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The U.S. Environmental Protection Agency Particulate Matter Health Effects Research Centers Program: A Midcourse Report of Status, Progress, and Plans

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In 1998 Congress mandated expanded U.S. Environmental Protection Agency (U.S. EPA) health effects research on ambient air particulate matter (PM) and a National Research Council (NRC) committee to provide research oversight. The U.S. EPA currently supports intramural and extramural PM research, including five academically based PM centers. The PM centers in their first 2.5 years have initiated research directed at critical issues identified by the NRC committee, including collaborative activities, and sponsored scientific workshops in key research areas. Through these activities, there is a better understanding of PM health effects and scientific uncertainties. Future PM centers research will focus on long-term effects associated with chronic PM exposures. This report provides a synopsis of accomplishments to date, short-term goals (during the next 2.5 years) and longer-term goals. It consists of six sections: biological mechanisms, acute effects, chronic effects, dosimetry, exposure assessment, and the specific attributes of a coordinated PM centers program. *Key words:* **acute effects, biological mechanisms, chronic effects, criteria pollutants, dosimetry, exposure assessment, infrastructure, morbidity, mortality, particulate matter.** *Environ Health Perspect* **111:1074–1092 (2003). doi:10.1289/ehp.5750 available via** *http://dx.doi.org/* **[Online 9 January 2003]**

Over the past 15 years, an ever-increasing number of epidemiologic studies have shown significant associations between the mass concentration of ambient air particulate matter (PM) and adverse respiratory and cardiovascular health effects. These effects include PM concentration-related excess rates of daily and annual mortality, hospital admissions, emergency room and clinician visits for respiratory and cardiac diseases, increased use of medications, and time lost from work and school. By the mid-1990s, the evidence for these associations was sufficiently compelling for the U.S. Environmental Protection Agency (U.S. EPA) to propose revised and more stringent National Ambient Air Quality Standards (NAAQS) for PM (NAAQS 1997). Their form and stringency were endorsed by the U.S. EPA Clean Air Scientific Advisory Committee (CASAC), a group of external scientific advisors whose charter was established by the Clean Air Act Amendments of 1977 (1991).

The revised PM NAAQS, promulgated in July 1997, retained with minor modification the previous daily maxima and annual average PM NAAQS for PM whose aerodynamic diameters were < 10 μm (PM_{10}). It also established new PM NAAQS for particles with aerodynamic diameters < 2.5 μm ($PM_{2.5}$), as excess mortality was found to be more strongly associated with $PM_{2.5}$ than with PM_{10}.

The U.S. EPA (and CASAC) acknowledged that the database supporting the judgments that $PM_{2.5}$ and PM_{10} exposures were likely causal factors for adverse health effects was not fully supported by, or consistent with, available knowledge of the underlying biological mechanisms. Among the likely factors for discrepancies between observations in human populations and corresponding observations in controlled animals and human studies are the following:

- Epidemiologic observations of adverse effects had largely been confined to subpopulations who may have been especially susceptible because of underlying preexisting disease or who were very young or very old. In contrast, because of practical and ethical considerations, most human clinical studies had examined effects in small groups of healthy individuals of intermediate ages. Similarly, most previous animal studies focused on healthy and younger animals and used exposures to concentrations that were much higher than those encountered in ambient air.
- Toxicological studies have been limited, as the most active components of PM remain a matter of speculation. Subsequent studies would need to use more realistic ambient mixtures, e.g., concentrated ambient air particles (CAPs) or laboratory-generated surrogates that focus on specific particle characteristics such as particle size [e.g., ultrafines (diameters less than 0.1 μm)] and/or chemistry [e.g., metals, polycyclic aromatic hydrocarbons (PAHs), quinones]. In addition, it is possible that responses may require a mixture of PM components and/or the simultaneous or sequential exposure to gaseous pollutants in the ambient air mixture [e.g., SO_2, NO_2, O_3, CO, and volatile organic compounds (VOCs)].
- No laboratory-based toxicological studies have been conducted involving chronic or even subchronic exposures to ambient air PM mixtures at concentrations at the upper end of the range of current U.S. ambient air concentrations.

As a result of the remaining scientific uncertainties, in 1998 Congress directed the U.S. EPA to substantially increase its level of funding on PM health effects research. It also mandated that a National Research Council (NRC) committee (i.e., the Committee on Research Priorities for Airborne Particulate Matter) be established to provide scientific oversight for the PM research. In the first of its three reports, the NRC Committee on Research Priorities for Airborne Particulate Matter recommended a multiyear research program that included the establishment of academically based research centers to create a comprehensive and integrated particle health effects research program. The PM centers were intended to foster interdisciplinary collaborations within and among institutions with extensive experience in air pollution health effects research. Research that arose through these collaborations were in turn intended to help the U.S. EPA address scientific issues about PM health effects in a timely and effective manner.

The U.S. EPA through its Science to Achieve Results (STAR) program for investigator-initiated research issued a request

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for proposals (RFPs) for centers for PM health effects research in 1998. It received and peer-reviewed 21 applications, and awarded five center grants in the summer of 1999. The successful applicants were (in alphabetical order)

- A California consortium, headquartered at University of California Los Angeles (UCLA; Los Angeles, California)
- Harvard University (Boston, Massachusetts)
- New York University (NYU; New York, New York)
- The University of Rochester (Rochester, New York)
- The University of Washington (Seattle, Washington).

The U.S. EPA in its RFP specified that *a*) each center have an external scientific advisory committee to ensure that its research program addresses important knowledge gaps and is scientifically sound (each of these committees include members from academic institutions, industrial companies, public interest groups, and some U.S. EPA scientists); *b*) center directors have an annual meeting with U.S. EPA scientists and administrators for the exchange of information, progress, and future plans, and for coordination of collaborative research; and *c*) each center have an outreach program to communicate to the public current knowledge of air pollution health effects and research progress being made to address knowledge gaps.

The centers have addressed these requirements and initiated collaborative activities. These include sponsorship of scientific workshops to further research in key areas such as characterizing cardiac health effects associated with PM exposures, assessing costs and health benefits of air pollution controls, examining the health impacts of gasoline emissions in California, and apportioning PM sources and their associated health effects.

Through their individual and collective activities during the initial years of the PM health effects research centers, real progress has been made toward the understanding of ambient air pollution health effects and addressing areas of remaining scientific uncertainty. The PM centers plan to continue to conduct both epidemiological and toxicological studies to enhance our understanding of the health effects associated with ambient PM exposures. Of particular interest will be investigations of chronic PM health effects. This research objective is consistent with the U.S. EPA multiyear plan for PM research as defined in its November 2001 presentation to the NRC Oversight Committee.

This report provides a synopsis of the centers' research accomplishments to date. Many of the research studies presented received major support from PM center funds. For others, primary support was from other sources, such as the National Institute of Environmental Health Sciences (NIEHS) and the Health Effects Institute (HEI), with the availability of PM center core services and interactions with other PM center investigators providing important supplemental support. This report consists of six sections. Sections 1–5 address issues relating to 1) biological mechanisms, 2) acute effects, 3) chronic effects, 4) dosimetry, and 5) exposure assessment. Section 6 summarizes the features of the PM centers program that have facilitated the harnessing of the skills and resources of the PM center investigators for collaborative research addressing the U.S. EPA PM NAAQS development. Each of the five key area sections begins with background summarizing the challenge faced by the PM centers as they began their centers program in terms of what was known and what goals were established to address key knowledge gaps. These background summaries are followed by an outline of progress that has been and is being made by PM center investigators in resolving knowledge gaps. The report also outlines short-term goals (during the 2.5 remaining years of center support) and long-term goals (beyond the initial 5 years of center support) for the PM centers.

The PM centers' approach has been highly interactive both within each center and across centers, combining research efforts on exposure/source characterization, epidemiological and toxicological effects, and biological mechanisms summarized in Figure 1. Continuous interdisciplinary dialogue among the PM center scientists assures that the total breadth of PM research is covered and that new research ideas have a sound scientific basis. This is accomplished partly through periodic meetings among center members from the different scientific disciplines represented in the centers. The exposure assessors provide data on specific ambient air PM constituents to toxicologists; toxicologists provide information on the relative toxicity of PM constituents to exposure assessors and epidemiologists; the *in vitro* toxicologists share insights with epidemiologists, clinicians, and animal toxicologists to help elucidate PM-specific response mechanisms. This kind of collegial interaction and sharing of research within and among the centers is a special feature of the PM centers program. The five research topics listed in the center section of Figure 1 are the areas jointly addressed by the five centers to obtain results useful for the regulatory needs of the U.S. EPA. Examples of progress in these areas are provided in this special report.

Biological Mechanisms for Particulate Matter Health Effects

Background

Justifications for the 1997 PM_{10} and $PM_{2.5}$ NAAQS were based primarily on a large and coherent epidemiological database of significant associations between ambient air PM concentrations and excess mortality and morbidity. Although the 1996 PM criteria document provided some support for biological plausibility of causal links between PM and health effects, evidence from controlled human and animal exposure studies was still largely unavailable. On the basis of this information gap, a major rationale for the establishment of the PM centers was to explore biological plausibility and mechanisms of PM-associated health outcomes. Over the past 2 years, substantial contributions have been made toward developing hypotheses based on experimental observations, in large part because of the research environment fostered by the structure of PM centers. The following section provides examples of how the PM centers program has contributed to the development and testing of plausible mechanistic hypotheses for the health effects of PM.

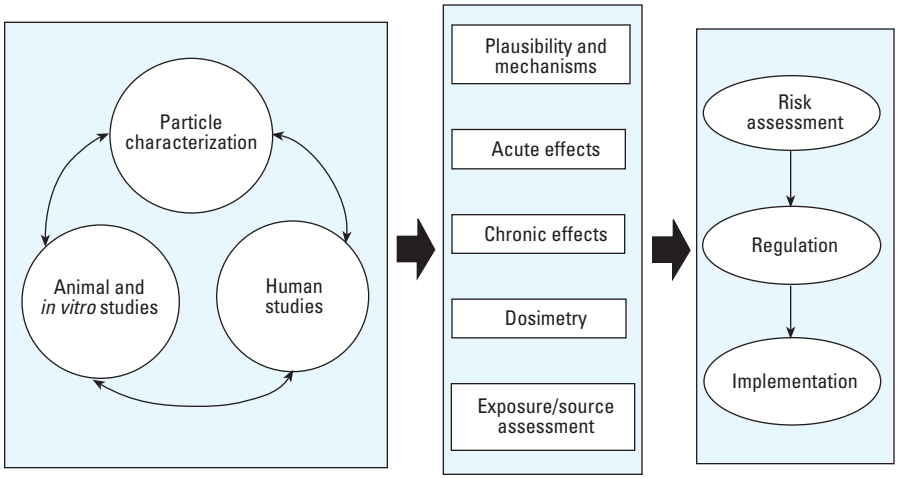

Figure 1. PM center integration of research disciplines.

Recently, several mechanistic pathways have been investigated that may link PM exposure with adverse health effects. Figure 2 highlights the complexity and interdependency of some of these pathways (Utell and Frampton 2000). The portal of entry for PM air pollution is the lung, and PM interactions with respiratory epithelium likely mediate a wide range of effects, as indicated by the central oval in Figure 2. These include respiratory as well as systemic and cardiovascular effects. However, PM, or its reaction products, may stimulate airway sensory nerves, leading to changes in lung function and in autonomic tone, which influence cardiac function. Ultrafine particles by virtue of their extremely small size may enter pulmonary capillary blood and be rapidly transported to extrapulmonary tissues such as liver, bone marrow, and heart, with either direct or indirect effects on organ function.

Progress in Biological Mechanism Research

This section is a discussion of mechanistic areas in which particular progress has been made by the PM centers in inflammation and immunity, mechanisms for cardiovascular effects, and the role of reactive oxygen species (ROS). This is not intended to be a comprehensive review but rather a summary of center research programs. Many important studies, particularly *in vitro* studies of PM mechanisms, are not addressed.

Inflammation and immunity. Airway injury and inflammation is a well-known consequence of toxic inhalation exposures. Previous studies involving animal models have shown that instillation or inhalation of particles such as diesel exhaust particles (DEPs) can cause inflammation and epithelial injury at high doses and concentrations. However, there was little evidence prior to the PM centers program that exposure to ambient concentrations of PM caused significant airway inflammation. The presence or absence of an inflammatory response is an important issue because inflammation may induce systemic effects, including an acute-phase response with increased blood viscosity and coagulability and possibly an increased risk for myocardial infarction in patients with severe coronary artery disease. In chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (COPD), inflammation is a key pathophysiologic feature. Chronic, repeated inflammatory challenge of the airways may result in airway remodeling that leads to irreversible lung disease. Thus, inflammation may be involved in both acute and chronic effects.

Following is a list of several key findings from PM center research about the role of inflammation and immunity in mediating the health effects of PM.

Particulate matter exposure and systemic markers of inflammation in humans. The PM centers in southern California and Rochester, New York, have collaborated in human clinical studies using identical crossover exposure protocols, subject recruitment criteria, and outcome measures. The California studies used CAPs at approximately 200 μg/m^3, and Rochester used laboratory-generated carbonaceous ultrafine particles at two concentrations—10 and 25 μg/m^3. Preliminary findings from both of these studies provide evidence for effects on systemic markers of inflammation and leukocyte recruitment (Boscia et al. 2000; Daigle et al. 2002; Frampton et al. 2001).

In both centers, subjects were exposed for 2 hr with intermittent exercise. Before and at intervals after exposure, symptom ratings, lung function testing, and phlebotomy were performed. Sputum induction was performed approximately 24 hr after exposure. In both centers, blood was analyzed for markers of systemic inflammation, acute-phase response, and blood coagulability. One such marker is soluble intercellular adhesion molecule-1 (sICAM-1), a transmembrane protein expressed on leukocytes and endothelial cells. sICAM-1 plays an important role in monocyte recruitment to atherosclerotic lesions and inflamed airways, where it is shed into plasma during leukocyte adhesion.

