations decreased with time post-exposure (3 wk and 2 and 6 mo). Thus, the translocation of UFP from the respiratory tract could lead to extra-pulmonary effects.

PM Center investigators have examined the effect of exposure to CAPs on the central nervous system. UFP can be transported from the nasal mucosa to the brain via the olfactory bulb, and from these there are transynaptic routes to CNS targets such as hypothalamus, substantia nigra, and olfactory cortex (59, 60). Murine exposure to CAPs for 4 hr/day over a two-week time period resulted in increased pro-inflammatory responses in the brain tissue (61). Significant increases in NF-κB were observed in cortical tissue after exposure to concentrated UFP or fine + UFP. This increase was accompanied by increased TNFα and IL-1α levels in brain tissue and is compatible with the role of this prescription factor in oxidative stress and inflammation. Veronesi and colleagues have also observed a reaction in the brain tissue of $ApoE^{-/-}$ mice exposed to CAPs for 5 months (62). Dopamineric neurons were stained immunohistochemically and image analysis demonstrated that neurons in the substantia nigra nucleus compacta portion of the brain were significantly reduced in CAPs-exposed relative to air-exposed Apo E-/- control mice. This was accompanied by significant increases ($p < 0.05$) in staining for astrocytes. Because these mice are characterized by elevated levels of oxidative stress in the brain, these findings suggest that exposure to ambient PM could contribute to neurodegeneration in susceptible individuals.

Table:

Table 1: Plausible Mechanisms to Explain PM Adverse Health Effects

- **Local Inflammation** → asthma, COPD, adjuvancy
- **Systemic inflammation** (from lungs as well as circulation) → ↑ CRP, atherosclerosis, blood coagubility
- **Autonomic nervous system activity** → Cardiac arrhythmias
- ↑ **clotting factors,** ↓ **fibrinolytic activity** → ↑ blood coagulation → MI, stroke
- ↑ **bone marrow production of myeloid lineage cells** → ↑WBC, including neutrophils, DC
- **Endotoxin-mediated cellular responses & inflammation** → airway & systemic inflammation
- **Irritant-type receptors** (e.g., vanilloid receptors) → airway hypereactivity, asthma
  Covalent modification of intracellular enzymes, mitochondrial membrane → ROS generation, cellular apoptosis
- **Phagocytic particle overload** → ↓ phagocytosis → ↑ respiratory infections
- **Free oxygen Radicals and Oxidative Stress** → Oxidative stress

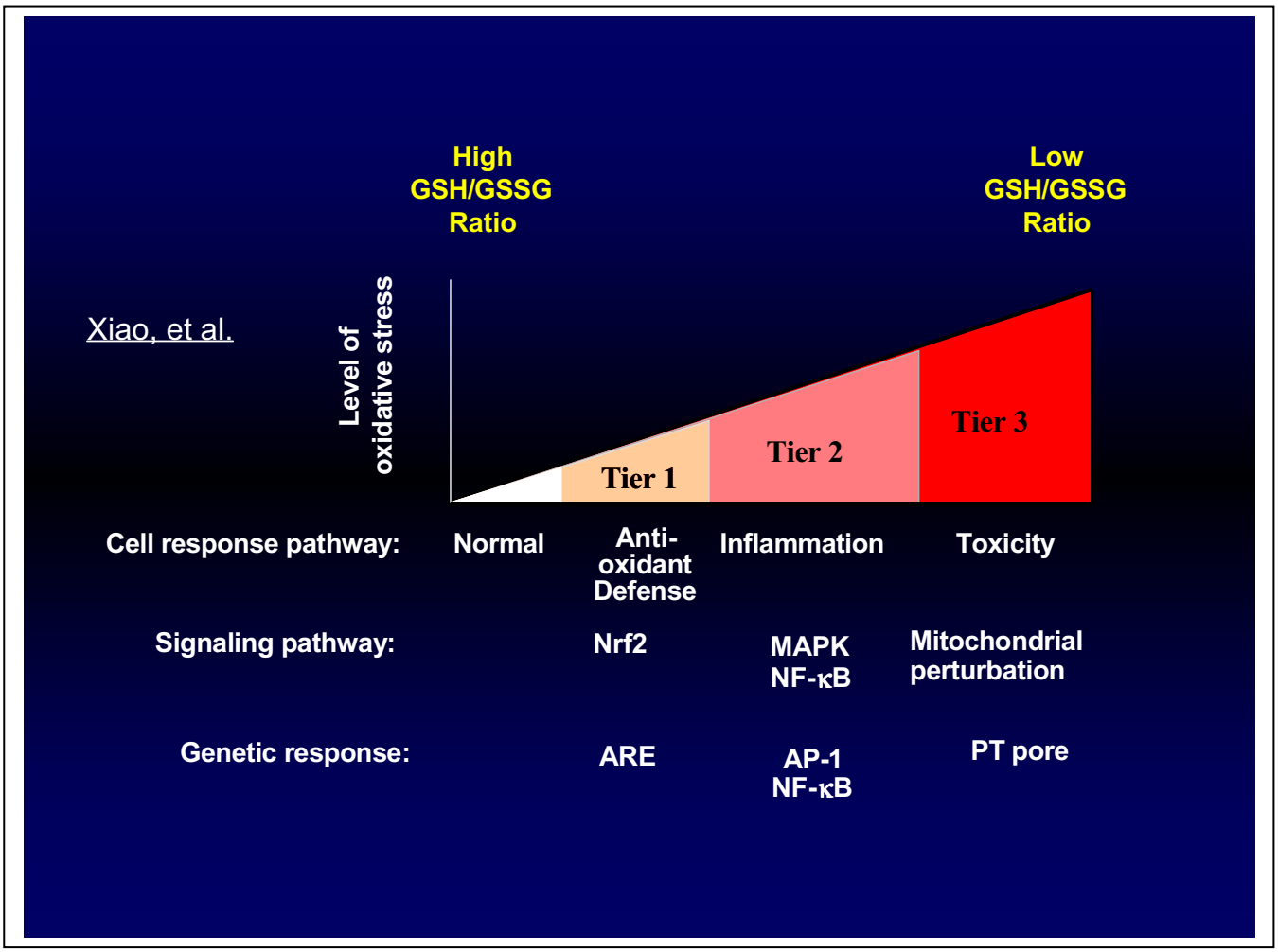

Figure 1: Hierarchical oxidative stress model in response to PM exposure. At a lower level of oxidative stress (tier 1), antioxidant enzymes are induced to restore cellular redox homeostasis. At an intermediate level of oxidative stress (tier 2), activation of MAPK cascades induce pro-inflammatory responses, e.g., cytokines and chemokines. At a high level of oxidative stress (tier 3), perturbation of the mitochondrial permeability transition pore and disruption of electron transfer result in cellular apoptosis or necrosis (adapted from 19).