In the southern California CAPs studies, sICAM-1 increased significantly ($p < 0.05$) after exposure to CAPs (relative to filtered-air controls) for the pooled healthy and asthmatic population. The effect was greater at 21 hr after exposure than at 4 hr. In the Rochester ultrafine particle studies, no significant change was found in sICAM-1 in the plasma of healthy subjects, but blood monocyte expression of ICAM-1 decreased immediately following particle exposure in a concentration–response fashion compared with air exposure. These studies suggest that exposures to either CAPs or ultrafine particles may initiate endothelial and leukocyte activation, with shedding of surface ICAM-1, a key initial step in leukocyte recruitment. These findings may have implications for cardiovascular and respiratory disease. In a major cardiac epidemiological study, plasma sICAM-1 levels were predictive of future coronary events (Ridker et al. 1998).

Particulate matter exposure and inflammation in animals. Studies in normal dogs exposed to Boston CAPs by inhalation showed increases in pulmonary inflammation by bronchoalveolar lavage and in circulating blood neutrophils associated with increases in specific ambient particle components. In these experiments, mean exposure doses were 203 and 361 μg/m^3 in the lavage and blood studies, respectively (Clarke et al. 2000). These PM center studies show that ambient particle components have significant pulmonary and systemic inflammatory potential. Similar findings have been reported in rats in PM center studies (Saldiva et al. 2002).

Effects of aging. Determining the mechanisms involved in increased susceptibility to PM comprises another goal of PM center research being explored at many levels. The role of aging is being examined, using animal and human exposure studies as well as *in vitro*

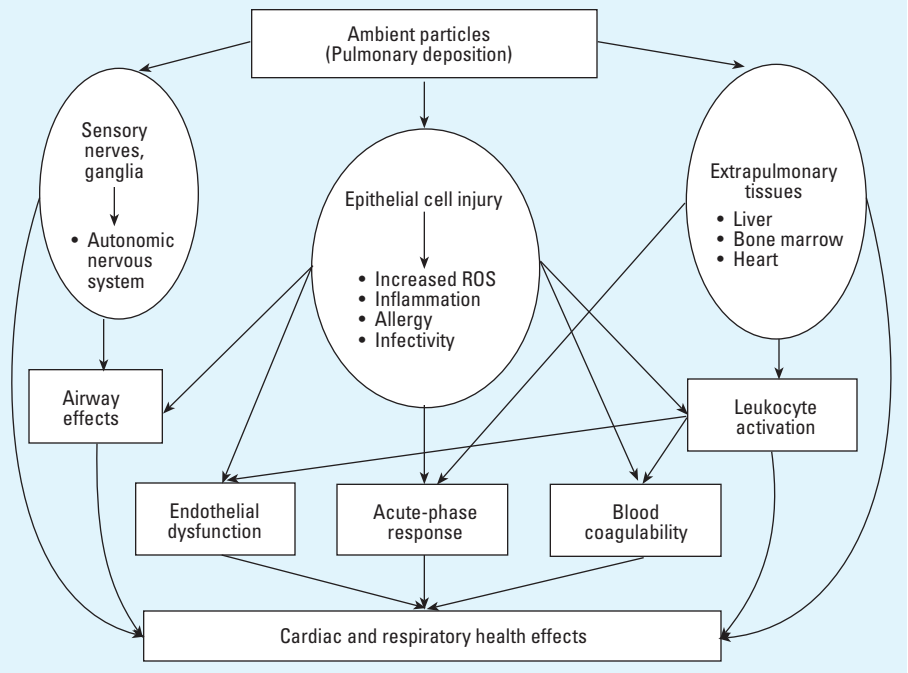

Figure 2. Hypotheses for health effects of PM.

models. Studies were conducted examining cytokine production by alveolar macrophages from aged rats and mice (> 20 months of age) after *in vitro* exposure to lipopolysaccharide (LPS) and PM. Macrophages from aged animals were incubated with endotoxin, with laboratory-generated mixed carbon/iron ultrafine particles, or with both. Baseline production of cytokines was elevated 30–50% in aged cells compared with cells from young animals (8–10 weeks of age). The response to LPS was enhanced at every dose in aged cells. The response to ultrafine particles containing iron was enhanced 2- to 3-fold in aged cells compared with young cells. Most significantly, in the aged animals, coadministration of ultrafine particles and LPS led to synergistic effects at the lowest ultrafine particle dose, whereas in young cells this was observed only at the highest PM dose (Finkelstein et al. 2002). These findings suggest a cellular basis for age-related increased susceptibility that may relate to increased susceptibility to oxidative stress. Alternatively, the results may be showing a lower threshold because of impaired protective mechanisms (e.g., antioxidant defenses). Investigations of these age-related differences are currently focusing on signal transduction mechanisms.

Effects on infection/pneumonia. Epidemiological studies have demonstrated that infection, specifically pneumonia, contributes substantially to the increased morbidity and mortality among elderly individuals after exposure to PM (Schwartz 1994), suggesting that inhaled PM can act as an immunosuppressive factor that undermines normal host pulmonary immune responses. A combination of particle concentrator technology and animal infectivity models is being used to investigate this hypothesis. A single 5-hr inhalation exposure of bacterially infected rats to New York City CAPs, at concentrations ranging from 65 to 150 μg/m^3, altered both pulmonary and systemic immunity and exacerbated the infection process in a time-dependent manner (Zelikoff et al. 1999). *Streptococcus pneumoniae*–infected rats exposed to PM demonstrated increased burdens of pulmonary bacteria, numbers of circulating white blood cells, extent of pneumococcal-associated lung lesions, and incidence of bacteremia, compared with air-exposed, infected control rats. Conversely, this same PM exposure resulted in decreased levels of lavageable polymorphonuclear neutrophils (PMNs), bronchus-associated lymphoid tissue, and proinflammatory cytokines [i.e., tumor necrosis factor-α, interleukin (IL)-1, and IL-6] in infected rats. Subsequent studies implicated the iron content in mediating these effects; many of the findings were reproduced with nose-only exposure to soluble iron but not with soluble forms of other metals (manganese, copper, or nickel). These findings suggest that PM exposure, and specifically the soluble iron component, may affect the host immune response during pulmonary infection and may help explain epidemiological observations. In addition to the effects of iron, PM center investigators have shown that DEPs and CAPs induce apoptosis in macrophages by an oxidative stress mechanism that is dependent on the organic chemicals in the PM. Macrophage apoptosis will lead to decreased phagocytic defenses in the lung.

Cardiovascular effects. Determining the mechanisms linking ambient PM to cardiovascular effects is one of the key challenges of the PM centers. There is growing clinical and epidemiological evidence that ambient air pollution can precipitate acute cardiac events such as angina pectoris, cardiac arrhythmias, and myocardial infarction, with the majority of excess PM-related deaths attributable to cardiovascular disease (CVD). The PM centers' approach to this issue is multifaceted and multidisciplinary. There are ongoing panel studies of susceptible subjects involving cardiovascular monitoring at three PM centers, animal exposure studies at four PM centers, and human clinical studies at three PM centers. Three PM centers share a cardiac monitoring and analysis protocol for human clinical studies.

A major step forward in this area was made with the convening of a workshop, "Cardiovascular Effects of Air Pollution: Potential Mechanisms and Methods of Testing," which met in Rochester, New York, in March 2001. The proceedings have now been published (Utell et al. 2002), and important research needs in this area have been identified. The workshop featured presentations by PM center and other investigators, along with clinical and research cardiologists. Each of the five U.S. EPA PM centers participated in the workshop along with representatives from the U.S. EPA. New hypotheses and research directions were developed, and practical issues of cardiac monitoring methodology currently in use at each PM center were reviewed and optimized. This workshop grew directly from one of the annual PM center directors meetings, where the need for intercenter collaboration on this issue was identified. The interchange of ideas served as an important turning point in our thinking about the mechanisms involved in cardiac effects, and new collaborative efforts were initiated.

Key observations in both human and animal studies of cardiovascular effects have been made since the PM center program was initiated. A few examples follow.

Human studies. Investigation of cardiovascular effects of PM has required multidisciplinary collaboration. For human exposure studies, analysis of cardiac monitoring includes a detailed analysis of heart rate variability (HRV) and repolarization intervals before, during, and for a period of 48 hr after exposure. In one study (Frampton et al. 2002), healthy subjects were exposed by mouthpiece for 2 hr with intermittent exercise on three separate occasions to air or ultrafine carbon particles at 10 and 25 μg/m^3. Frequency-domain analysis of the continuously recorded electrocardiogram (EKG) indicated that response of the parasympathetic nervous system was blunted during recovery from exercise immediately after exposure to ultrafine particles, compared with air. This diminished vagal response was not observed 3.5 hr later. Monitoring also indicated that exposure to ultrafine particles altered cardiac repolarization, as indicated by the corrected QT interval (QTc) on the cardiogram. The increase in QTc following exercise during air exposure was blunted with PM exposure, and this persisted to at least 21 hr after exposure. This change in repolarization was not explained by changes in heart rate (Frampton 2001; Zareba et al. 2001). It is plausible that ultrafine particle exposure imposes an effect on repolarization, either through an indirect effect via the autonomic nervous system or by directly affecting ion channel function in ventricular myocardium through a yet unknown mechanism. This observation is important because changes in the QT interval have been implicated in susceptibility to cardiac arrhythmias in patients with heart disease.

These human clinical studies are complemented by a major panel study involving patients with preexisting coronary artery disease in Erfurt, Germany. Analysis of the EKG recordings and blood parameters are under way, including detailed analyses of HRV and repolarization, and acute-phase proteins using methodology identical to the human clinical studies. These clinical/epidemiological/toxicological collaborations are examples of how the PM centers program has fostered research among diverse disciplines and locales.

Animal studies. Rats and mice are being instrumented for continuous cardiac and blood pressure monitoring. Algorithms have been developed for analysis of heart rate and blood pressure variability using continuous 24-hr recordings in up to eight rodents simultaneously (Couderc et al. 2002). A crossover study with aging, spontaneously hypertensive rats exposed to carbonaceous ultrafine particles is ongoing.

PM center investigators are evaluating both inflammation and cardiovascular effects in animal models. The hypothesis being tested is that inhaled PM causes release of inflammatory mediators from cells in the lung that then become bloodborne and target the cardiovascular system. The research plan uses transgenic mouse strains with specific cardiovascular

genetic alterations to create susceptibility models. Initial studies have been using a mouse model of atherosclerosis, the apolipoprotein E–deficient mouse generated via a targeted disruption of the mouse *apo-E* gene. The deficiency of apo-E leads to a spontaneous hypercholesterolemia. The nonhypertensive animals form atherosclerotic lesions throughout the vasculature, which resemble, in part, human atherosclerotic lesions at 3–5 months of age. Animals are instrumented and monitored for blood pressure and heart rate using radiotelemetry. Individual mice were dosed with 125 μg Washington, DC, urban dust in 50 μL saline by oropharyngeal aspiration into the lungs. Heart rate was reduced after exposure to PM in normal and in apoE-/- mice. ApoE-/- mice showed a trend to increased blood pressure and increased variability of blood pressure after PM exposure that did not differ significantly from that of the normal mice (Luchtel et al. 2002). Additional experiments are being performed as the control and apoE-/- mice age, and studies involving more realistic exposures are being planned.

In another PM center study, male Fischer 344 rats 18 months of age with implanted EKG transmitters were used to determine the effects of PM on the frequency of spontaneous arrhythmias. As old rats (18 months of age) were found to have many spontaneous arrhythmias, a standardized definition for each type of arrhythmia was developed, and a procedure for quantifying the frequency of spontaneous arrhythmias was established. Rats were exposed to New York City CAPs or filtered air for 4 hr. The rats were exposed twice with a crossover design so each rat could serve as its own control. The CAP concentration was 160 μg/m^3 and 200 μg/m^3 for the first and second exposures, respectively. EKG tracings demonstrated a significant increase in the frequency of supraventricular arrhythmias after exposure to CAPs compared with filtered air–exposed control animals. The same rats were also exposed twice to 1 ppm SO_2 and twice to air in a repeated crossover design. No significant change in the frequency of any category of spontaneous arrhythmia after exposure to SO_2 (or filtered air) was observed (Nadziejko et al. 2001).

The effects of PM on myocardial ischemia have also been the focus of PM center research. Inhaled PM exacerbated ischemia in a clinically relevant model of coronary artery occlusion in conscious dogs. Exposures to CAPs significantly increased peak electrocardiographic ST-segment elevation during a 5-min coronary artery occlusion compared with sham exposures in two different protocols using conscious dogs (Godleski et al. 2000; Wellenius et al. 2003). The relationship of ambient particle components to the degree of ischemia in dogs is the focus of ongoing PM center research (Wellenius et al. 2003). Other PM center studies have used an improved model of myocardial infarction in the rat and demonstrated increased ventricular arrhythmias with aerosol exposure to oil fly ash particles but not to carbon black particles (Wellenius et al. 2002). This model of myocardial infarction is being used to study the effects of combinations of CAPs and carbon monoxide. In other studies of the vascular response to inhaled particles, a variety of cell and molecular biologic methods have been used.

Reactive oxygen species. A major finding has been that PM generates ROS, which provide proinflammatory stimuli to bronchial epithelial cells and macrophages. These cellular targets respond with cytokine and chemokine production, which can enhance the response to allergens. PM may therefore act as an adjuvant that strengthens the response of the immune system to environmental allergens. Hallmarks of allergic inflammation include increased immunoglobin E (IgE) production, eosinophilic bronchial inflammation, airway hyperresponsiveness, and an increase of nitric oxide (NO) in exhaled air.

This hypothesis is being tested using *in vitro* and animal studies (Whitekus et al. 2002). In one study using an allergic mouse model, animals were exposed at high doses to nebulized DEPs (2000 mg/m^3) for 1 hr, followed by nebulized antigen [ovalbumin (OVA)] for 20 min daily for 10 days. A control group received saline instead of DEPs followed by OVA. To determine the role of ROS, the same exposure groups were pretreated every day with intraperitoneal *N*-acetylcysteine (320 mg/kg). Control animals received saline intraperitoneally. Two days after the last exposure, blood was obtained and assayed for IgE and IgG1, and the lungs were assayed for carbonyl proteins and lipid peroxides. DEPs markedly enhanced the antibody response (Figure 3) and lipid peroxidation, and these effects were abrogated by antioxidant treatment. Follow-up studies using more realistic exposure levels of DEPs and/or CAPs are being planned. Furthermore, human nasal challenge studies confirmed the role of DEPs as an adjuvant in already established allergic responses, as well as in exposure to neoallergens. Taken together, these findings may explain the increased number and severity of asthma attacks in an urban setting after a surge in PM levels, and may implicate DEPs as a factor in asthma exacerbations.