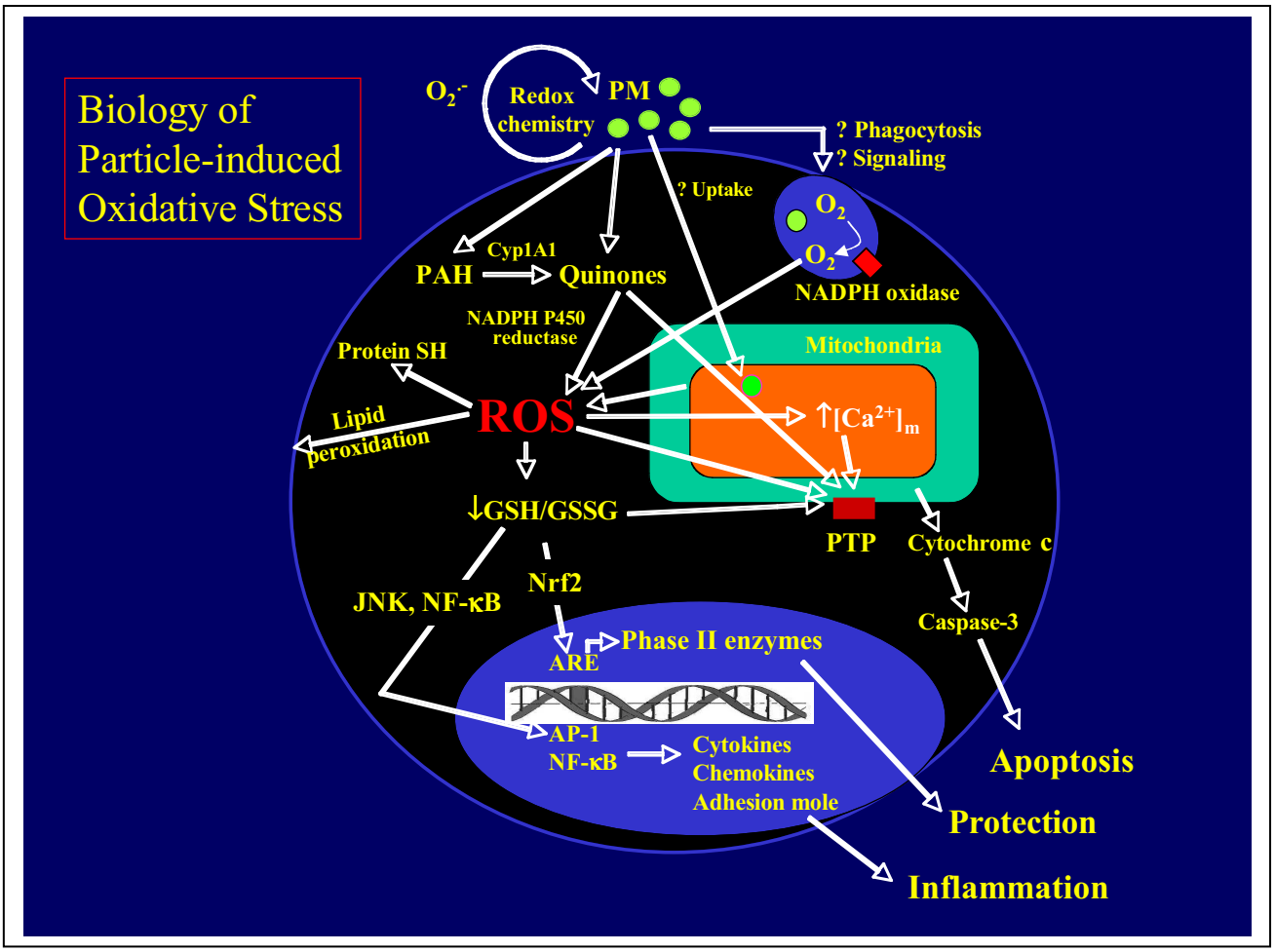

Figure 2: Mechanisms of particle-induced ROS generation, mitochondrial targeting and the biological effects of oxidative stress. Sources of ROS production and their effects on cells. Quinones can redox cycle to produce ROS in the endoplasmic reticulum under the catalytic influence of NADPH-cytochrome P450 reductase. Phygocytosis can induce the assembly and activation of NADPH oxidase to produce superoxide. PM can interfere in electron transduction in the mitochondrial inner membrane as well as in the perturbation of the PT pore to generate ROS. ROS leads to lipid peroxidation in the cell membrane can crosslink protein SH groups and induce redox equilibrium through a depletion of GSH. Depending on the level of oxidative stress this could induce Nrf2 release to the nucleus, activation of MAPK and NF-κB signaling cascades or cytotoxicity, Nrf2 interaction with the ARE leads to the expression HO-1 and other phase II enzymes at low level of oxidative stress, while MAPK and NF-κB signaling cascades lead to pro-inflammatory responses (e.g., cytokine production) at higher levels of oxidative stress. At the highest oxidative stress level, ROS can lead to opening of mitochondrial PT pore, followed by cytochrome c release, caspase-3 activation and apoptosis or necrosis.

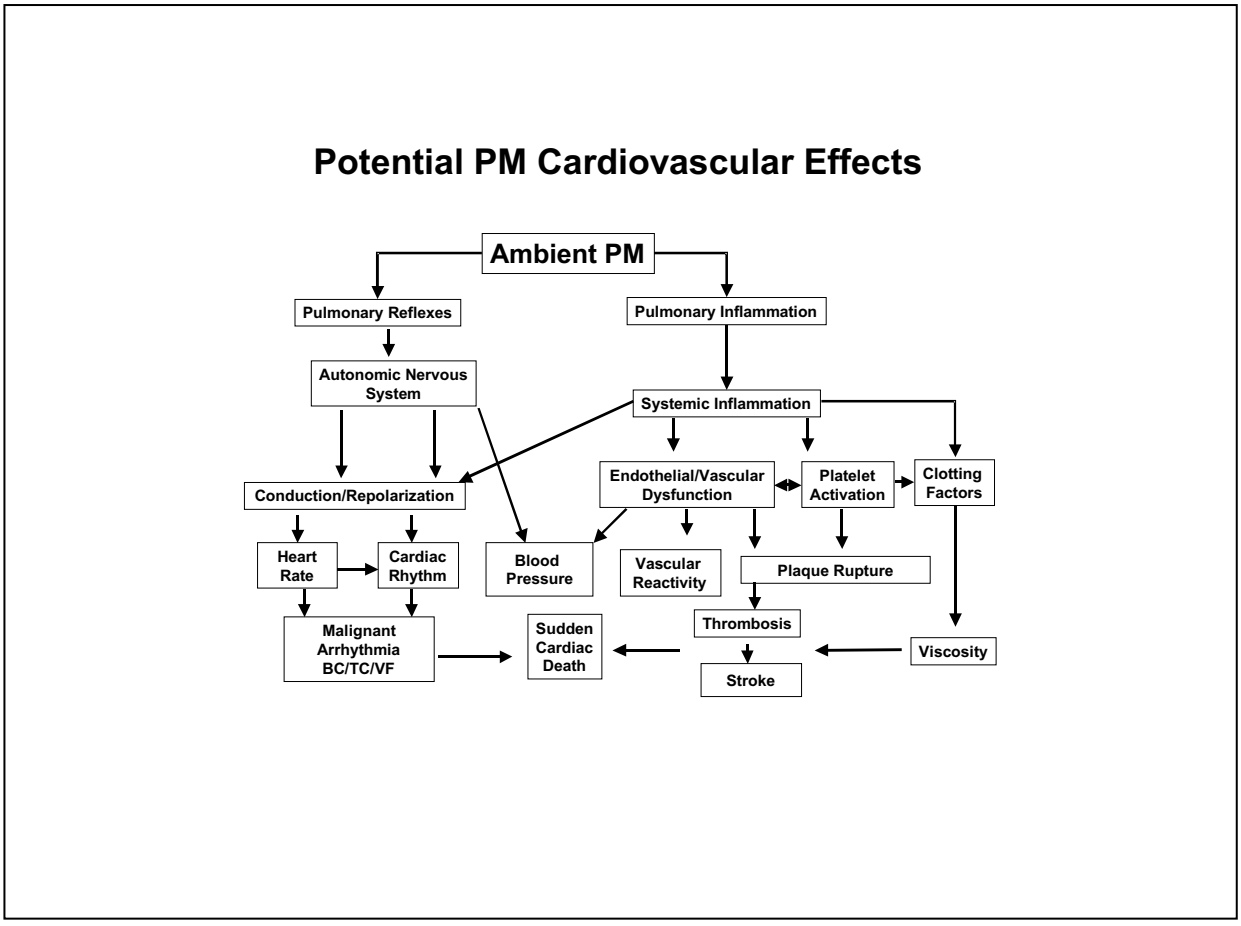

Figure 3: **Potential PM Cardiovascular Effects**

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PM Exposure Assessment Research: Supplement to *Accomplishments of the Particulate Matter (PM) Centers (1999-2005)*

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This document summarizes the progress made by the EPA Particulate Matter (PM) Centers during their first 6 years in identifying and understanding human exposures to PM air pollution since the mid-course report prepared in 2002 (Lippmann et al., 2003). When the PM Centers were designated in 1999, the primary body of evidence for personal and population-based exposures to ambient air PM consisted, in large measure, of research conducted as part of the Harvard Six Cities Study and the U.S. EPA Particle Total Exposure Assessment Methodology Study (Clayton et al., 1993), and we concluded that the pre-existing PM exposure database was inadequate for investigating associations between personal exposures and outdoor concentration data from compliance based monitoring sites. In the first phase of the PM Centers program there was substantial work to understand and assess potential flaws and weaknesses in this body of evidence, and to develop an understanding of the spatial and temporal aspects of exposures to PM of ambient air origin. In the more recent work, the PM Centers research has substantially expanded the range of PM mass and PM component concentration variations in community air for PM_{10}, $PM_{2.5}$, $PM_{1.0}$, and for $PM_{0.1}$ (ultrafine PM, or UFP).

FROM SOURCES TO EXPOSURE

Outdoor Exposure

PM_{10} and $PM_{2.5}$

Ito et al. (2001) showed that monitor-to-monitor correlations of ambient air concentrations of criteria pollutants are relatively high (~0.6) over a scale of 100 miles in the North-Central region of the United States (US) for PM_{10}, O_3, and NO_2, but only ~0.3 for CO and SO_2 using a nationwide database (Ito et al., 2005a). The overall rank in monitor-to-monitor correlation, on the average, was: O_3, NO_2, PM_{10}, (0.7~0.8) > CO (~0.6) > SO_2 (~0.4). Both the separation distance and regional variation were important predictors of the correlation. For PM_{10}, for example, the correlation for the monitors along the East Coast was higher by ~0.2 than for monitors in western regions.

Some previous research suggested a relationship between coarse mode (>2.5μm), accumulation mode (0.1 to 1.0μm) and inter-modal (1-2.5 μm) PM. For coarse, inter-

modal, and fine PM collected across four sites in the Los Angeles (LA) Basin, the studies showed that the inter-modal PM consists of a significant portion of particles that are similar in chemical composition to PM_1. These results indicated that a PM_1 standard would not constitute an unambiguous separation of coarse and fine mode PM in this urban air shed (Geller et al., 2004).

$PM_{2.5}$ Components

Spatial variability of PM components was examined using a mobile lab at 6 locations within an area of New York City (NYC), the South Bronx, where high concentrations of diesel trucks and waste transfer facilities are located (Maciejzyk et al., 2004), as well as at the Hunter College School of Health Sciences on the east side of midtown Manhattan. $PM_{2.5}$, black carbon (BC), and gaseous pollutants were measured at 6 locations for three four-week intervals each during the period of April 2001–February 2003. Although the median daily $PM_{2.5}$ concentrations agreed within 20%, the median hourly BC concentrations were higher at all South Bronx sites, ranging from 2.2 to 3.8 $\mu g/m^3$, compared to 1.0–2.6 $\mu g/m^3$ at Hunter College. Continuous Aethalometer BC measurements at additional 27 sampling sites in the South Bronx along major highways indicated that the BC concentrations varied within each site, depending on time-of-day, with a large spatial variability from site-to-site.

Ito et al. (2004) used EPA's $PM_{2.5}$ speciation data for NYC to evaluate the potential exposure error associated with each source type, and found the strongest temporal correlations across the three monitors for the individual PM components related to secondary aerosols (e.g., S, NH_4^+). Source-apportionment of these data identified four major source/pollution types: 1) secondary (largely regional) aerosols; 2) soil; 3) traffic-related; and 4) residual oil burning/incineration, at each of three monitoring sites. The estimated source-apportioned $PM_{2.5}$ mass generally showed the highest monitor-to-monitor correlation for the secondary aerosol factor (r range = 0.72 to 0.93). The correlation for the more localized traffic-related factor was more variable (r range = 0.26 to 0.95).

PM size and chemical composition were extensively characterized in the LA Basin, showing that the PM physical and chemical properties depend greatly on locations and season. UFP concentrations were found to be the highest at the source sites, characterized as being near fresh vehicular emission sources. Mass concentrations in the accumulation mode ($0.1 < d_p < 2.5$ μm) were lower in winter than in summer, especially at the receptor sites. PM concentrations in the coarse thoracic particulate matter (CTPM) range ($2.5 < d_p < 10$ μm) were lower in winter, and were composed mostly of nitrate (NO_3^-) and crustal elements (Fe, Ca, K, Si, and Al). In the accumulation mode, NO_3^- and organic carbon (OC) were predominant, with higher NO_3^- levels found at the receptor sites. The UFP consisted of mostly OC, with higher wintertime levels at the source sites, due to the enhanced condensation of organic vapor from motor vehicles at lower temperatures. Conversely, higher UFP OC levels at the receptor areas were due to secondary organic aerosol formation by photochemical reactions, as well as increased advection of polluted air masses from upwind areas (Sardar et al., 2005a).