ROS associated with exposure to PM may play a role in cardiovascular effects. 9,10-Phenanthroquinone (9,10-PQ) is a potent inhibitor of neuronal form of nitric oxide synthase (NOS). 9,10-PQ also inhibits the endothelial form of NOS, which plays a critical role in vascular tone, thereby causing the suppression of NO-dependent vasorelaxation of aorta and significant increase in blood pressure in rats. Therefore, quinones and other compounds producing ROS, for example, nitro-PAHs, may contribute to diseases related to vascular dysfunction caused by exposure. In addition to the production of ROS, quinones, PAHs, nitro-PAHs, and related compounds may also undergo electrophilic addition to macromolecules producing complementary toxicity.

The key role of the PM centers in facilitating this line of investigation on ROS has been the collaboration between scientists with diverse expertise. For example, the collective expertise in the southern California PM center facilitated the use of CAPs to replace DEPs for mechanistic *in vitro* and *in vivo* studies. Asthma animal models are now being used to compare the prooxidative and proinflammatory effects of CAPs collected on California freeways and various source-receptor sites. In addition, human panel studies and CAPs exposure studies now typically include the role of oxidative stress in airway inflammation (e.g., assays for NO and CO content in the expired air, and measures of cytokines in induced sputum, blood, and breath condensate).

Another important development by the PM centers is collaboration among chemists, engineers, and biologists in exploring how chemical constituents of CAPs contribute to ROS generation and inflammation. An important observation has been that organic components present in the organic carbon fraction generate ROS through their ability to undergo redox cycling. The *in vitro* reactions correlate well with the ability of organic PM components to generate oxidative stress in epithelial cells and macrophages. Preliminary evidence indicates that PAHs and their oxidized derivatives (quinones and ketones) play a key role in ROS generation at the cellular level. The *in vitro* toxicity studies predict a hierarchical or stratified oxidative stress response in which the biological effects range from *a*) protective (e.g., expression of antioxidant enzymes); *b*) proinflammatory (e.g., production of cytokines

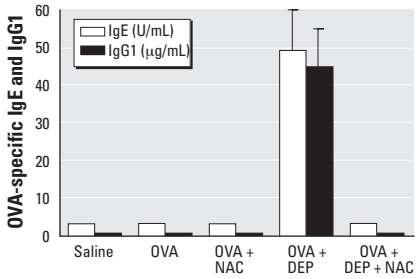

Figure 3. Thiol antioxidant interferes in the adjuvant effects of DEPs during OVA sensitization in a murine allergic inflammation model.

and chemokines); and *c*) cytotoxic (e.g., cellular apoptosis and necrosis), depending on the level of oxidative stress. The ability to relate the inherent redox-cycling and oxidative stress capabilities of a PM sample to specific biological effects allows a more rational interpretation of the *in vivo* toxicity data being generated in the community, freeway, and source/receptor studies.

Short-Term Particulate Matter Research Goals in Biological Mechanisms

Objectives of the PM centers during the remaining funding period will include a greater utilization of intercenter collaborations. In addition to the collaborative efforts described in this section, new interactive center initiatives will elucidate mechanisms of PM effects. These include sharing of PM collected from various sources at different PM centers for use in animal and *in vitro* studies, sharing of exposure technology, and developing common laboratory protocols.

An important objective will be to identify additional mechanistic links. For example, neural pathways may play a role in mediating cardiovascular effects. If studies currently under way confirm that PM affects autonomic regulation of cardiovascular function, we will need to further define the mechanisms by which those effects are initiated, including the cells and cell mediators responsible. Research on these mechanisms will provide vital clues about individual susceptibility and potential approaches to the prevention of adverse health effects.

Animal studies based on the hierarchical or stratified oxidative stress model are being planned and will consider the possible identification of susceptible human subjects with weakened oxidative stress defenses. This could involve polymorphisms of the heme oxygenase 1 gene, which is a very sensitive antioxidant defense mechanism that protects cells against redox-cycling DEPs chemicals and contributes to CO and NO production during *in vivo* DEP exposure. The elucidation of susceptible individuals who can be studied with rational end points will enhance epidemiological studies and will also help to monitor the impact of regulatory measures to reduce adverse health effects.

A continuing research objective is to further examine the role of PM composition, size, surface area, gaseous co-pollutants, and other factors in mediating effects. This involves a variety of experimental approaches, from detailed morphologic and chemical analysis of ambient air PM to *in vivo* and *in vitro* exposure studies using both CAPs and laboratory-generated PM of carefully defined composition. A related longer-term goal is to determine the role of complex mixtures in eliciting health effects using factor analysis to identify the sources of PM and associated gaseous air pollutants.

Determining the mechanisms underlying individual susceptibility to PM effects is another major objective under way in each of the PM centers. Host susceptibility factors being investigated include age, gender, underlying disease, infections, and genetic factors.

A key goal of PM centers research is to develop biologic markers of specific mechanistic pathways that can be used to link findings from animal, human, and epidemiological studies. Many examples are currently being investigated, including plasma sICAM-1 and monocyte ICAM-1 as indicators of enhanced leukocyte endothelial interaction, and measurement of changes in HRV as a measure of cardiovascular autonomic effects.

Long-Term Particulate Matter Research Goals in Biological Mechanisms

One major long-term objective of the PM centers is to determine the mechanisms involved in chronic health effects of ambient PM exposure. Epidemiological studies have indicated that exposure to $PM_{2.5}$ leads to a shortening of life span, and this finding was a major impetus in the establishment of the annual average $PM_{2.5}$ NAAQS. Does $PM_{2.5}$ exposure exacerbate underlying disease or contribute to the genesis of disease (or both)? Some of the key findings currently being investigated in the PM centers, and summarized here, have implications for chronic (long-term) effects.

For example, one hypothesis is that recurrent inflammatory or allergic challenges to the airway leads to airway remodeling, a key feature in the development of irreversible airways obstruction in asthma and COPD. Second, there is growing evidence that atherosclerotic CVD is an inflammatory process. PM centers are testing the hypothesis that immune or inflammatory effects of PM exposure may promote or accelerate atherosclerosis. Third, diabetes is associated with severe, accelerated atherosclerotic vascular disease and increased susceptibility to infection, and a recent study identified diabetics as particularly susceptible to the health effects of PM. Determining the mechanisms involved in this susceptibility of diabetics may help shed new light on PM effects mechanisms in general.

Ambient air PM contains carcinogens, and recent epidemiological evidence indicates that mortality due to lung cancer is increased in relation to $PM_{2.5}$ exposure. Investigating the PM components and mechanisms for carcinogenesis will be an important long-term goal of the PM centers.

Finally, major long-term goals of the PM centers are to contribute new scientific data and risk assessment tools and information that will assist in refining air quality standards for PM, and to evaluate the public health benefits of reductions in PM exposure. This will involve more comparisons of PM potency across animal, clinical, and *in vitro* end points coupled with ever-increasing specificity as to aerosol characteristics, leading to better indicators of biological mechanism and links of particular sources of PM to specific health effects.

Acute Health Effects of Particulate Matter

Background

The acute health effects of PM exposures have been extensively examined by a large number of epidemiological studies conducted worldwide. These studies have consistently shown significant associations between daily average ambient PM concentrations and corresponding cardiopulmonary mortality, morbidity, and functional impairments. When the U.S. EPA promulgated its 24-hr $PM_{2.5}$ NAAQS in 1997, it relied primarily on this large body of epidemiological data relating PM exposures to daily deaths and hospital admissions. However, critics of the revised PM NAAQS raised a number of key issues in their challenges to the credibility of, or need for, a new 24-hr $PM_{2.5}$ NAAQS, such as *a*) the associations represented deaths of frail individuals whose deaths were brought forward by only a few days or weeks (harvesting), and thus had little public health significance; *b*) particles originating from different sources have varying toxicities; therefore, the relative health impacts of particle sources should be assessed prior to regulating their emissions; *c*) the associations were potentially confounded by season, weather, and other gaseous pollutants; *d*) the associations were implausible because ambient $PM_{2.5}$ concentrations were not appropriate surrogates of personal $PM_{2.5}$ exposures; *e*) the associations were implausible because there was limited support from controlled human and animal studies; *f*) the identification of populations susceptible to $PM_{2.5}$ health effects is necessary prior to promulgating a new $PM_{2.5}$ standard; and *g*) the exposure–response curves, showing no thresholds, were unlikely from a biological standpoint.

Many of these issues have been addressed in recent epidemiological and toxicological investigations examining the acute health effects associated with short-term PM exposures. Collaborations between epidemiological and toxicological communities have led to the development of common study hypotheses and common health end points such as lung function, arterial oxygen saturation, heart rate, HRV, blood pressure, tissue biomarkers of effects, exhaled nitric oxide (eNO), cardiac dysrhythmias, and respiratory symptoms. As a result, our understanding of

ambient PM acute health effects has been advanced. For example, results from recent controlled laboratory studies have shown that short-term exposures to CAPs, compared with artificially generated particles, result in acute health effects comparable to those reported in the epidemiological literature. These toxicological findings provided some evidence about biological plausibility and mechanisms that will be of paramount importance to our efforts to examine the validity of the observed epidemiological study findings.

Progress Made in Acute Effects Research

Since the establishment of the PM centers, substantial progress has been made in our understanding of PM health effects. Collectively, the PM centers have addressed a large number of scientific issues regarding acute PM health effects. These investigations are broadly categorized as either observational or controlled studies. It has recently been reported that the S-Plus Software (MathSoft, Inc., Cambridge, MA) fails to adequately meet the convergence criteria nominally applicable to multiply smoothed data in studies of acute health effects of air pollution (Dominici et al. 2002). Center investigators who have used this software have reanalyzed many of their recent studies using the new default criteria of Dominici et al. (2002), and although small changes in effect size estimates have been made, none of them necessitated changes in the conclusions drawn from these studies.

Observational studies. **Harvesting.** If air pollution–related deaths mainly affect already frail or sick individuals whose deaths are being brought forward by only a few days (the harvesting hypothesis), then PM-related mortality would have little or no importance on total mortality rates. Studies examining the harvesting hypothesis were conducted by the PM centers. Using a series of moving averages of mortality and exposure data (7-day, 15-day, etc.), Schwartz (2000a) examined whether the strength of the associations changed over the various averaging periods. On the basis of the harvesting hypothesis, one would expect that estimated PM-mortality associations would become weaker as the averaging periods increased. However, the results from this analysis showed that the association between PM_{10} and mortality remained significant, and the estimated relative risks were, in fact, higher for the longer averaging periods. Similar results were found for hospital admissions data (Schwartz 2001). An alternate analytical approach was used in a 10-city meta-analysis study, where averaging periods were increased incrementally from 1 to 45 days. Results from this analysis showed that the PM effect increased by a factor of 2.5, again suggesting significant shortening of life (Zanobetti et al. 2002). Similar analyses are currently being conducted to examine cause-specific mortality.

Threshold/exposure response. Analytical methods were developed to combine smoothed exposure–response curves from multiple locations to examine whether a threshold in the PM_{10} and daily death relationship exists. Results from multiple cities in the United States and Spain suggest that the PM_{10}-mortality relationships are linear down to the lowest observed exposure concentrations, supporting the no-threshold hypothesis (Schwartz and Zanobetti 2000; Schwartz et al. 2001). Similar results were found in subsequent studies of $PM_{2.5}$ and mortality in six U.S. cities and of PM_{10} and hospital admissions. In the PM_{10}-hospital admissions study, the methodology was modified to examine sources of heterogeneity in the exposure–response relationship by calculating a random slope for each city. In the near future, we plan to apply the random slope model to mortality data and to examine other responses to PM exposures, such as EKG changes.

Particle-specific associations. PM center investigators have examined the relationship between mortality and source-specific PM concentrations. Laden et al. (2000) used source-apportionment techniques to group elemental PM concentrations from six U.S. cities into a small number of categories, or "factors." These factors were attributed to different PM sources such as vehicular emissions, oil combustion, and soil. For each factor, a daily score was calculated. Subsequently, excess daily mortality was regressed against the daily scores using multiple regression analyses. Significant associations were found between mortality and the traffic and coal combustion factors, with the largest effect size for the traffic factor. No significant associations were observed for the oil and soil factors. Mar et al. (2000) applied factor analysis to $PM_{2.5}$, PM_{10}, $PM_{10-2.5}$, sulfates, non–soil $PM_{2.5}$, organic carbon, elemental carbon, total carbon, gaseous co-pollutants, and cardiac mortality data in Phoenix, Arizona. Results from this study showed that combustion-related pollutants and sulfates were associated with cardiovascular mortality. Source-specific effects were also examined by Janssen et al. (2002), who used source emission and home characteristics to explain observed variability in the city-specific PM_{10}-hospital admissions coefficients. As shown in Figure 4, the PM_{10} coefficient for CVD-related hospital admissions increased with the fraction of PM_{10} emissions from traffic-related sources, suggesting higher relative risks from PM_{10} for cities with a greater number of traffic-related PM_{10} sources.

Susceptibility. Several studies have shown higher PM-associated health risks for susceptible subpopulations such as the elderly or those with existing cardiopulmonary diseases. PM center investigators have attempted to identify important susceptible subpopulations. Results from these studies have shown that socioeconomic factors and race do not affect susceptibility to PM-associated mortality (Zanobetti and Schwartz 2000); however, females were shown to be at greater risk.

The elderly have long been thought to be particularly susceptible to PM pollution. Consequently, several panel studies have focused on examining the impact of PM exposures on the elderly. In Seattle, Washington, the relationship between outdoor PM concentrations and cardiopulmonary health effects was examined for three panels of elderly subjects (healthy, with CVD, and with COPD). Results from this study showed that during paced breathing exercises, an increase in outdoor $PM_{2.5}$ (lagged by 1 hr) was associated with a decrease in the median of high frequency heart rate variability (HF HRV) in subjects with CVD (Sullivan et al. 2002). Decreases in HF HRV were also observed for 4- and 24-hr lagged periods. No effects were observed either for healthy subjects or those with COPD. However, results from these studies have not been entirely consistent, as a recent study showed no significant association between $PM_{2.5}$ exposure (0- to 2-day lag) and coached FEV_1 (forced expiratory volume in 1 sec) for subjects with COPD or CVD. In contrast, local outdoor $PM_{2.5}$ concentration was associated with a decrement in FEV_1 for normal subjects without reported COPD or CVD (Trenga et al. 2002).