The effects of atmospheric transport on the size distribution of PM–bound species, such as polynuclear aromatic hydrocarbons (PAHs), elemental carbon (EC), OC, $SO_4^=$, and NO_3^- were also reported in the LA Basin. For October - February, the size

distributions of PAHs were similar, but were drastically different from those in March to July. A significant fraction of the PAH and the NO_3^- mass was in the coarse mode, as compared with the previous period. The correlation of temperature with the concentration of PAHs in the less volatile or PM-phase group was negative, consistent with increased partitioning from the vapor-phase with decreasing temperature. During all seasons, the form and shape of the EC size distributions did not vary much, and were distinguished by prominent mass in the UFP and accumulation modes. For the individual modes of the major species measured, the highest correlations were found in the UFP for $SO_4^=$ and EC, suggesting increased atmospheric transport of vehicular emissions from the urban downtown LA region (Miguel et al., 2004).

Measurements of daily size-fractionated ambient PM_{10} metals were conducted at source (Downey) and receptor (Riverside) sites within the LA Basin. All crustal species were predominantly in particles > 1 μm. At the source site, potentially toxic metals (e.g., Pb, Sn, Ni, Cr, V, and Ba) were predominantly partitioned (70–85%, by mass) in the particles < 1 μm. The receptor site exhibited coarser distributions for almost all particle-bound metals. $PM_{2.5}$ metal concentrations at that site were due to a few local emission sources and transport from urban LA. CTPM metal concentration trends were governed by variations in the wind speeds in each location, whereas the diurnal trends in the $PM_{2.5}$ metal concentrations were a function of prevailing meteorological conditions and upwind sources (Singh et al., 2002).

A nationwide cross-sectional source apportionment used the 2000 to 2003 EPA $PM_{2.5}$ speciation quarterly average concentrations for over 200 US locations. The eight largest pollution source categories were: traffic, coal combustion, secondary sulfates, soil, salt, residual oil combustion, metals, and steel production. Of these, traffic was a major contributor to the total $PM_{2.5}$ throughout the US, with the highest levels observed along the West Coast, especially in California. However, other components were often more regional, or limited in scope; for example, coal combustion effluents and secondary sulfates appeared to affect most of the Northeast US, while the soil levels were most elevated in the southwest of the country. The residual oil combustion component was found to be associated mainly with the deep US seaports, where electric power plants burn residual oil, and containerized cargo ships burn extremely polluting "bunker" fuel oil. Wood burning was a major component of $PM_{2.5}$ in selected cities in the Pacific Northwest (Kim et al., 2003, 2004; Maykut et al., 2003; Larson et al., 2004).

Reactive oxygen species (ROS), a term used to collectively describe oxygen-containing species with strong oxidizing ability, include molecules like hydrogen peroxide (H_2O_2), ions like the hypochlorite ion (OCl^-), hydroxyl (OH) radical, and the superoxide anion (O_2^-). One hypothesis for the adverse health effects of airborne PM is that ROS, formed by metals acting as catalysts for Fenton reactions, at concentrations that cause inflammation, lead to systemic dysfunctions (Stohs and Bagchi, 1995). Docherty et al. (2005) investigated the role of organic peroxides in secondary organic aerosol (SOA) formation from biogenic monoterpenes such as α-pinene, and concluded that SOA was predominantly organic peroxides, which contributed anywhere between 47% and 85% of the total SOA mass. Venkatachari *et al.* (2005; 2006) determined diurnal concentrations of particle-bound ROS for various size fractions of the aerosol, ranging from 10 nm to 18 μm in Rubidoux, CA in July 2003, and in Flushing, NY during the period of January and early February 2004, using a MOUDI™ cascade impactor. In

Rubidoux, California, ROS concentrations correlated moderately with the ambient O_3 concentrations, smaller particles were observed to have higher ROS concentrations, and the general magnitude of ROS concentrations were found to be at least an order of magnitude higher than those observed in studies in Taipei (Hung and Wang, 2001). In NYC, correlations of the PM ROS concentrations with the intensity of photochemical reactions and gas phase radical concentrations were found to moderate factors affecting PM ROS concentrations. Lower concentrations of ROS were found in NYC than those in Rubidoux, CA.

Ultrafine Particles (UFP)

Increasing epidemiological and toxicological evidence links cardiopulmonary health effects with UFP exposures. The method of Dillner et al. (2005) was used to measure the composition of UFP at two sites in Houston, TX; one surrounded by refineries, chemical plants, and vehicular and commercial shipping traffic, and the other, 25 miles inland, which was surrounded by residences, light industrial facilities, and vehicular traffic. Twenty-four hour size-segregated ($0.056 < D_p < 1.8$ μm) PM samples were collected. Inductively coupled plasma/mass spectroscopy (ICP/MS) was used to quantify 32 elements with concentrations as low as a few picograms per cubic meter (pg/m^3). Concentrations of PM mass, $SO_4^=$ and OC were often not significantly different from each other, and had smooth uni-modal size distributions, indicating the regional nature of these chemical species. Concentrations varied widely, and often showed sharp peaks for diameters between 0.1 and 0.3 μm and in UFP, suggesting that the sources were local, high-temperature processes. The clustered elements were generally attributed to different sources (such as automobile catalysts, fluid catalytic cracking unit catalysts, fuel oil burning, a coal-fired power plant, and high-temperature metal working) at the two sites during each sampling day, indicating the diversity of local sources that impact metals concentrations in the region.

Motor vehicles may be the primary direct emission sources of UFP to urban atmospheres, especially in the LA Basin. It is important to understand how UFP behave after being emitted and transported. Understanding the characteristics of UFP volatility and UFP penetration into indoor environments is also a vital information need.

Utilizing a mobile particle concentrator, the physical and chemical PM characteristics and volatility on/near freeways, the impact of mobile sources on indoor environments, and PM characteristics and emission factors in roadway tunnels with light-duty or heavy-duty vehicles were determined. Relative concentrations of CO, EC and UFP number decreased exponentially and tracked each other well with distance from a freeway (Zhu et al., 2002a; 2002b; 2004). In-vehicle measurements showed a 5-fold higher particle numbers and EC concentrations compared to those measured in typical urban background areas (Westerdahl et al., 005), and that PM emissions from vehicles are externally mixed; i.e., different particles of the same size can have different chemical compositions (Kuhn et al., 2005a). Between 70-90% of the particles, and 10-30% by mass consisted of semi-volatile material originating from condensation of organic vapors from fuel and lubricating oil (Kuhn et al., 2005a). The non-volatile portion consists primarily of EC, which is often coated with more volatile organic species. The volatility of the surface coatings explains the more rapid decay in their concentration with respect to distance from a roadway, as compared to that of non-labile PM species (such as EC) or

gaseous co-pollutants, such as CO and NO_x, the concentrations most affected by atmospheric dilution (Zhang et al., 2004; 2005). These studies also showed that the volatile components of PM may be present in its gaseous phase in indoor environments, causing particle shrinkage and/or complete evaporation as they infiltrate indoors (Zhu et al., 2004 b; Kuhn et al., 2005b). In future research, given that the majority of people's exposure during commuting will be dominated by these particles, it would be useful to know whether the non-volatile or semi-volatile material is more toxic.

A better understanding of UFP characteristics and their volatility would allow for a narrowing of the search for the most toxic PM components. Current particle traps for Diesel engines remove non-volatile soot particles, but not the precursors of the smaller semi-volatile particles. An unintended result of any reduction of the larger, non-volatile particles from the exhaust may be a potential increase in the formation/emission of the smaller, semi-volatile PM, as seen in experiments performed at the Caldecott tunnel, in which size fractionated emission factors were determined for heavy and light duty vehicles and compared to those of previous studies in the same location (Geller et al., 2005).