Other PM center–sponsored studies examining whether individuals with preexisting disease are at increased risk from PM exposures are currently under way. Data collection and analyses for a study in Erfurt, in eastern Germany, of patients with preexisting CVD or COPD is currently being conducted to examine whether ultrafine particles exacerbate CVDs through different mechanisms than $PM_{2.5}$ (Wichmann and Peters 2000). Analyses of 683 EKG recordings and blood parameter analyses are ongoing. Fieldwork for the study

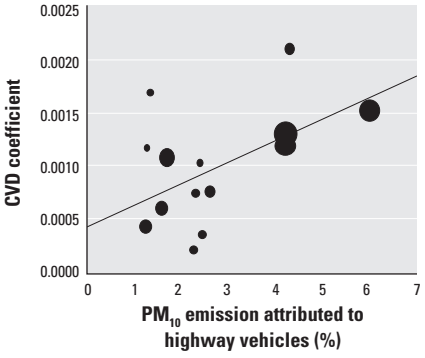

Figure 4. Association of motor vehicle PM_{10} and CVD hospital admissions. The size of the symbol reflects the size of the population group studied.

on COPD patients started in October 2001 and was completed in spring 2002.

Children may also be particularly susceptible to PM exposures. Koenig et al. (2002) conducted a study of 16 children (6–12 years of age) with mild to moderate asthma living in Seattle. Daily samples of eNO, a marker of pulmonary inflammation, were collected for each of these children for up to 10 consecutive days. Results from this study showed that an increase in $PM_{2.5}$ was associated with an increase in eNO, suggesting that $PM_{2.5}$, at ambient concentrations, can act as an inflammatory agent.

In an additional study conducted in Seattle, 133 children (5–13 years of age) with asthma were observed for an average of 58 days (range 28–112 days) while they were screened for enrollment in the Childhood Asthma Management Program study (Yu et al. 2000). In single-pollutant models, the population average estimates indicated an increase of 18% in the odds of an asthma symptom corresponding to a 10 μg/m^3 increment in same-day PM_{10} and an increase of 11% for PM_{10} lagged 1 day.

More recently, diabetics have been identified as an important susceptible population. In several single-city studies, the risk of PM-associated hospital admissions for heart disease for diabetics was double that for the general population (Zanobetti and Schwartz 2001, 2002). In addition, diabetics had an increased risk of PM-associated mortality (Bateson and Schwartz 2001). Individuals with other preexisting diseases were also at higher risk. Respiratory illness, for example, modified the risk of cardiovascular hospital admissions, whereas heart failure was found to modify the risk of COPD hospital admissions (Zanobetti et al. 2000).

Confounding. A new hierarchical model was used to examine the potential confounding effect of gaseous co-pollutants (Schwartz 2000b). The results of this study suggest that the association between PM_{10} and daily deaths was not confounded by gaseous air pollutants. A subsequent examination of the season-specific relationship between PM and mortality data for Philadelphia, Pennsylvania, confirmed that mortality was predominantly associated with $PM_{2.5}$ and not SO_2 (Schwartz 2000b). Additional information about confounding was provided by exposure studies, which showed that ambient gaseous air pollutant concentrations are not well correlated with their respective personal exposures but are significantly correlated to personal PM exposures, suggesting that the ambient gaseous concentrations are acting as surrogates for personal exposures to PM (Sarnat et al. 2001; also see "Exposure Assessment").

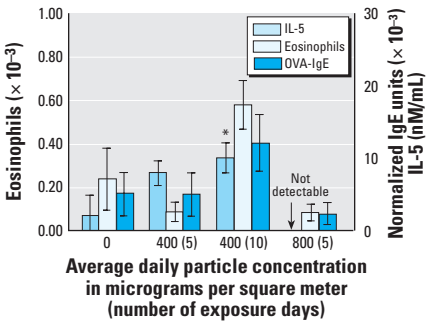

Figure 5. Responses of mice exposed approximately 50 meters downwind of freeway to CAPs and OVA. Group sizes = 9. Error bars represent mean ± SE.
*Significantly different from control ($p < 0.05$).

Controlled animal and human studies. A large number of controlled exposure studies in humans and animals have been conducted by the PM centers in an effort to investigate the acute biological responses induced by PM exposures. Some of these studies have used laboratory-generated PM, whereas others have relied on particle concentrators developed by aerosol scientists at the PM centers to specifically serve the needs of their health effects colleagues. This makes it possible to simulate upper-bound $PM_{2.5}$ concentrations on a regular and prolonged basis to examine the cardiac and pulmonary function, response biomarkers, and other health end points before, during, and after exposures.

Results from these studies have repeatedly shown associations between $PM_{2.5}$ exposures and a variety of acute responses, providing direct evidence that $PM_{2.5}$ is biologically active at current peak exposure levels. As described in "Biological Mechanisms for Particulate Matter Health Effects," health outcomes that may warrant further examination in epidemiological studies include heart rate, HRV, QTc interval changes, arrhythmias and ischemia, blood ICAM-1 and IL-6, and oxidant stress markers. Some brief descriptions follow controlled acute health effects of animals and humans conducted by the PM centers.

Animal studies. Ovalbumin-sensitized mice were exposed in a specially equipped van located 50 meters downwind of a Los Angeles freeway. Groups of mice were exposed to CAPs at two concentrations (400 μg/m^3 and 800 μg/m^3) for 5 or 10 days. Control mice were exposed to purified air under the same conditions. All mice received an inhalation challenge of OVA 2 weeks after their last CAPs or air exposure. As illustrated in Figure 5, eosinophils and OVA-IgE were increased, relative to controls, after the 5- and 10-day exposures at 400 μg/m^3 ($p = 0.05$ for 10-day exposure) but not after exposure to 800 μg/m^3 for 5 days. The mice exposed to 800 μg/m^3 for 5 days were not different from controls and were suppressed with respect to eosinophils and IgE compared with 400 μg/m^3 for 10 days. These preliminary results are consistent with a multiphasic immunological response to CAPs. It is possible that exposures to high CAPs concentrations can have cytotoxic effects that suppress allergic responses, whereas exposures at lower CAPs concentrations can stimulate allergic responses.

The age-resolved response of sensitized rodents was examined using 6-hr exposures to laboratory-generated ultrafine carbon particles (100–150 μg/m^3) with and without O_3 co-exposures (Elder et al. 2000, 2002). Results showed that age, ultrafine particle concentration, ozone concentration, and sensitizing agents were significantly associated with cellular and biochemical lung lavage analysis and oxidative stress-related parameters of lavage and blood cells, and acute-phase proteins. Compared with young animals, old rodents were at increased risk for greater oxidative stress from combined ultrafine carbon and ozone exposure.

Human studies. Eleven human volunteer subjects who were either healthy or had asthma were exposed to inhaled carbon ultrafine particles by mouthpiece for 2 hr (~ 2 × 10^6 particles/cm^3; count median diameter = 22 nm) (Frampton et al. 2002). Healthy subjects showed no significant response to exposure, as determined by symptoms, spirometry, pulse oximetry, exhaled NO, or sputum cell differential (counts compared with air control). PMN expression of ICAM-1 and leukocyte function–associated antigen-1 decreased 3.5 hr after exposures but to a greater degree after ultrafine particle exposure than filtered air exposure.

For healthy subjects ($n = 12$), an ultrafine count concentration–response decrease in soluble CD40L was seen compared with air exposure ($p = 0.006$) (Daigle et al. 2002). An interim analysis was performed on eight subjects with asthma. CD40L expression on monocytes, a marker of adverse inflammatory responses, increased after PM exposure relative to air exposure ($p = 0.047$). There were no changes in lung function or airway production of NO. These observations are the first evidence of CD40–CD40L changes following exposures to ultrafine particles and are consistent with previous findings of altered leukocyte-endothelial interactions after such exposures.

Human volunteers were exposed to $PM_{2.5}$ CAPs in Los Angeles. Exposure to $PM_{2.5}$ induced decreased HRV in healthy young adults; increased blood ICAM-1 (indicating increased inflammation and coagulability), which was similar in healthy and asthmatic adults; marginally significant increases in serum IL-6, an acute-phase reactant, in asthmatics; transient decreases in arterial oxygen saturation in elderly subjects; decreased HRV in elderly subjects; a greater number of supraventricular and ventricular ectopic heartbeats in elderly subjects; and increased IL-6 in induced sputum, suggesting increased airway inflammation.

As many of these studies continue, their results are beginning to elucidate the roles of key exposure variables on biological responses. These findings not only support the epidemiological observations but also provide new information about the biological outcomes that can be measured in field studies.

Short-Term Goals in Acute Effects

During the next several years, the PM centers will continue to develop and apply new analytical techniques to address important scientific issues relating to harvesting, confounding, exposure–response relationships, and susceptibility. The novel statistical approaches developed for evaluating harvesting will be applied to additional U.S. populations for a variety of health outcomes. In addition, these new methodologies will be used to examine the relationship between acute and subacute effects. These investigations will, in turn, provide valuable insights for our efforts to quantify chronic PM health effects.

Furthermore, we will continue to investigate the potential for confounding by gaseous co-pollutants and the exposure–response relationships in a variety of urban environments with different mixtures of gaseous and particulate pollutants.

Over the past few years, the PM centers have been successful in identifying susceptible populations. These studies suggest that individuals with cardiac and pulmonary diseases are more vulnerable to PM exposures, and diabetics have been identified as an important susceptible population. Observational studies will continue to be critical in our efforts to understand susceptibility. Indeed, on the basis of the results of previous observational studies, PM centers have been able to develop animal models replicating human susceptibility.

Investigation of the relationship between particle composition or source characterization and adverse health outcomes will continue to be a main focus of the PM center research agendas, and the data now being generated by the U.S. EPA speciation and Supersite monitoring programs will be used in this endeavor. We will also investigate the impact of DEP exposures on the exacerbation of asthma. Similarly, we will examine the relationship between freshly generated ultrafine particles from mobile sources and children's acute health effects, using the residence location relative to major interstate roadways as a marker of exposure.

The PM centers observational studies will be supplemented by a series of animal toxicology studies that will also be completed during the next few years. The main objective of these controlled studies will be to investigate the pulmonary and cardiovascular effects induced by exposures to concentrated fine and ultrafine particles and gaseous co-pollutant mixtures. Of particular interest will be identification and evaluation of susceptible models. For example, we will conduct studies exposing mice and rats to concentrated fine and ultrafine particles at varying distances from high-volume interstate highways in southern California to determine the effects associated with freshly generated vehicular emissions. In addition, collaborative studies will be performed to examine the variability in the PM-induced biological responses in animal hosts (e.g., pulmonary immunomodulation) as a function of PM composition. Toward this end, PM centers will collect and exchange $PM_{2.5}$ samples containing particles of substantially different physicochemical characteristics.

Collectively, the acute observational and controlled investigations will enable us to enhance our understanding about the nature of transient cardiac function changes associated with short-term peaks in PM exposures; the role of underlying ischemia on associations between short-term peaks of PM exposure and incident myocardial infarction; and the role of underlying respiratory disease on associations between PM exposures and HRV.

Long-Term Goals in Acute Effects

We anticipate that many of the scientific issues addressed in the above list of short-term goals will not be adequately resolved during the next 2–3 years. Therefore, it is likely that research on many of these issues will comprise much of our long-term agenda on acute PM health effects. Among these issues will be research-investigating factors, both environmental and genetic, that explain human susceptibility. In addition, we expect that future collaborative investigations between epidemiologists and toxicologists will lead to the development of better biomarkers of PM effects. Finally, PM center efforts aimed at quantifying the acute health effects of PM exposures will ultimately assist in the creation of new chronic PM health effects models.

Chronic Health Effects of Particulate Matter

Background

As discussed in the previous section, a plethora of PM acute health effects studies have been conducted to date. In contrast, much less information is available on chronic effects associated with PM exposures. This is because of the complexity and cost of chronic effect studies.

The most frequently cited PM chronic health effect studies are the Harvard Six Cities Study (Dockery et al. 1993) and American Cancer Society (ACS) Cohort Study (Pope et al. 1995). They found increased mortality rates (decreased life expectancy) in cities with higher average ambient $PM_{2.5}$ and sulfate concentrations. Differences in city-specific mortality rates were not explained by data on personal risk factors. Because these studies provided most of the justification for the annual $PM_{2.5}$ NAAQS in 1997, they generated a number of concerns that were expressed in public comments that questioned *a*) the validity of underlying study findings due to the lack of public access to raw data; *b*) the adequacy consideration of city-specific characteristics and other alternative explanations of the observed differences in mortality rates; *c*) the adequacy of controls for the individual characteristics such as age, smoking, occupation, obesity, and socioeconomic factors; and *d*) the appropriateness of ambient PM measurements as surrogates of community personal exposures.

In addition to mortality in adults, increased respiratory effects in children associated with long-term PM exposures were reported. The Harvard Six and Twenty-four Cities cross-sectional studies observed higher rates of respiratory symptoms (Dockery et al. 1996) and lower lung function (Raizenne et al. 1996) for children in cities with higher average $PM_{2.5}$ and acidic sulfate concentrations. Concerns expressed in public comments at CASAC reviews of the PM Criteria Document and Staff Paper were similar to those expressed for the mortality studies, except for the influence of smoking and occupation.

There were effectively no animal studies of mortality or other morbidity end points of chronic PM exposures consistent with the epidemiological findings. Long-term studies had shown increased lung cancer in rats exposed to very high concentrations of DEPs or carbon particles (possibly related to the particle clearance overload they produced).

Because of the high visibility of the Six Cities and ACS cohort studies and their influence in the standard setting process, the HEI undertook sponsorship of a comprehensive independent validation and reanalysis of these studies. The HEI project (Krewski et al. 2000) validated the quality of the original annual mortality study data, replicated the original findings, and demonstrated the validity and robustness of Six Cities and ACS Mortality studies findings. For both of the cohorts studied, PM-associated mortality risk was highest for individuals with less than high school educations.

Data from other chronic exposure mortality studies have been limited. Survival analyses of nonsmokers in California (the Adventist Health and Smog Study) reported increased mortality associated with $PM_{2.5}$ concentrations (Abbey et al. 1999). Preliminary analyses of male veterans being treated for hypertension reported no statistically significant increased mortality associated with fine particle concentrations (Lipfert et al. 2000).

Progress Made in Chronic Effects

Annual mortality cohort studies. Two of the PM centers have been engaged in continued follow-up of the original Six Cities and ACS cohorts, respectively, for an additional 9 years. New data on occupation and other individual characteristics as well as new analytic methods developed in the HEI reanalyses were applied in both studies. These recent findings are illustrated in Figure 6. The updated ACS study involves the analysis of more extensive $PM_{2.5}$, sulfate, and gaseous co-pollutant data. For the Six Cities cohort, $PM_{2.5}$ and sulfate concentrations continued to be associated with decreased survival and with increased mortality from cardiovascular and pulmonary causes (Laden et al. 2001). In addition, increased lung cancer deaths were significantly associated with $PM_{2.5}$ and sulfates in the extended follow-up. For the ACS cohort, $PM_{2.5}$ and sulfate also continued to be associated with increased cardiovascular and pulmonary mortality, and there was also an excess of lung cancer (Pope et al. 2002). The excess risks in the ACS cohort were seen only among individuals with less than high school educations in these extended analyses.

Effects of subchronic exposures. Recent PM center analyses have attempted to bridge the time spans for health effects observed in studies of acute daily exposures versus long-term chronic exposures. Analyses of the association between daily mortality and hospital admissions with PM concentrations during the preceding weeks to months have highlighted the importance of subchronic exposures. In Boston, for example, an increase in the 2-day average $PM_{2.5}$ of 10 mg/m^3 was associated with an increase in mortality by 2.1%, while for the same increase in monthly average, mortality increased by 3.8% (Schwartz 2000a). In the ACS cohort study, this same increase in annual average $PM_{2.5}$ was associated with a 6.8% increase in mortality .

Children's lung growth cohort studies. Studies of chronic effects of PM exposure on the respiratory health of children have been conducted by two of the PM centers. Recently reported results from the Children's Health Study (CHS) in southern California showed that $PM_{2.5}$ is significantly associated with slower growth of lung function in children residing in communities with higher than average annual $PM_{2.5}$ concentrations. Furthermore, children who have moved from the high PM areas into areas with lower PM did not recover their lost lung function but had subsequent rates of lung function growth equal to that of the children in their new communities. Children who moved from low PM areas to communities with higher PM levels had less lung function growth than children who remained in the low PM areas (Avol et al. 2001). The results of these analyses have provided a basis for follow-on studies of intracommunity variations in response that are supported by PM center funds. The focus of these recent and future studies is on proximity to heavily traveled express roads as an independent risk factor.

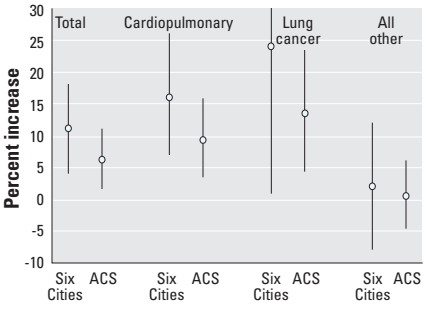

Figure 6. Mean increase and 95% confidence intervals for annual mortality rate increases per 10 µg/m^3 increment of $PM_{2.5}$ for the Six Cities and ACS cohorts based on 16 years of mortality data.

These results have also stimulated PM center plans for follow-up studies of the cohorts of children from the Six Cities and Twenty-four Cities studies. Fourth and fifth grade children in the Twenty-four Cities study showed lower lung function associated with community-specific $PM_{2.5}$ concentrations. Many of these children have now been retested as mature twelfth graders, and their lung growth will be compared with community air pollution levels as was done in the southern California study.

Short-Term Goals in Chronic Effects

The follow-up and data analysis on the Six Cities and ACS study participants will continue to assess source-specific characteristics of PM responsible for chronic mortality (longevity reduction) effects. This will include the assessment of individual characteristics (disease state, socioeconomic status, smoking, occupational exposures) that make study participants more susceptible to effects of PM on survival. Air pollution will be treated as a time-dependent covariate in the Six Cities cohort to determine how much of the association with mortality in the prospective cohort study is due to exposure in the past year as opposed to over a lifetime. Estimates of the distribution of years of life lost associated with PM air pollution in the Six Cities cohort will also be computed. Further studies of the ACS cohort longevity reduction will focus on the relationship between mortality and PM composition and/or source contribution. This will be accomplished by using particle speciation data, both the historic dichotomous sampler network data and the new speciation site data, and source apportionment techniques. The association between $PM_{2.5}$ and/or sulfate and local airport visibility will be determined for each community where suitable data are available. City-specific relationships between pollution and visibility measurements will be used to estimate $PM_{2.5}$ levels prior to and after the limited period for which direct measurement data are available in an effort to investigate the relative roles of past and current exposures.

Analyses of data previously collected on cohorts of children from studies performed by PM center investigators (Twenty-four Cities and southern California CHS) will be pooled to assess effects of PM from different source classes (e.g., vehicular vs. power plant) and composition (e.g., nitrate vs. sulfate). Each of these cohort studies assessed fourth and fifth grade school children using the same questionnaires and pulmonary function measurements. The Twenty-four Cities study assessed the effects of sulfate particles from coal-fired power plants. The southern California study assessed the effects of nitrate particles from vehicular traffic. The combined data set will assess chronic health effects in these children versus $PM_{2.5}$ mass and composition data.

A new epidemiological study of participants in the National Heart, Lung, and Blood Institute Women's Health Initiative Observational Study (93,000 women 50–79 years of age from 40 centers around the country) is currently under way. The women are being observed for the occurrence of CVD and will be matched with community air pollution levels from ambient monitors. The effects of chronic exposure to ambient PM on the incidence of CVD will be assessed in this cohort using a proportional hazards model approach.

In prior epidemiological studies in Erfurt and Augsburg, Germany, acute effects of ultrafine particles on daily mortality (Wichmann et al. 2000) and on myocardial infarction (Peters et al. 2001) were observed. Cohorts that were established in these cities will be used in the future to investigate whether there are long-term effects of exposures to ultrafine particles.

A PM center subchronic animal inhalation study using New York CAPs began in the spring of 2002. Normal and cardiac disease–susceptible mice will be exposed for 6 hr/day, 5 days per week, for 6 months, with or without intermittent ozone exposure. Cardiac and respiratory function will be monitored periodically, and biochemical and genetic biomarkers of responses will be investigated in tissues and lung lavage cells from the exposed animals. In another study, mice will undergo infectious bacterial challenge prior to CAPs exposures to determine possible PM exposure-related enhancement of their susceptibility to infection. These studies will thereby assess health end points being evaluated during acute CAPs exposures of animals at several of the PM centers and of humans at the U.S. EPA Clinical Studies Laboratory in

Chapel Hill, North Carolina, and determine whether such acute effects progress to chronic changes that could lead to enhanced susceptibility to PM exposures.

Long-Term Goals in Chronic Effects

Based on previous experience, repeated follow-up of existing cohorts can be of paramount importance in understanding the association between chronic PM exposures and human health. Results from the Six Cities and Children's Health studies, for example, have enabled us to understand the effects of air pollution on children's lung growth. Collectively, the PM centers have already investigated numerous cohorts that span ages, ethnicities, and geographic locations and that are exposed to varying PM levels and compositions. Health and exposure history data for these cohorts are also available. Information from these numerous study locations will be combined to increase the statistical power of existing chronic health effects models. The range of data will, in turn, allow us to better examine the role of PM composition, gaseous co-pollutants, ethnicity, exposure history, age, and climate on chronic PM health effects. Therefore, it would be advantageous for the PM centers to allocate a large fraction of their resources during the next 5–10 years to continue with the follow-up for these existing cohorts.

As new scientific information emerges, providing better indicators of air pollution health effects, questionnaires and medical examinations will be amended accordingly. For example, the early cohort studies of populations initially recruited in the mid-1970s focused on the impact of air pollution exposures on respiratory health. Therefore, investigations centered on collecting detailed information on respiratory health. Recent studies have demonstrated the influence of particles and air quality on cardiovascular health. Correspondingly, information on cardiac health has assumed a more central role in current studies. Although limited information currently exists on the effect of air pollution among susceptible populations, future knowledge may require a better understanding of factors predisposing these individuals to adverse health outcomes. Of particular interest is the gene–environment interaction. Research that integrates the findings from the CHS with new information on traffic density and the organic constituents in traffic-related PM will also be pursued as a long-term goal.

For chronic studies using CAPs-exposed animal models, the specific hypothesis to be addressed in future years will be based, in part, on the findings of the first subchronic exposure study to be conducted in New York. This study will examine the possible acceleration of atherosclerotic plaque development, increased reactive or obstructive airway disease, reduced lung capacities, enhanced susceptibility to infectious agent exposures, and altered gene expression. Moreover, efforts will be made to determine whether variations in responses during the exposures can be related to the day-to-day variations in the chemical composition of the CAPs. Based on the results from this study, the following parameters may be considered in future chronic exposure studies: *a*) including additional animal models to replicate human susceptibility; *b*) increasing exposure durations; and *c*) examining coexposures to CAPs with one or more gaseous co-pollutants. Of additional importance will be the investigation of chronic health effects thresholds by conducting subchronic exposure studies at multiple PM concentrations to determine the existence of thresholds, if any, and replication of one or more of these studies in areas where the PM composition is different. Finally, we expect that the findings from the controlled animal studies will assist in further refining study designs for our human chronic health effects investigations.

Dosimetry

Background

A critical link in the evaluation of the relationship between individual exposures to PM and health responses is dosimetry. Dosimetry research investigates not only the amounts and distribution of the deposited PM in the respiratory tract but also the pathways by which this material is translocated to other sites in both the respiratory tract and to more distal organ systems. Furthermore, knowledge of inter- and intraspecies differences is critical to our effort to extrapolate results from experimental studies to the population at large.

Prior to the establishment of the PM centers, studies of PM dosimetry in lung airways concentrated largely on healthy adult humans, although there were a few studies of children and adults with chronic respiratory disease. However, very little research has examined deposition in animal models that are currently being used in studies of PM health effects as surrogates of susceptible human populations. Mathematical deposition models derived from experimental data had been developed and were largely used for predicting total and regional deposited doses. Mechanisms for clearance of deposited particles had also been studied, and major clearance pathways in the respiratory tract regions had been well described. However, several major gaps in our understanding of particle deposition and clearance remained, including detailed analyses of size-resolved PM deposition and clearance, especially for ultrafine particles, and clearance mechanisms for PM components as well as their translocation pathways.

Research addressing these gaps is important for the interpretation of toxicological and epidemiological findings suggesting effects on cardiopulmonary systems, which could be due to indirect (via biological mediators initiated from acute pulmonary effects) or direct or neuronal function changes. The multidisciplinary teams of PM centers investigators provides a great advantage in addressing these questions.

Progress Made in Dosimetry

Significant advances have been made at the PM centers in a number of critical areas. These include development of techniques for the production of hollow lung airway casts of potentially susceptible people and evaluation of dosimetry of specific fractions of ambient PM that may be especially toxic. There has also been work on dosimetry in humans and in animal models being used in PM studies, using both ambient PM and surrogate particles.

To address the paucity of data regarding PM deposition in the lungs of people with preexisting pulmonary disease, a pilot PM center project investigated the potential for retrieval of morphometric data from three-dimensional images of tracheobronchial airways obtained *in vivo* by X-ray computerized tomography (CT). The study also explored the potential for the use of stereolithography (STL) to produce hollow airway casts of normal and abnormal lung airways for the determination of site-specific deposition and for experimental verification of particle deposition models. A volumetric rendering of the interior surface of a hollow airway cast (used in previous studies) was generated, producing a surface representation of the airway tree. These three-dimensional images were then converted to an STL file format required for the rapid prototyping of airway casts. The STL unit uses a computer-controlled arm connected to a plastic extrusion device to build volumetric structures layer by layer. Figure 7 demonstrates the close concordance between an original hollow airway cast and the replicate made of it by STL (Won et al. 2000a, 2000b).

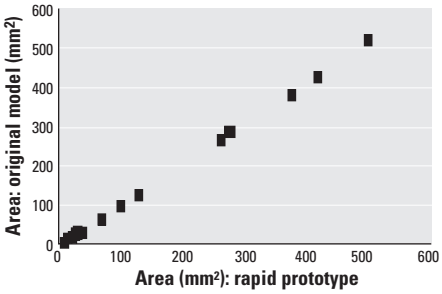

Figure 7. Cross-sectional luminal airway surface of original hollow airway cast made from a normal human tracheobronchial tree in square millimeters versus corresponding airway surface in square millimeters of a replica produced by STL using data obtained by CT scanning of the original cast.

Thin, multislice, helical CT scanning allows for the acquisition of high-resolution volumetric image data sets of the lung during a breath-hold, or at multiple phases within a respiratory cycle for children, the elderly, and people of all ages having asthma or chronic obstructive lung disease. From these scans, hollow airway casts that include five or six bronchial generations can be created and replicated for use in studies of inhaled particle deposition in replicate casts of both healthy and diseased airways, using realistic air flow rates.

In another study, PM center methodology was developed to determine total lung deposition of CAPs in dogs. These studies are relevant to animal models of cardiac disease used in the PM center research. The methods included the use of a breath-by-breath respiratory monitoring system that provided both rate and tidal volume as well as measurement of inhaled and exhaled particle number, classified by size. These measurements used both a TSI Scanning Mobility Particle Sizer for particles in the ultrafine and smaller fine ranges, and a TSI Aerosizer (TSI, Inc., Shoreview, MN) for particles in the fine particle size range. The canine studies showed deposition of ambient particles followed a pattern similar to that described in the International Commission on Radiological Protection (ICRP) human particle deposition model (ICRP 1994) but was slightly greater than that predicted by the ICRP model. This method was subsequently used in a study of six human subjects breathing ambient air without any enhancement of particle concentration. In all subjects, total respiratory deposition fraction (DF) for each measured size range (> 10 nm) followed the same pattern predicted by the ICRP model, and in some individuals, deposition exceeded the predicted fraction. Human studies of ambient particle deposition are important to validate deposition models.