Studies in Southern California showed that UFP size distributions at source sites were generally unimodal, with a mode diameter of 30–40 nm, and without significant monthly variations, but were bimodal at receptor sites, with a significant increase in the accumulation mode sizes in summer. Afternoon periods in the warmer months were characterized by high number counts, while mass and EC remained low, suggesting the formation of new particles by photochemistry. Particle mode diameters ranged from 30 nm up to above 100 nm, a result not seen in other urban or rural areas where mode diameters are generally less than 50 nm. UFP concentrations and size distributions were influenced by long-range advection and photochemical processes, as well as by vehicular emissions, which have previously been assumed to dominate day-to-day UFP levels (Fine et al., 2004a).

The very small mass of UFP has posed a great challenge in determining their size-dependent chemical composition using conventional aerosol sampling technologies. The use of two technologies in series, the USC Ultrafine Concentrator described by Kim et al. (2001a, b), and the MSP NanoMOUDI, made it possible to overcome these problems. UFP were measured at source and receptor sites during three consecutive 3-hour time intervals (i.e., morning, midday and afternoon). A distinct mode in the 32-56 nm size range was most pronounced in the morning. While the mass concentrations at the source site decreased with time, the levels measured at Riverside, CA (a "receptor" site) were highest in the afternoon, with a minimum at midday. At that site, UFP EC and OC concentrations were highly correlated during the morning period, but collapsed later in the day in this area (Geller et al., 2002).

The PM Center exposure research was coupled with toxicological and health effects studies that demonstrated increased toxicity in terms of the oxidative potential of UFP, compared to other PM size fractions on a mass basis, as measured by a variety of *in vitro* bioassays (Li et al., 2003; Xia et al., 2004; Cho et al., 2005) as well as by *in vivo* concentrated ambient particle studies (CAPs) conducted in the vicinity of freeways (Campbell et al., 2005; Kleinman et al., 2005), as described in the health effects section.

Since UFP originates from vehicular emissions the concentrations of gases such as CO, NO, or NO_2 that also originate from traffic sources have be used as surrogate

measures of UFP. The validity of that assumption was tested at five sites in the LA Basin. There was an overall lack of significant associations between hourly and 24-hr particle number (PN) versus gaseous co-pollutant concentrations, which may be attributable to the differences in the sources and formation mechanisms responsible for generating these pollutants in the LA Basin. These findings also imply that potential confounding effects of co-pollutants will not affect epidemiologic analysis seeking to link UFP to health effects because of the general lack of associations between PN and co-pollutant concentrations (Sardar et al., 2005b).

Improved characterization of individual UFP became possible through improvements in the Aerosol Time-of-Flight Mass Spectrometer (ATOFMS) described by Su et al. (2004). They use a more efficient ATOFMS, for the on-line detection and determination of the size and chemical composition of single fine (100-300 nm) and UFP (<100 nm) particles. Polystyrene latex spheres (PSL) were used to characterize: the particle sizing efficiency, particle detection efficiency, and particle beam profile, and to perform instrument calibration. At PN concentrations of <20 particles/cm^3, the particle sizing efficiencies were ~0.5% for 95 nm, and ~47% for 290-nm PSL particles, while the particle detection efficiencies were ~0.3% for 95 nm, and 44% for 290-nm PSL. This represents an increase by 3 orders of magnitude in detection efficiencies for smaller particles over the conventional ATOFMS. In addition, the beam profiles for PSL followed a Gaussian distribution, with a full width at half-maximum of ~0.35 μm. The resulting higher detection efficiencies allow the ATOFMS to obtain higher temporal resolution measurements of the composition of individual fine and UFP, as demonstrated in initial ambient measurements in La Jolla, CA. At typical ambient PN concentrations of 102-103 particles/cm^3, ~30 000 particles with aerodynamic diameters of <300 nm were detected with average 24-h hit rates of 30% for PM between 50 and 300 nm. This advancement allows high temporal resolution measurements of the composition of smaller particles with higher efficiency in order to chemically characterize individual fine and UFP.

Source Apportionment

The PM Centers sponsored a Workshop on Source Apportionment for Particulate Matter Health Effects in May 2003 to evaluate the consistency of the various source apportionment methods for assessing source contributions to daily $PM_{2.5}$ mass-mortality associations. Seven groups of investigators, using various methods, estimated source apportionments of $PM_{2.5}$ mass samples collected in Washington, DC and Phoenix, AZ. Apportionments were evaluated for their respective associations with mortality using Poisson regressions, allowing a comparative assessment of the extent to which variations in the apportionments contributed to variability in the source-specific mortality results. Analyses indicated that source types were significant predictors of relative risks, whereas apportionment group differences were not, adding only ~15% to the mortality regression uncertainties (Thurston et al., 2005). Other Workshop results investigations of inter-method variability in the associations for Washington (Ito et al., 2005b) and in Phoenix (Mar et al., 2006).

Hopke et al. (2006) presented the results of the source apportionment inter-comparison, and reported that there was good agreement among the major resolved source types. Crustal (soil), sulfate, oil combustion, and salt were the sources that were

most unambiguously identified (generally highest correlation across the sites). Traffic and vegetative burning showed considerable variability among the sites, and there was variability in the ability of the methods to partition the motor vehicle contributions between gasoline and diesel vehicles. However, when the total motor vehicle contributions were estimated, good correspondence was obtained among the results. The source impacts were especially similar across various analyses for the larger mass contributors (e.g., the standard error (SE) for Washington, DC, for secondary sulfate, was 7%, and for traffic it was 11%; in Phoenix, the secondary sulfate SE was 17%, and was 7% for traffic). Especially important for time-series health effects assessment, the source-specific impacts were found to be highly correlated across analysis methods/researchers for the major components (e.g., mean analysis to analysis correlation, $r > 0.9$ for traffic and secondary sulfates in Phoenix, and for traffic and secondary nitrates in Washington. The mean sulfate r-value was > 0.75 for Washington. Overall, although this inter-comparison suggested areas where further research is needed (e.g., better division of traffic emissions between diesel and gasoline vehicles), they provided support for the contention that $PM_{2.5}$ mass source apportionment results are consistent across users and methods, and that today's source apportionment methods are sufficiently robust for application to $PM_{2.5}$ health effects assessments.

By examining the seasonal, temporal, spatial, size-fractionation, and inter-correlations of individual organic compounds, the sources and atmospheric fate of these tracers can be better understood and their utility as molecular markers can be assessed. Southern California PM Center investigators used a high-flow rate, low pressure-drop UFP separator to collect sufficient mass for organic speciation of UFP and accumulation mode aerosol on a diurnal basis. Sampling was conducted at two sites (source and receptor oriented) over two seasons (summer and winter). Hopanes, used as organic markers for vehicular emissions, were found to exist primarily in the UFP mode. Levoglucosan, an indicator of wood combustion, was quantified in both size ranges, but more was present in the accumulation mode particles. An indicator of photochemical secondary organic aerosol formation, 1,2 benzenedicarboxylic acids, was found primarily in the accumulation mode and varied with site, season, and time of day, as one would expect for a photochemical reaction product. These data will be used to assess the concentration of specific PM sources to personal exposure and ultimately to health effects in upcoming epidemiological and toxicological studies in the LA Basin (Fine et al., 2004b).