In vivo deposition studies of ultrafine particles are currently being conducted in humans and rats. For the human studies, a system was developed for mouthpiece exposures, with alternating rest and exercise every 15 min. Total respiratory DFs for particle number and mass were determined down to 7-nm particles. Ultrafine carbon particles (22 nm) were generated. Eleven nonsmoking, healthy subjects and 11 nonsmoking subjects with mild asthma were exposed for 2 hr. The total respiratory deposition of inhaled ultrafine particles was high relative to fine particles in healthy subjects (DF = 0.63 ± 0.03), and was further increased during exercise (DF = 0.83 ± 0.04), with enhanced deposition in asthmatics (rest DF = 0.77 ± 0.05; exercise DF = 0.86 ± 0.04). These results suggest that asthmatics may be at greater risk from exposure to ultrafine particles because of increased deposition in central airways.

Deposition rates for size-resolved ultrafine particle data showed that 7-nm particles had the highest total deposition (~ 80%). Deposition efficiency (DE) for 100-nm particles was ~ 45%. These deposition rates are in good agreement with the ICRP particle deposition model (ICRP 1994). This model predicts that ambient ultrafine particles of 20 nm will have the highest overall DE, as well as the highest deposition in the gas-exchange region (Chalupa et al. 2002a, 2000b; Daigle et al. 2003; Frampton et al. 2000).

Experiments in rats were performed using two types of ultrafine ^{192}Ir particles and carbon particles containing the stable isotope ^{13}C particles. The basic design of both rat studies was the same, i.e., exposure to the ultrafine particles followed by serial sacrifice in the postexposure period and analysis of excised lungs and extrapulmonary organs for ^{13}C and ^{192}Ir, respectively. However, there were major differences. The ^{13}C exposure occurred for 6 hr in compartmentalized whole-body exposure chambers, whereas the ^{192}Ir exposures occurred via a 1-hr intratracheal inhalation in anesthetized rats.

For the ^{13}C exposures, the median particle size was in the range of 20–29 nm. Exposure concentrations ranged from 80 to 180 μg/m^3, and organ burdens were determined in the first series of experiments at 0.5, 18, and 24 hr after exposure. Six unexposed rats served as controls. Lung lobes, liver, heart, kidney, and brain sections (olfactory, cerebrum, cerebellum) were analyzed for ^{13}C by isotope ratio mass spectrometry (MS). On average, approximately 9 ng ^{13}C/g lung per μg/m^3 of exposure was found in the lung, much less than expected based on predictive lung deposition models. Significant amounts of ^{13}C had accumulated in the liver by 0.5 hr postinhalation only at the high exposure concentration of 180 μg/m^3, whereas by 18 and 24 hr postexposure, all liver samples showed significantly increased ^{13}C. Indeed, the amount of total ^{13}C in the liver at those time points exceeded the amount retained in the lung, demonstrating a fast translocation of ultrafine carbon particles to the liver. Other extrapulmonary organs analyzed for ^{13}C did not show significant increases by 24 hr postexposure (Oberdörster et al. 2002).

Results were different for ultrafine Ir particles. Ir was found, in pretest studies, to be essentially insoluble. In these studies, the median particle size was approximately 15 nm. Gamma-spectroscopic analysis for ^{192}Ir was performed at time points ranging from 6 hr to 7 days postexposure. The ultrafine ^{192}Ir particles, after an initial fast tracheobronchial clearance, were essentially retained in the lung; only minute amounts were found in extrapulmonary tissues [~ 0.5% in bone and soft tissue (muscle), < 0.1% in liver, and even less in heart and brain]. Soluble Ir was excreted via urine (60% over 7 days), 8% remained in the lungs, and 10% was retained in both soft tissue and bone (Kreyling et al. 2002). These results show that translocation of poorly soluble ultrafine particles to extrapulmonary sites appears to be highly dependent on the chemical nature of the particles. Carbon particles were to a large degree translocated to the liver, whereas metal iridium particles were not. These results raise a number of questions that need to be addressed in future studies, including the evaluation of pathways for ultrafine particle translocation (lymphatic, blood vessels, gastrointestinal tract, nasal vs. tracheobronchial vs. alveolar region) and also binding of ultrafines to proteins.

Because currently available inhaled particle dose models provide estimates of averaged doses (i.e., environmental aerosol concentrations), they do not take into account the true PM heterogeneity of tissue doses that occur in the lungs. Higher-than-average doses can be expected for those people exposed near local particle sources, those who have high specific ventilations (ratio of air intake to body mass), and those who have heterogeneous deposition of particles within various regions of the respiratory tract. Thus, exercising adults and children with lung diseases who live near heavily used freeways may be receiving greatly elevated particle doses at numerous sites within their lungs. Similarly, the available software used for computing inhaled particle deposition cannot identify local populations of cells that may be of toxicological interest. The numbers of cells that must be significantly damaged to seriously impact vulnerable individuals is not yet known. These issues were addressed by a PM center workshop in October 2001 that developed a framework for estimating expected local tissue dose concentrations. This framework was applied to southern California Supersite data sets, and local doses to sites containing the highest exposed 100 cells at each bifurcation in the tracheobronchial tree were calculated for heavily exposed individuals. These local bifurcation area doses were compared with the doses applied to cells in *in vitro* studies of PM effects. The high *in vitro* doses corresponded to estimated airway bifurcation PM doses potentially occurring in the lungs of individuals living in the Los Angeles basin.

Short-Term Goals in Dosimetry

In a successor project to the pilot feasibility study for the production of hollow airway casts that faithfully reproduce the *in vivo* dimensions, *in vivo* studies are being conducted of particle deposition in anesthetized sheep at the facilities of study collaborators at

the University of Iowa (Iowa City, Iowa) using 5-μm diameter radio-opaque droplets. This involves development of analytical programs to examine the CT scan data. The second phase will be to examine changes in the deposition pattern and efficiency when inhalation is done at different points of the inspiratory cycle. The computer-controlled respirator can produce almost any breathing pattern and can key the aerosol delivery to any point in the respiratory cycle of the anesthetized sheep. When quantitation has been accomplished *in vivo*, hollow airway casts of the sheep lung will be produced using the methods developed in the initial PM center pilot study. PM deposition patterns and efficiencies will then be determined using an artificial thorax and methods and models previously used at NYU to measure particle deposition in the hollow-cast systems. Successful development of the test aerosol as well as quantitation methods will ultimately allow future exploitation of these techniques for *in vivo* studies in both healthy people and those with compromised lung function. In addition to determining DE and pattern studies, PM center studies will explore the potential for identifying bolus deposition in the sheep lung and the extent to which assumptions of standard bolus deposition experiments are valid. The system will allow testing of the hypothesis proposed by C. Kim and colleagues at the U.S. EPA—that regional deposition can be accurately assessed in people via a series of bolus inhalation experiments (Kim 2000).

Other PM center dosimetry studies will continue to focus on ambient particles and their diurnal and temporal variation in concentration and composition. The number of subjects will be expanded as well as the number of repetitions per subject. This will permit definition of the range of individual differences in particle DFs, including male–female and age differences. In addition, the relationship of DF to day-to-day compositional differences will be explored to determine the factors important in variations in DF.

Research will continue on ultrafine particle deposition, disposition, and chemistry. Investigations will include the continued study of susceptible groups (e.g., COPD, elderly) in terms of total respiratory tract deposition; the effects of changes in tidal volume and respiratory rate in deposition; deposition efficiencies in different anatomical compartments of the respiratory tract; clearance and translocation pathways via the respiratory and gastrointestinal tracts; translocation following the initial phase of accumulation in the liver; the liver as a storage organ for distribution to other organs; the mechanisms of particle translocation; the role of particle chemistry; the fate of organic carbon particles; the efficiency of ultrafine particle translocation along sensory nerves in the conducting airways; and the long-term consequences of ultrafine particle translocation to extrapulmonary organs like the heart.

Answers to these questions are important for the interpretation of results of toxicological and epidemiological studies with respect to effects of inhaled particles on the cardiovascular system that could be due to indirect (via biological mediators initiated from acute pulmonary effects) or direct (ultrafine particles interacting with cardiac or endothelial cells) or neuronal functions effects.

To better understand the generalizability of controlled animal PM exposures to human health, the regional inhaled particle depositions in mice from the freeway study will be determined. Toward this end, replica airway casts of 30 Balb/c mice (15 controls and 15 sensitized) will be prepared, and morphometric measurements of airways will be conducted. The mice will be exposed to three sizes of electrically discharged fluorescent monodisperse polystyrene latex particles. Their lungs will be digested, and particles deposited in the lungs will be recovered and counted using a fluorescence-equipped microscope. These measurements will be used for examining the toxicologic responses of the sensitized model, as well as for performing extrapolations to humans.

Long-Term Goals in Dosimetry

The results of the *in vivo* particle deposition studies in sheep will provide a firm basis for a study of human subjects. The future human *in vivo* studies will focus on *a*) application of accepted mathematical dosimetry models to individuals, using the morphometric data retrieved from the images. This will be assisted by respiratory function test results that will be available for these individuals; *b*) measurement of detailed particle deposition data for a range of breathing patterns and particle sizes in hollow-cast models of the airways of individuals representative of the various groups of interest; and *c*) reconciliation of the results calculated from the current models with experimental data to produce verified empirical deposition models that can be used to better predict inhaled dose on the basis of metrics of exposure to ambient PM.

Future research to relate airborne PM to improved dosimetric models will be directed toward defining the population receiving the highest local doses in their tissues, and defining the appropriate particle doses for use in *in vitro* mechanistic studies. Research is also needed to address *a*) the environmental heterogeneity of PM-associated pollutants; *b*) how much time susceptible subpopulations spend in such high-concentration areas; *c*) ventilation rates of those exposed; *d*) heterogeneous deposition patterns of inhaled particles; *e*) dose implications of impaired clearance (as is seen in some disease states) and sequestration of particle associated substances; *f*) vulnerable target cells in the tissues; and *g*) realistic target particle doses that can be used for the design and interpretation of *in vitro* mechanistic research.

Exposure Assessment

Background

As noted in the section "Acute Health Effects of Particulate Matter," results from daily mortality and morbidity time-series analyses provided much of the scientific basis for setting the PM standards in 1997. These epidemiological studies typically used PM mass concentrations measured at outdoor monitoring sites as surrogates of population exposures to ambient air PM. The extent to which outdoor measurements accurately reflect PM exposures has been the subject of considerable scientific debate. Results from early exposure studies, such as those conducted as part of the Harvard Six Cities Study and the U.S. EPA Particle Total Exposure Assessment Methodology Study (Clayton et al. 1993), for example, suggested that personal PM exposures may differ substantially from outdoor concentrations because of contributions from indoor sources. Cross-sectional analyses of these data showed weak associations between daily outdoor PM concentrations and corresponding personal exposures, which were attributed to intersubject variability and the limited number of measurements (~ 1–2 days) for each individual. Consequently, the existing PM exposure database was considered inadequate for investigating associations between personal exposures and outdoor concentrations, and longitudinal exposure studies were proposed.

Upon completion of the 1997 PM NAAQS review and prior to the establishment of the five PM centers, a series of longitudinal PM exposure studies were funded by the U.S. EPA, HEI, Electric Power Research Institute, U.S. Department of Energy, and American Petroleum Institute. The main objective of these studies was to investigate associations over time between personal PM exposures and outdoor PM concentrations, with specific focus on characterizing exposures for individuals thought to be especially susceptible to PM-associated health effects. U.S. EPA-sponsored research projects were conducted by groups from Harvard University, NYU, and the University of Washington. The three research groups collaborated closely in the design and preparation of field studies and shared similar sampling procedures and questionnaires in an effort to create compatible data sets. As part of these early longitudinal studies, the exposures of several cohorts of susceptible individuals, including senior citizens, children, and

individuals with COPD or CVD, were measured for periods ranging from 1 to 2 weeks. Field studies were conducted during both the summer and winter and in a variety of urban and suburban environments including Atlanta, Georgia; Baltimore, Maryland; Boston, Massachusetts; Nashville, Tennessee; New York, New York; and Seattle, Washington. Although analyses of the entire body of data are still in progress, several papers have been published and have been cited in the 2001 PM Criteria Document external-review draft. The U.S. EPA Office of Research and Development is planning to use this rich database to develop both acute and chronic exposure models.

A major finding of these studies is that stronger personal-outdoor PM correlations exist when data are analyzed by individual, over time. However, the degree of this association varies by individual, with some individuals having significant associations and others not. A major deficiency in exposure assessment research, which still persists, relates to the lack of accurate chronic exposure models to be used by epidemiological studies.

Progress Made in Exposure Assessment

The findings from the longitudinal PM exposure studies, described above, were critical to the evolution of our collective research efforts on PM exposure assessment. Results from the centers research pertaining to PM exposures are presented briefly below, with many of the findings already published, in press, or submitted for publication in peer-reviewed journals.

Considerable research was conducted to identify factors that contribute to observed differences between outdoor concentrations of PM and corresponding population exposures. The differences have traditionally been referred to as "exposure error" because PM epidemiological studies typically use outdoor PM concentrations as surrogates of exposure.

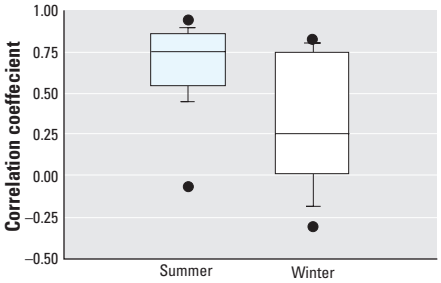

Figure 8. Distribution of subject-specific correlation coefficients: ambient $PM_{2.5}$ concentration versus personal $PM_{2.5}$ exposures in Baltimore, Maryland, 1998–1999. $n = 14$ for both sampling seasons (Sarnat et al. 2000). These box plots show median values and the 25th and 75th percentiles. The vertical lines extend to the 10th and 90th percentiles and the circles indicate extreme values.

PM centers exposure research efforts have focused on the following topics:

- Characterization of spatio-temporal variability of PM components and gaseous co-pollutants measured at centrally located outdoor sites as a function of site characteristics using the entire U.S. air monitoring network. The models will enable epidemiologists to quantify the effects of exposure error on health risk estimates.
- Assessment of the contribution of outdoor and indoor PM sources on personal exposures. This area of research is critical for determining the relative toxicities of PM of outdoor and indoor origin.
- Measurement of exposure to specific toxic PM components and gaseous co-pollutants. This information will be of great value to epidemiologists and toxicologists in their efforts to identify the causal agents of air pollution–related toxicity.

Investigation of the association between outdoor particulate matter concentrations and corresponding personal exposures. Ambient $PM_{2.5}$ concentrations were significant predictors of corresponding personal exposures over time for a cohort of healthy senior citizens (Figure 8) (Sarnat et al. 2000). Although the strength of these associations varied by individual and season, the results suggest that for certain individuals, ambient $PM_{2.5}$ concentrations are appropriate surrogates of personal $PM_{2.5}$ exposures. These findings are consistent with results from recent longitudinal PM exposure studies conducted in Boston (Rojas-Bracho et al. 2000) and the Netherlands (Janssen et al. 1998). When the subject-specific data were aggregated and analyzed together (i.e., cross-sectionally), the association between personal exposures and outdoor concentration was weaker, further highlighting the inadequacy of cross-sectional analytical methods for assessing true personal-ambient $PM_{2.5}$ associations for time-series studies.

Because most of these subjects spent the majority of their time (> 95%) indoors during both seasons, seasonal differences in personal-ambient associations were likely due to changes in indoor ventilation conditions between the summer and winter. Summertime records of open window status provided direct evidence of the effect of indoor ventilation on personal-ambient $PM_{2.5}$ associations, with ambient concentrations shown to be strong predictors of corresponding personal exposures for subjects who spent the majority of their time in well-ventilated indoor environments. This was illustrated in the plot of personal $PM_{2.5}$ exposures on ambient $PM_{2.5}$ concentrations, which had a slope that was close to 1 and a limited amount of scatter. Personal-ambient $PM_{2.5}$ associations were also significant for subjects who spent the majority of their time in poorly ventilated indoor environments, but the association was much weaker. The plot of personal exposures on ambient concentrations for poorly ventilated environments had a slope well below 1, with a considerable amount of scatter due to the influence of poor ventilation on both reducing the penetration efficiencies of ambient particles and increasing the contribution of indoor source particles on personal exposures.

Spatial variability of outdoor particulate matter concentrations. Some of the interpersonal differences in personal-outdoor PM associations may be due to the spatial variability in outdoor PM concentrations. Ito et al. (2001) used nationwide PM_{10} and gaseous pollutant measurements to examine relationships between concentration measured at various urban and suburban sites. Figure 9 shows a smoothed monitor-to-monitor temporal correlation for air pollution and weather variables as a function of distance in seven northern and

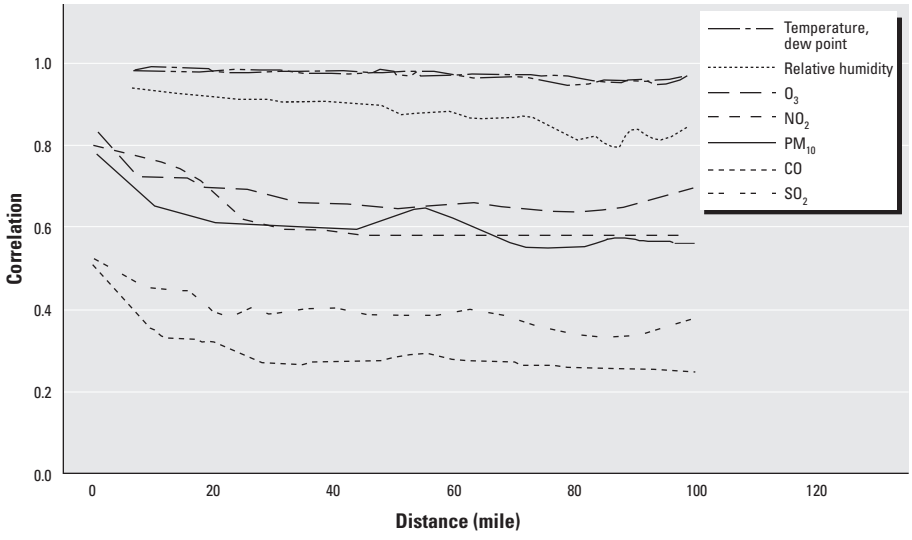

Figure 9. Smoothed monitor-to-monitor temporal correlation in seven north-central U.S. sites.

central U.S. states. As shown, monitor-to-monitor correlations varied by pollutant. Stronger spatial correlations were found for PM_{10}, NO_2, and O_3 compared with those found for CO and SO_2. $PM_{2.5}$ data will be incorporated within this analysis as it becomes available.

Previous studies in the Northeastern United States have shown that mean daily $PM_{2.5}$ concentrations are reasonably uniform within large Eastern U.S. metropolitan areas such as Washington, DC, Philadelphia, and Baltimore. Findings from PM center-supported studies further indicate that there is relatively little variation in ambient $PM_{2.5}$ concentrations between the center of New York City and a rural upwind location (Figure 10). Together, these results suggest that most of the $PM_{2.5}$ in the Northeastern United States originates from distant upwind sources (Thurston et al. Unpublished data). However, this may not be the case in other locations in the United States. $PM_{2.5}$ mass concentrations in Seattle exhibited modest, yet significant, spatial variability within a radius of 20 km. These differences were associated with both proximity to major highways and the elevation of the monitoring location (Goswami et al. 2002). In Los Angeles, $PM_{2.5}$ and PM_{10} concentrations measured at various distances from highways (10–1,000 meters) showed little spatial variability. However, particle number and black carbon concentrations decreased rapidly with distance from highways (Zhu et al. 2002) (Figure 11).

The spatial variability of reactive chemical species in $PM_{2.5}$, such as quinones and their precursors in the Los Angeles basin, showed considerable variability from west to east, reflecting the direction of the prevailing winds. The naphthoquinones decreased, but the concentration of 9,10-phenanthroquinone increased. As quinones are known to be toxic, their concentrations in PM fractions may be a more sensitive measure of exposure. Levels of quinones in the outlying areas were, as expected, extremely low.

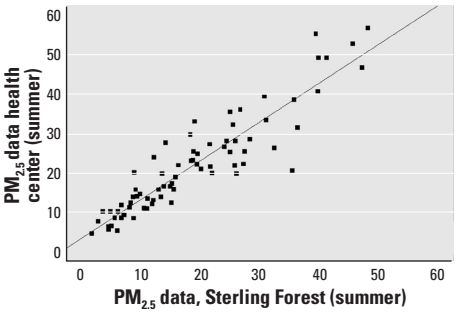

Figure 10. Most NYC summer $PM_{2.5}$ mass at health center (in Manhattan) is explained by regional $PM_{2.5}$ at Sterling Forest, a state park northwest of Manhattan. $y = 0.9895x + 3.1262$; $R^2 = 0.843$.

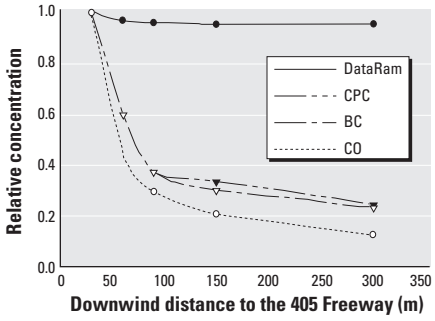

Figure 11. Relative $PM_{2.5}$ mass [measured by DataRam nephelometer (MIE, Inc., Bedford, MA)], particle number (measured by CPC), black carbon (BC), and carbon monoxide (CO) concentrations versus downwind distance from the Los Angeles 405 freeway (Zhu et al. 2002).

Impact of indoor particulate matter sources on personal exposures. As shown in the earlier cross-sectional PM exposure studies, variability in personal-outdoor PM associations is due, in part, to contributions from nonambient particle sources (Ozkaynak et al. 1996). PM center–supported longitudinal exposure studies have provided additional evidence of the impact of non–outdoor PM sources for both cohorts of healthy individuals and susceptible subpopulations. Results from Seattle and Baltimore showed that nonambient $PM_{2.5}$ sources contributed 49 and 67% of the total personal $PM_{2.5}$ exposures, respectively (Allen et al. 2002; Sarnat et al. 2000). In Seattle the pediatric subjects experienced significantly higher exposures to particles of indoor origin than did adults. Their average fraction of ambient PM contribution to total human exposures was no higher, however (Allen et al. 2002). In Baltimore, indoor ventilation influenced nonambient PM contributions to personal exposures, where nonambient $PM_{2.5}$ sources contributed 29, 30, and 45% to total personal $PM_{2.5}$ exposures for subjects spending their time in well-ventilated, moderately ventilated, and poorly ventilated indoor environments, respectively.

Variability of outdoor particulate matter penetration efficiencies. People spend the majority of their time indoors. Therefore, quantifying the effective penetration efficiencies of outdoor particles into indoor environments is critical for understanding the variability of personal-outdoor PM relationships. Until recently, particle penetration efficiencies were thought to be constant and were often assumed to be 100%. As a result, personal/outdoor concentration ratios higher than one were assumed to be indicative of the influence from non–outdoor PM sources. Results from current PM center exposure studies have shown that particle penetration efficiencies vary substantially by residence type (Larson et al. 2001). In Seattle for example, particle penetration efficiency exhibited significant interhome and intrahome variability for the 30 homes monitored. Because home characteristics, including ventilation, vary by season and locale, these findings may be used to explain some of the heterogeneity in PM-associated risk factors in various epidemiological studies, as reported in the section "Acute Health Effects of Particulate Matter."

Measurement of personal exposures to specific toxic particulate matter components. Identifying the specific component(s) of outdoor PM responsible for the numerous observed adverse health effects remains an important objective of our exposure assessment research. Results presented in "Biological Mechanisms for Particulate Matter Health Effects" of this review suggest that specific components of outdoor PM, such as transition metals, ultrafine particles, and PAHs, may be related to allergic airway disease and cardiovascular effects.

Funding has facilitated the development of novel personal multiple pollutant sampling methods currently being used in many of the PM center field studies (Figure 12) (Demokritou et al. 2001). The Multiple Pollutant Personal Sampler provides data on concurrent size- and species-resolved personal PM exposures as well as exposures to numerous gaseous co-pollutants (NO_2, O_3, SO_2, and numerous VOCs). To date, the Multiple Pollutant Personal Sampler has been used in the Baltimore; Atlanta; Steubenville, Ohio; and Los Angeles field studies. Other methods are currently under development at the PM centers to examine exposures to specific PM components.

The PM centers are conducting a series of studies to identify and quantify specific toxic PM components as well as examine their spatial and temporal distributions. Organic PM constituents such as quinones may play a particularly important role in generating oxidative stress, inflammation, and immunomodulating effects in the lungs and airways. Currently, PM seasonal and spatial distributions of outdoor PAH, aldehyde, and quinone concentrations are being characterized for 12 southern California communities participating in the CHS (Cho et al. Unpublished data). Center investigators expect to link spatial intercommunity variability of specific organic (i.e., quinones) and carbonyl concentrations with reported respiratory health effects of school children.

Measurement of personal exposures to gaseous co-pollutants. Ambient concentrations of PM and its gaseous co-pollutants are frequently correlated, making it difficult for epidemiological investigations to determine whether observed PM-health effect associations are confounded by these co-pollutants. The role of gaseous co-pollutants as potential confounders or surrogates of personal $PM_{2.5}$ exposures was investigated for cohorts of

healthy senior citizens, children, and individuals with COPD living in Baltimore (Sarnat et al. 2001). Investigators used the Multiple-Pollutant Personal Sampler, which allowed for associations between personal particulate and gaseous exposures to be examined directly. Consistent with results from previous monitoring studies, strong correlations existed between ambient $PM_{2.5}$ and personal gaseous co-pollutant concentrations (i.e., O_3, NO_2) (Figure 13). In contrast, weak correlations were found between personal $PM_{2.5}$ and personal gaseous co-pollutant exposures, suggesting that the gaseous co-pollutants are unlikely confounders of $PM_{2.5}$. Finally, strong correlations between personal exposures to $PM_{2.5}$ and ambient concentrations of the co-pollutants existed, indicating that the gaseous co-pollutants may serve as appropriate surrogates of personal $PM_{2.5}$ exposures.