FROM EXPOSURE TO HEALTH EFFECTS

Personal Exposure

$PM_{2.5}$

A number of PM Center studies were conducted to obtain a better understanding of the relationship of personal exposures to ambient and indoor concentrations of $PM_{2.5}$. A large panel study of Seattle residents (Liu et al., 2003) provided important information about daily average exposures of at-risk groups to $PM_{2.5}$ and also about the relationship between personal exposures to $PM_{2.5}$ and simultaneous measurements at fixed-site community monitors. This 3-year study involved 108 individuals with and without

chronic obstructive pulmonary disease (COPD), coronary heart disease (CHD), and asthma. Overall, the elderly healthy, COPD and CHD subjects had similar exposures. All three groups had lower $PM_{2.5}$ exposures than the asthmatic children. Within a given group, the $PM_{2.5}$ exposure varied with the subject's residences, due to the different particle infiltration efficiencies of different buildings. Although a wide range of longitudinal correlations between central site and personal $PM_{2.5}$ measurements were found, the longitudinal correlation for any given subject was closely related to the particle infiltration efficiency, F_{inf}, of their residence.

Allen et al. (2003) used a recursive mass balance model to estimate the 10-day average F_{inf} for individual residences of panel subjects. The average F_{inf} was 0.65 ± 0.21 for 44 residences. F_{inf} differed significantly by season, being higher in the non-heating season. For the 44 study residences, outdoor-generated particles accounted for an average of $79 \pm 17\%$ of the indoor $PM_{2.5}$ concentration, with a range of 40 to 100% at individual residences. In a subsequent analysis of 62 residences of panel subjects, F_{inf} was found to vary by: residence type (group homes > private residences); the presence of an air cleaner; and meteorological conditions (temperature and rainfall) (Koenig et al., 2005). The Seattle studies found that total personal exposure was poorly predicted by stationary outdoor monitors among persons whose $PM_{2.5}$ exposure was dominated by non-ambient sources, for example, those living in tightly sealed homes, those who cook, and active children (Allen et al., 2003).

Using a similar approach, Wu et al. (2004, 2005) studied children living in Alpine, CA. The contributions to the children's hourly, personal $PM_{2.5}$ exposure from outdoor sources, indoor sources and personal activity were 11.1, 5.5, and 10.0 $\mu g/m^3$, respectively, when the modeling error was minimized. The high $PM_{2.5}$ exposure to personal activities was attributed to the children's more active lifestyle as compared with those of older adult subjects in previous studies.

Sheppard et al. (2005a, b) conducted a series of simulated acute health effects studies to examine the consequences of using the ambient concentrations measured at fixed sites in place of personal exposures measurements. They included important model parameters based upon the Seattle panel study results (Liu et al., 2003; Allen et al., 2003, 2004), including the distributions of C_0, C_i, φ, and E_{ig}. Assuming that φ (ambient $PM_{2.5}$ contribution fraction) does not vary with individual over time, they found no noticeable impact on the estimation of the effect estimate from the time-series model, even under the most restrictive condition that E_{ig} is independent of E_{ag}. However, when the value of φ for each individual was allowed to vary with season, the time-series health effect estimates changed. They concluded that understanding the temporal variability in φ is important to interpreting the results of time-series studies. They also concluded that the suggestion of using total personal exposure as the exposure metric of interest for acute time-series studies (NRC, 1998; Zeger et al., 2000) is not realistic, because daily personal measurements are needed for the entire study population. Using only a few individuals to estimate the daily average population exposure results in a highly attenuated health effect estimate, but it is possible to correct for this measurement bias using a measurement error model.

The relationship between ambient concentrations and personal exposures to $PM_{2.5}$ and gases was examined using data from Boston, MA and Steubenville, OH. These studies used similar methods and study designs to measure, over 24 hr periods, indoor,

outdoor, and personal $PM_{2.5}$ and gaseous levels ($PM_{2.5}$, SO_4^{2-}, EC, O_3, NO_2, and SO_2) In Boston, study participants included 20 healthy senior citizens and 23 schoolchildren, while in Steubenville, study participants included 10 senior citizens. In both studies, personal exposure and ambient concentration data were analyzed using correlation and mixed model regression analyses to examine relationships between ambient and personal $PM_{2.5}$ and gaseous exposures.

Similar results were observed in Boston and Steubenville. In Boston, substantial correlations were found between ambient $PM_{2.5}$ concentrations and corresponding personal exposures over time. Additionally, these results supported the earlier finding that summertime gaseous pollutant concentrations may be better surrogates of personal $PM_{2.5}$ exposures, especially personal exposures to $PM_{2.5}$ of ambient origin, than surrogates of personal exposures to the gases themselves. $PM_{2.5}$ health effects studies that include both ambient $PM_{2.5}$ and gaseous concentrations as independent variables must be analyzed carefully and interpreted cautiously, since both parameters may be serving as surrogates for $PM_{2.5}$ exposures (Sarnat et al., 2006).

Similarly, in Steubenville, strong associations were found between ambient $PM_{2.5}$ concentrations and corresponding personal exposures as well as between ambient O_3 and NO_2 and their corresponding exposures. These associations, in particular those for O_3, were highest for individuals spending the majority of their time in highly, as compared to poorly, ventilated environments. In cross-pollutant models, significant associations between ambient $PM_{2.5}$ concentrations and personal gas exposures were found, with particularly strong associations between ambient SO_4^{2-} and personal O_3, and between ambient EC and personal NO_2. Findings that ambient gas concentrations reflect corresponding personal exposures have implications for air pollution epidemiology, suggesting that confounding of PM-associated health effects by gaseous pollutants may occur given the often strong correlations among the ambient pollutants. Furthermore, findings that ambient $PM_{2.5}$ may represent exposures to both $PM_{2.5}$ and gases, suggest that time-series health studies based on 24-hour ambient concentrations may not be able to separate the independent effects of $PM_{2.5}$ and gases.

$PM_{2.5}$ Components

In addition to particle mass, a subset of the Seattle panel filters were analyzed for selected chemical species and positive matrix factorization was then used to identify five contributing sources: vegetative burning, mobile emissions, secondary sulfate, a source rich in chlorine, and a source of crustal-derived material. Vegetative burning contributed the majority of mass and BC in all samples. The indoor/outdoor ratios for vegetative burning and secondary source contributions varied significantly by residence, in agreement with the infiltration efficiencies derived using the recursive mass balance model approach (Allen et al., 2003). Personal exposure to the combustion-derived particles was correlated with outdoor sources, whereas exposure to the crustal and Cl-rich particles was not. Personal exposures to crustal source particles were strongly associated with personal activities, especially time spent at school among children, in agreement with a follow-up panel in Seattle (Jansen et al., 2005) that measured indoor, outdoor and personal levels of BC on a daily basis in adult subjects with asthma and/or COPD. There were good correlations between daily measures of indoor, outdoor and personal BC, but poor correlations between outdoor and personal PM_{10}.