Short-Term Goals in Exposure Assessment

Exposure studies conducted to date have provided a solid basis for future research. Goals of the PM centers during the next 2–3 years include the following:

- Complete exposure studies currently being conducted in St. Louis, Los Angeles, Steubenville, and Seattle. These additional data sets will enable us to further characterize personal PM exposures in locations with diverse study populations and meteorological and air quality conditions. Of particular importance are potential differences in exposures among susceptible subpopulations. To date, cohort-specific differences in PM exposures have not been examined because of sample-size limitations.
- Examine associations between personal exposures to PM and its gaseous co-pollutants in various locations throughout the United States. Results from Baltimore provide important initial information concerning the role of gaseous pollutants as confounders and/or surrogates of PM. Exposure data from studies in Los Angeles, Atlanta, Steubenville, and St. Louis will enable us to examine these relationships in additional urban environments.
- Continue the characterization of short-term (24-hr) PM exposures and identify factors that influence their relationship to outdoor concentrations. This information will assist in creating models of short-term personal exposures to be used in estimating chronic exposures to PM.
- Collect data on outdoor PM concentrations and personal exposures to specific PM components such as metals, PAHs, elemental and organic carbon, sulfate, and nitrate as part of our ongoing field studies. In addition, efforts will continue to expand the range of personal exposure measurements, including ultrafine particles and carbon monoxide.
- Determine concentrations of quinones, carbonyls, and PAHs in ambient air samples at selected sites in the Los Angeles basin. The objective is to provide quantitative chemical data on specific chemical entities relevant to human health and to the development of a model for atmospheric changes in the chemical constituents of air pollution.
- Continue data analyses for the investigation of exposure error using air pollution data from 1985 to 2000 for the entire United States. More detailed characterization and

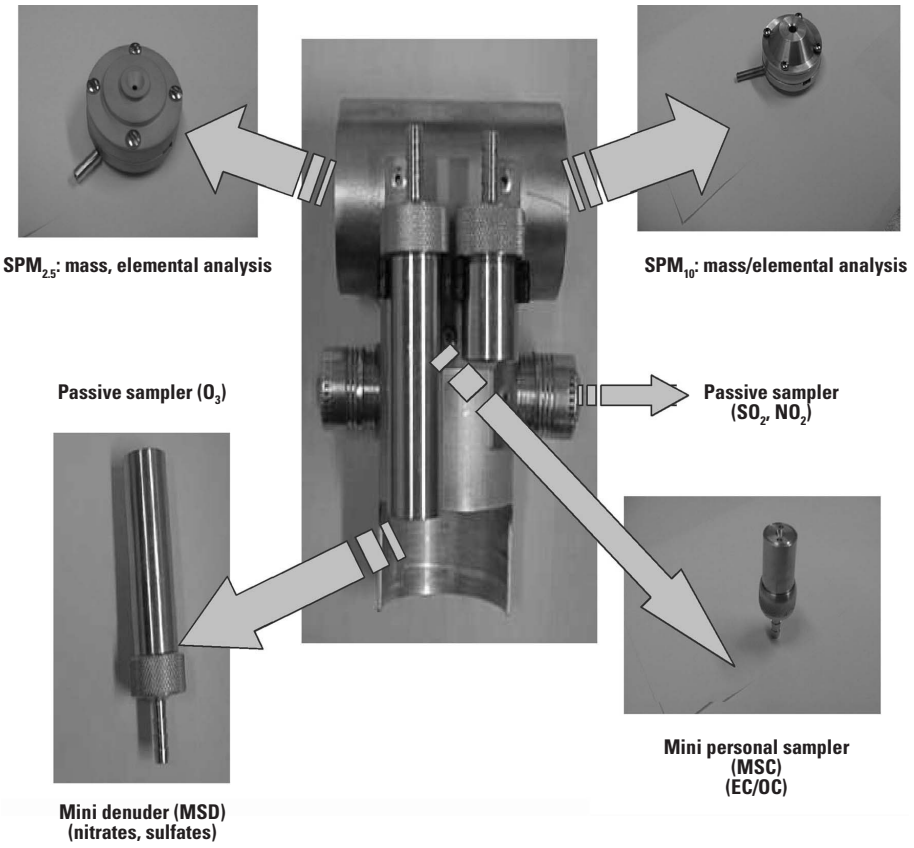

Figure 12. Diagram of the multipollutant personal sampler (Demokritou et al. 2001). Abbreviations: EC, elemental carbon; MSC, mini personal sampler; MSD, mini denuder; OC, organic carbon; SPM, suspended particulate matter.

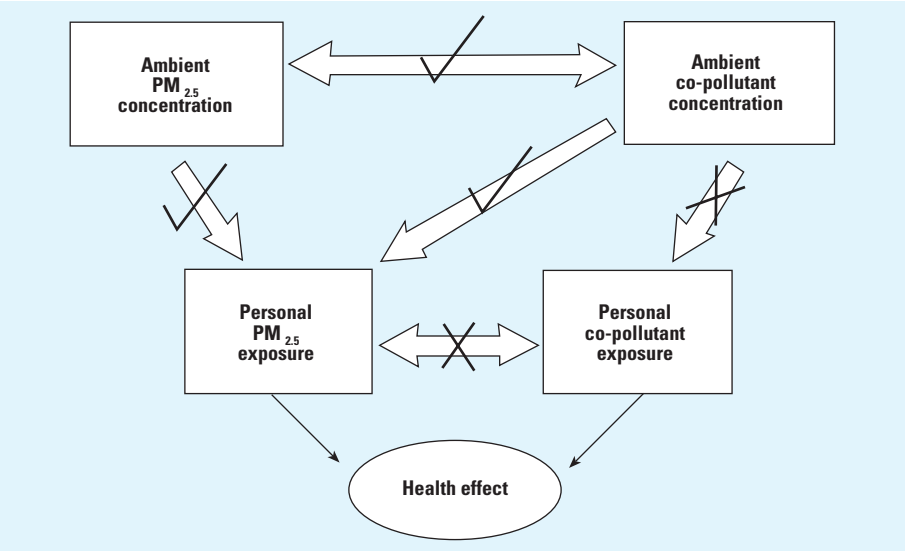

Figure 13. Summary of associations between ambient concentrations and corresponding personal exposures and among pollutant exposures and concentrations in Baltimore, Maryland (Sarnat et al. 2001). "✓" indicates strong correlations; "×" indicates weak correlations.

comparison of PM_{10} and $PM_{2.5}$ data will be conducted in areas with multiple monitors.
- Continue collection of outdoor PM samples to be used in toxicological studies of fine and ultrafine ambient particles. Initial studies to examine the hypothesis that freshly generated fine plus ultrafine emissions from a freeway with heavy diesel traffic increases the severity of asthma and inflammatory responses in a sensitive animal model are under way.
- Validate and refine models of local within-community exposures based on already available data sets on traffic patterns in metropolitan areas and further assessment of the chemical composition of traffic-associated particulate exposures.

Long-Term Goals in Exposure Assessment

The PM centers' long-term goals involving exposure assessment include the following: *a*) Development of chronic PM exposure models. Chronic exposure models will be based on relevant advances in short-term exposure models, reexaminations of historical and newly generated data on air pollution composition and levels, time–activity information, and indirect measures of PM exposure, such as covarying gaseous pollutant concentrations; geographical information system data, and housing characteristics may also be used as input data for these chronic exposure models. Individual or population estimates of long-term exposures will be used by epidemiological studies to assess chronic health effects. Of particular importance will be the development of chronic exposure models for specific toxic components of PM. *b*) Development and validation of source-apportionment techniques to link health outcomes with specific PM source types. The NYU PM center in collaboration with colleagues at the other PM centers and at the U.S. EPA will host a source apportionment workshop to standardize source-apportionment methodologies for PM epidemiological studies. *c*) Development of new and more sensitive biomarkers of PM exposure. This will enable us to assess human exposures for prospective population studies in a timely and cost-effective manner. Some preliminary work on a urinary biomarker for exposure to wood smoke is under way (Dills et al. 2001).

Coordination of Research within the Particulate Matter Centers Program

Background

Research centers are most productive where there is a well-defined set of scientific questions, and where the approaches needed to answer those questions cross traditional disciplinary boundaries of chemists, engineers, aerosol scientists, toxicologists, epidemiologists, pulmonologists, cardiologists, immunologists, molecular biologists, statisticians, and experts in exposure and risk assessment.

Going beyond what individual centers can accomplish, the U.S. EPA–sponsored PM Health Effects Research centers, with encouragement from the U.S. EPA, have created a coordinated PM centers program that keeps each center aware of work in progress and plans for the other centers, enabling them to more fully address the critical knowledge gaps confronting the U.S. EPA.

Linking the activities of the five PM centers and the U.S. EPA labs on a conceptual and operational basis to maximize productivity and relevance of the scientific findings represents a new direction for university-based research, with long-term significance. For example, for biological mechanisms, investigators from all five PM centers participated in discussions about unifying hypotheses of biological mechanisms for PM health effects, which include inflammation and immunity, mechanisms for cardiovascular effects, and the role of ROS.

There are other valuable features of the PM centers programs.
- Acceleration of the research process. In a rapidly evolving field such as PM research, investigators need to initiate new projects that may have a significant impact on the field. Conducting hypothesis-generating studies can be of paramount importance. The PM centers program has used pilot project funds to expedite the process of conducting exploratory research.
- Use of science advisory committees. The PM centers have effectively used groups of experts to oversee their research programs, and most of the PM centers' external science advisory committees include researchers from other PM centers. This has helped to coordinate research efforts among the PM centers, avoid duplication, and share resources.
- Leveraging funds to start new research initiatives. The PM centers can provide seed funds for future research initiatives and create opportunities for young investigators to be integrated into the overall research of the PM centers.
- Development of new technologies. The PM centers have the resources to develop complex new technologies that are available to the individuals in PM centers as well as to other affiliated research groups. The creation of the new ultrafine concentrator and multipollutant sampler are examples of innovative technology that derived from the creation of the PM centers. Oversight of the application of new technologies across the PM centers facilitates their validation and optimal utilization.
- Flexibility to reallocate funds as needed and to change research direction. The PM centers have sometimes been faced with unexpected research opportunities and/or expenses. On the basis of the input from their science advisory committees, the PM centers can reduce or expand activities in certain projects in favor of others.

Particulate Matter Center Strengths

Each of the PM centers has particular strengths, as outlined below.

Strengths of the NYU PM center include a capacity to conduct studies of the acute and cumulative effects of concentrated ambient air particles on cardiac and respiratory system effects in laboratory animals; a focus on particle dosimetry in the respiratory tract in both normal and abnormal human lungs; capabilities for the generation of exposure atmospheres of freshly formed ultrafine and fine monodisperse aerosols of acid aerosols, elemental and organic particles, and metal oxides; and an array of inhalation chambers and head-only exposure chambers for controlled exposure studies.

Strengths of the Rochester PM center include an interdisciplinary approach in ambient characterization studies, epidemiological studies, clinical studies, animal studies, and *in vitro* mechanistic studies; a focus on the role of ultrafine particles compared with other particle-size fractions; and experience in performing studies using laboratory-generated surrogate particles as well as real-world particles.

Strengths of the southern California PM center include a well-defined focus on mobile source pollution and health effects, with emphasis on investigation of the biological mechanisms of PM effects in relation to PM characteristics; emission sources and related adverse health effects; and the varying spatial and temporal patterns of ambient PM and copollutants and resulting health effects, with emphasis on field measurements and modeling.

Other strengths include the uniqueness of the Los Angeles basin airshed and close interaction with the Los Angeles Supersite; expertise from five leading universities in the region as well as the University of California Davis (Davis, California) and Michigan State University (East Lansing, Michigan); extensive ties with the CHS; the availability of mobile ultrafine, fine, and coarse concentrators for sample collection and *in vivo* animal and human clinical studies; and a focus on ROS acting as electrophilic agents and playing a central role in allergic airway disease including asthma and cardiovascular effects through their ability to generate oxidative stress, inflammation, and immunomodulating effects in the lungs and airways.

Strengths of the University of Washington PM center include experience in exposure assessment; experience in studies of acute health effects; a mouse model of CVD; a new chronic cohort mortality study (the Women's Health Initiative); and access to an airshed in

the western United States without the sulfate common to the east and with a higher contribution from vegetative burning.

Strengths of the Harvard PM center include close interaction among biological, epidemiological, exposure, and engineering research groups, which has led to the development of sensitive animal models based on our epidemiology studies—in turn, the controlled toxicology studies have developed refined outcomes that are currently being used in epidemiological and exposure group studies (Steubenville and St. Louis); experience in conducting panel studies examining the relationships between outdoor and indoor PM concentrations and corresponding personal exposures; development of statistical models for use in time-series analyses addressing harvesting, confounding, and dose–response relationships; a focus on methods development for measuring continuous sulfate and nitrate concentrations, personal particulate, and gaseous exposures; a PM concentrator for coarse, fine and ultrafine particle modes; and development of novel inhalation technologies for exposing individuals to ambient concentrations of PM and improving dosimetric techniques in animal exposure models.

Particulate Matter Center Interactions

PM center personnel have met on an annual basis since the inception of the centers. At the first PM center directors meeting, the directors and their colleagues described the research planned or under way within their respective PM centers. This was a period of familiarization and was characterized by informative reports. At the third meeting in Boston (July 2001), there was a sea change in the approach of the PM centers to their mission. It became apparent that there needed to be greater interaction across PM centers in an intellectual context as well as greater collaboration in a wide range of research areas. There was a clear need expressed to communicate more effectively on an ongoing basis and to interact with the U.S. EPA in a collaborative context to assist in the U.S. EPA research program more fully.

Since July 2001, the PM center directors and selected colleagues have held conference calls on a regular basis to discuss directions in research and collaborations. These discussions have resulted in development of both short- and long-term research goals, with intercenter collaborations as an important mechanism in achieving these goals. In this report, both short-term and long-term research goals are described for the PM centers overall and form the basis for collaboration and interaction over a second 5-year period of funding. There are now plans for a number of joint research efforts and workshops. The PM center directors believe that funding of individual research grants should continue through the STAR program and other sources, e.g., NIEHS and HEI, and one challenge would be to find a way to link the research of individual investigators and research teams to the unified efforts of the PM centers. We believe that a series of defined workshops organized by the PM centers would bring together investigators from the PM centers and the STAR program to discuss research findings in the context of the U.S. EPA regulatory needs and to seek out opportunities for further collaboration. This approach would be best organized through a joint U.S. EPA-centers effort. The ability to link a wide range of investigators from around the country, both intellectually and practically, represents a clear advantage of the PM centers structure.

Particulate Matter Centers Research and Its Implications for Public Health and Regulatory Policy

Using complementary approaches, the centers are addressing key policy issues such as

- What properties of PM are responsible for health effects? This issue was identified by the NRC committee as of critical importance. In testing hypotheses concerning PM toxicity and attribution to specific constituents or characteristics, work in the PM centers is addressing particle mass versus surface area versus number concentration (ultrafines); the role of transition metals; and the contributions to toxicity of acidity versus organic compounds versus elemental carbon. Toxicological approaches are employing exposures to CAPs or laboratory-generated PM in humans and animals; *in vitro* approaches are using a number of cell lines and specific particles; and epidemiological studies are focusing on different PM mixtures. This work should contribute not only to our understanding of mechanisms of response but also to development of more targeted control strategies.
- Are there specific risk factors or effect modifiers for PM effects? This is another high priority issue. Using a variety of approaches, PM center programs are examining risk factors such as age, gender, nutrition, and preexisting cardiopulmonary disease. All of the PM centers are currently conducting work to identify and characterize susceptible populations, a clear prerequisite of any regulatory policy.
- What types of PM pose the greatest health risks? What effects are directly attributable to PM, and what health responses may be modified by other air pollutants? These are areas of intense research focus within the PM centers using the different research strengths housed within each of them. It is the output from these integrated programs that will help set the next PM NAAQS and address issues raised by the public, industry, government, and public advisory bodies such as CASAC.

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