Lippmann et al. (2005) measured personal PM_{10} exposures for a cohort of elderly COPD patients in New York City for twelve days in both summer and winter, and compared them with levels of $PM_{2.5}$ and PM_{10} measured indoors, outdoors, and at a community air quality monitor. For all pollutant measures, personal concentrations tended to be higher and more variable than corresponding indoor and ambient concentrations in both seasons, and the mean indoor concentrations tended to be higher than co-located outdoor concentrations. Particle concentrations showed some degree of seasonal variation, and had larger variability in summer months compared to winter months. Indoor and personal concentrations were higher in the summer as compared to winter for each of the measured pollutants. In contrast, summer and winter outdoor measurements (residential outdoor and central site) tended to be comparable.

Data from Boston were analyzed to characterize the relationships between personal, home indoor, home outdoor and ambient levels of SO_4^{2-}, EC, and $PM_{2.5}$ for a panel of sensitive individuals with either chronic CVD or COPD. Four main factors that were likely to affect personal exposures were investigated: time spent in key microenvironments, such as the home; infiltration into the home; spatial variability in home outdoor concentrations; and measurement error. This investigation was based on simultaneous 24-hour integrated personal, home indoor, and home outdoor $PM_{2.5}$, SO_4^{2-}, EC, O_3, SO_2 and NO_2 concentrations that were measured in 25 single-family homes in the Boston, MA area (Brown, 2006).

Ambient SO_4^{2-} was strongly correlated with personal and home indoor SO_4^{2-} for all individuals without an indoor source of SO_4^{2-}. Associations were not as strong for EC and $PM_{2.5}$, likely due to local and indoor sources of these pollutants. While the strength of the associations for SO_4^{2-} varied between subjects and by season, outdoor or ambient SO_4^{2-} accounted for approximately 80% or more of the variability in personal and indoor SO_4^{2-} concentrations. Housing conditions, as indicated by the high indoor-outdoor SO_4^{2-} correlations, tended to be quite similar day-to-day, indicating that home indoor and home outdoor levels correspond consistently regardless of the differences in the absolute levels in the two microenvironments. While ambient levels and indoor source contributions of

$PM_{2.5}$ can vary by day, the infiltration into homes appears to be relatively constant, at least during a one-week monitoring period (Brown, 2006).

Contrary to the results for SO_4^{2-}, EC showed relatively weak associations between personal/indoor EC levels and outdoor/ambient levels. This result was likely due to indoor and local source generation of EC. Indoor EC concentrations explained only 50% of the variation in corresponding personal exposures, likely the result of exposures to EC that occurred outside the home, or of greater imprecision in the EC measurement method as compared to those for SO_4^{2-} and $PM_{2.5}$. Additionally, indoor-outdoor ratios were higher and more variable for EC than SO_4^{2-}. This difference in ratios could include different infiltration rates, or a greater contribution of indoor sources of EC as compared to SO_4^{2-}. Since relatively few homes had indoor-outdoor EC ratios greater than 1, indicating few indoor EC sources, the results suggest that differences in SO_4^{2-} and EC infiltration was the more important factor. Differences in their infiltration may be related to corresponding differences in their particle size distributions (Seinfeld and Pandis, 1998). The results also indicate greater spatial variability in EC-related PM than for $PM_{2.5}$ or SO_4^{2-}, for which outdoor concentrations were relatively uniform (Brown, 2006).

Particle infiltration is a key determinant of the indoor concentrations of ambient air PM. To address the issue of the influence of $PM_{2.5}$ composition on infiltration, a comprehensive indoor air monitoring study was conducted in 17 Los Angeles area homes (Sarnat, 2006). In this study, indoor/outdoor concentration ratios during overnight (non-indoor source) periods were used to estimate the fraction of ambient $PM_{2.5}$ remaining airborne indoors, or the particle infiltration factor (F_{INF}), for $PM_{2.5}$, its non-volatile (i.e., BC) and volatile (i.e., NO_3^-) components, and particle sizes ranging between 0.02 and 10 μm. F_{INF} was found to be highest for BC (median = 0.84) and lowest for NO_3^- (median = 0.18). The low F_{INF} for NO_3^- was likely due to volatilization of NO_3^- particles once indoors, in addition to depositional losses upon building entry. In addition, it was found that the F_{INF} for $PM_{2.5}$ (median = 0.48) fell between those for BC and NO_3^-, reflecting the contributions of both particle components to $PM_{2.5}$. F_{INF} varied with particle size, air exchange rate and outdoor NO_3^- concentrations. The F_{INF} for particles between 0.7-2.0 μm in size was significantly lower during periods of high, as compared to low, outdoor NO_3^- concentrations, suggesting that outdoor NO_3^- particles fall in this size range, and that its volatilization influenced the size distribution of indoor particles. This study demonstrated that infiltration of $PM_{2.5}$ varies by component, and is lowest for volatile species such as NH_4NO_3. Thus, indoor $PM_{2.5}$ of ambient origin may differ from that outdoors with respect to composition and size distribution, especially when the outdoor concentration of volatile particle components is high. In addition, based on these results, SO_4^{2-} particles may not be suitable proxies of particles of outdoor origin in areas with high concentrations of volatile $PM_{2.5}$. Particle composition, therefore, may influence the ability for outdoor $PM_{2.5}$ concentrations to represent indoor and thus personal $PM_{2.5}$ exposures, and can ultimately influence observed epidemiologic relationships based on ambient monitoring data.

A study to evaluate contributions of vehicle generated UFP to indoor environments in close proximity to freeways in the absence of known indoor aerosol sources found that PN concentration I/O ratios showed a strong dependence on particle sizes, and was influenced by different ventilation mechanisms. Highest I/O ratios (0.6–0.9) were usually observed for larger UFP (70–100 nm), while the lowest I/O ratios (0.1–

0.4) occurred typically around 10–20 nm. The size distributions of indoor aerosols showed less variability than those of outdoor freeway aerosols. The penetration factors and deposition rates also varied significantly, depending on particle size, and agreed with literature data and theories for particles greater than 20 nm. For particles less than 20 nm, I/O ratios, penetration factors, and deposition rates did not follow the expected trend based on theoretical prediction, as a result of the unique, semi-volatile, nature of freeway UFP. Sub-50 nm particles form mobile sources are semivolatile, thus shrink to a smaller size (or evaporate completely) as they infiltrate indoors (Zhu et al., 2005).

GENERATION AND CHARACTERIZATION OF PM FOR LABORATORY EXPOSURES

Model Particles

To perform human/animal exposure studies, there is a need for methods that can be used to generate high number concentrations of UFP with controllable compositions. The Palas spark discharge generator (Palas GFG 1000) generates "soot-like" particles for such studies. It is important to assess the chemical variability and reproducibility of the UFP produced using such techniques. Su et al. (2005) performed an on-line assessment of the chemical variability of individual UFP and fine (50–300 nm) particles produced by a Palas generator. The aerodynamic size and chemical composition of ^{12}C and ^{13}C elemental carbon (EC), composite iron–carbon (Fe-^{12}C), and welding particles were analyzed using an ATOFMS. When using ^{12}C electrodes, EC particles were produced with sizes peaking in the UFP mode and 96% of the mass spectra containing distinct C_n^+ (n = 1–3) envelopes at m/z 12, 24, and 36. In contrast, the mass spectra of the particles generated from ^{13}C labeled graphite electrodes showed 73% of the particles producing EC carbon ion cluster patterns at m/z 13 ($^{13}C^+$), 26($^{13}C_2^+$), and 39 ($^{13}C_3^+$), with additional OC species. Observed differences between the ^{12}C and ^{13}C particle spectra are most likely due to their different surface properties, with ^{13}C particles more effectively adsorbing semivolatile organic species originating in the particle-free dilution air. Homogeneous metal particles were also generated from Fe-^{12}C and welding rods with almost all (92% and 97%, respectively) of the spectra showing reproducible Fe/Mn/Cr and Fe/^{12}C ion ratios.

Particle Concentrators

The size, concentration enrichment, and chemical composition of coarse-mode (> 2.5 μm) and fine-mode (< 2.5 μm) particles within the non-concentrated and concentrated flows of a coarse particle concentrator used for human exposure studies have been characterized using an ATOFMS (Moffett et al., 2004) for fixed time intervals over the course of three days. The coarse particle concentrator was intended to concentrate ambient particles in the $PM_{10-2.5}$ size range before sending them into a human exposure chamber. Based on the ATOFMS results, it was found that there was no change in the composition of the ten major particle types observed in the upstream and downstream flows of the concentrator under normal operating conditions. Furthermore, no new particle types were detected downstream that were not detected upstream. A characterization of the aerosol chemical composition and its dependence on sampling

conditions was also discussed. Aerosol size distributions were measured with three aerodynamic particle-sizing (APS) instruments sampling simultaneously from different regions of the concentrator. The APS size distributions were used to scale ATOFMS data and measure the ambient concentration factors for the coarse particle concentrator and the exposure chamber. The average concentration factor (ratio of inlet number concentration to the outlet number concentration) for the particle concentrator was 60±17 for the 2.5–7.2 μm size range before dilution and transport to the exposure chamber. It was observed that not only were coarse particles being concentrated, but PM2.5 was being concentrated as well, with concentration factors ranging from 2–46 for aerodynamic particle sizes from 0.54–2.5 μm.

UFP concentrators have also been used in PM health effects studies in an effort to control exposure levels to ambient aerosols over a broad enough concentration range. An ATOFMS was used to characterize individual concentrated UFP and fine (100–300 nm in aerodynamic diameter) PM from several UFP concentrators (Su et al., 2005). Experimental results showed that particles undergo chemical changes during the enrichment processes at super-saturation ratios of 3.0 or lower. Comparing the relative fractions of particle types in concentrated versus non-concentrated ambient airstreams, a decrease was observed in nominally fresh EC particles relative to EC particles coated with OC after undergoing the concentration process. An increase in the number fraction of aromatic- and polycyclic aromatic hydrocarbon-containing particles was also observed in both the UFP and the fine mode. Such changes are attributable to gas-to-particle partitioning (e.g. water-soluble organic compounds) onto pre-existing UFP and fine particles during the particle enrichment process, which involves super-saturation, condensation, desolvation, and evaporation for particle growth and size restoration. In addition, during the morning hours in Rochester, NY, aqueous phase sulfur chemistry occurred in the concentrated particles, as indicated by the presence of hydroxymethanesulfonate (HMS), an indicator commonly used to indicate fog processing in ambient aerosols.

Two different particle mass spectrometers, the Aerodyne (AMS) and the UC Davis RSMS-3, were used to evaluate the performance of the Versatile Aerosol Concentration Enrichment System (VACES) developed by USC. The Rapid Single-particle Mass Spectrometer (RSMS-3) experiments were conducted as part of the EPA Supersite program in Pittsburgh during March 2002. RSMS-3 hit rate increases were measured, and possible particle composition changes introduced by the VACES were examined in the single particle mass spectra. Both ambient and concentrated carbonaceous and ammonium nitrate composition distributions were indistinguishable with RSMS-3, suggesting that VACES introduces an insignificant artifact for those particles (Zhao et al., 2005). The effect of concentrating semi-volatile aerosols using the VACES and the AMS during measurements of ambient aerosol in Pittsburgh, PA was also investigated. It was found that the shape of the SO_4^{2-} mass- weighted size distribution was approximately preserved during passage through the concentrator for all the experiments performed, with a mass enhancement factor of about 10 to 20 depending on the experiment. The size distributions of OC, ammonium and NO_3^- were preserved on a relatively clean day (SO_4^{2-} concentration around 7 $\mu g/m^3$), while during more polluted conditions the concentration of these compounds, especially NO_3^-, was increased at small sizes after passage through the concentrator. The amount of the extra material, however,

was found to be rather small: between 2.4% and 7.5% of the final concentrated $PM_{2.5}$ mass is due to "artifact" condensation (Khlystov et al., 2005).

A subchronic animal inhalation study using an ambient particle concentrator addressed the issues of composition and sources of ambient $PM_{2.5}$, as well as the relationship of these $PM_{2.5}$ characteristics to the cellular response of human bronchial epithelial cells (Maciejczyk and Chen, 2005). An *in vitro* exposure technique was used to compare the daily variations of the responses of cells to fine CAPs collected from a rural area upwind of New York City for the period of 9 a.m. to 3 p.m. on weekdays only, March–September 2003. Chemical composition data for CAPs were modeled using factor analysis, with Varimax orthogonal rotation, to determine four particle source categories contributing significant amount of mass to CAPs at Sterling Forest (Tuxedo, NY). These source categories are: (1) regional secondary sulfate characterized by high S, Si, and OC; (2) resuspended soil characterized by high concentrations of Ca, Fe, Al, and Si; (3) oil-fired power plants emissions of the eastern United States identified by presence of V, Ni, and Se; and (4) unknown other sources. To estimate the mass contributions of each individual source category, the CAPs mass concentration was regressed against the factor scores. Regional SO_4^{2-} was the largest contributor to mass (65%), followed by soil (20%), residual oil combustion (2%), and the other sources contributing 13%.

CONCLUSIONS

The research conducted at the EPA PM Centers on exposures has provided a substantial body of new data indicating that:

- Spatial correlations over large metropolitan areas are relatively high (~0.6) for O3, NO2, PM_{10} and $PM_{2.5}$
- Spatial correlations over large metropolitan areas are relatively low (~0.6) for CO and SO2.
- Spatial correlations over short distances can be very low for black carbon and for UFP
- Semi-volatile components in ambient air can move from fine PM to coarse PM over time, especially in warm months
- Reactive oxygen species (ROS) are found in particles ranging in size from UFP to coarse PM, and are found in larger concentrations influenced by photochemical reactions
- PM source categories were shown to be significant predictors of relative risks in epidemiological studies
- $PM_{2.5}$ exposures of asthmatic children were higher than those of healthy people, and subjects with coronary heart disease and chronic obstructive pulmonary disease.

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