The PM Centers Program 2005-2010

Overviews and Abstracts

November 30 - December 1, 2005

Research Triangle Park, NC

Swine Photo Courtesy of USDA NRCS.

PM Centers Kick-Off Meeting

U.S. Environmental Protection Agency
Main Campus, Building C
Auditorium A & B
Research Triangle Park, NC

November 30 – December 1, 2005

Agenda

Day 1 – Wednesday, November 30

8:00 a.m. – 8:45 a.m.	**Breakfast and Registration**
8:45 a.m. – 9:00 a.m.	**Welcome** Gary Foley, Director, EPA National Center for Environmental Research
9:00 a.m. – 9:30 a.m.	**Goals for the Meeting: Promoting Collaboration in the PM Research Program** Dan Costa, EPA National Program Director for Air Research
9:30 a.m. – 10:00 a.m.	**San Joaquin Valley Aerosol Health Effects Center Overview** Tony Wexler, Center Director
10:00 a.m. – 10:30 a.m.	**Harvard University PM Research Center Overview** Petros Koutrakis, Center Director
10:30 a.m. – 10:45 a.m.	**Break**
10:45 a.m. – 11:15 a.m.	**Southern California Particle Center Overview** John Froines, Center Director
11:15 a.m. – 11:45 a.m.	**University of Rochester PM Research Center Overview** Günter Oberdörster, Center Director
11:45 a.m. – 1:15 p.m.	**Lunch (PM Center Directors meet together for lunch)**
1:15 p.m. – 1:45 p.m.	**Johns Hopkins PM Research Center Overview** Jon Samet, Center Director
1:45 p.m. – 2:15 p.m.	**EPA Research Laboratories: PM Research Overview** Dan Costa, EPA National Program Director for Air Research

Wednesday, November 30, 2005 (continued)

2:15 p.m. – 2:45 p.m.	**PM Science/Policy Futures: Deconstructing a Multiple Pollutant** John Bachmann, Associate Director for Science/Policy and New Programs EPA Office of Air Quality Planning and Standards
2:45 p.m. – 3:00 p.m.	**Break**
3:00 p.m. – 4:30 p.m.	**Breakout Session One — small groups meet on the following research topics:** Susceptible Populations (animal and human) Discussion Leaders: Diane Gold/Harvard and Günter Oberdörster/Rochester Panel and Controlled Exposure Studies (design, measurements, end points, populations) Discussion Leaders: Mark Frampton/Rochester and Ralph Delfino/SCPC Application of "OMICS" Technology to Toxicology Studies Discussion Leaders: Joe G.N. Garcia/Hopkins and Tony Huang/EPA Mechanisms/Oxidative Stress Discussion Leaders: Andre Nel/SCPC and Andy Ghio/EPA Chronic Effects Discussion Leaders: Doug Dockery/Harvard and Charlie Plopper/UC Davis Source and Source-Oriented Sampling Research Discussion Leaders: Costas Sioutas/SCPC and Michael Hays/EPA Source Apportionment (different approaches, how to strengthen use of models with atmospheric science) Discussion Leaders: Mike Kleeman/UC Davis and Linda Sheldon/EPA
4:30 p.m. – 5:30 p.m.	**Plenary: Report Back From Breakouts and Discussion**
5:30 p.m. – 6:30 p.m.	**Reception (at EPA)**
6:30 p.m.	**Group Dinner (at EPA)**

Day 2 – Thursday, December 1

8:00 a.m.	**Breakfast**
8:15 a.m. – 8:20 a.m.	**Introductions** Robert Devlin, Chief Clinical Research Branch, National Health and Environmental Effects Research Laboratory, EPA
8:20 a.m. – 8:40 a.m.	**National Monitoring Strategy and Implications for Health Studies** Phil Lorang, Acting Group Leader, Ambient Air Monitoring Group EPA Office of Air Quality Planning and Standards
8:40 a.m. – 9:00 a.m.	**Air Quality Data Base for Health Effects Studies** Geoffrey Sunshine, Health Effects Institute
9:00 a.m. – 10:30 a.m.	**Breakout Session Two: Multi-Disciplinary Components/Sources-to-Effects Research** Multi-disciplinary groups meet to discuss assigned questions (see next page)
10:30 a.m. – 10:45 a.m.	**Break**
10:45 a.m. – 11:45 a.m.	**Plenary: Report Back From Breakouts and Discussion**
11:45 a.m. – 1:00 p.m.	**Lunch**
1:00 p.m. – 1:15 p.m.	**Plenary: Directions to Collaborative Groups** Robert Devlin, Chief Clinical Research Branch, National Health and Environmental Effects Research Laboratory, EPA
1:15 pm. – 2:15p.m.	**Small Group Discussions to Begin Collaboration Planning** Meeting participants gather in small groups of their choosing to discuss specific collaborations in more concrete terms.
2:15 p.m. – 3:00 p.m.	**Panel of PM Center Directors and EPA – Meeting Highlights and Next Steps**
3:00 p.m.	**Meeting Adjourns**

Breakout Group Questions, Thursday, 9:00 a.m.

Group A — Discussion Leaders: John Godleski/Harvard and Patrick Breysse/Hopkins

What are the best approaches to identify/attribute sources (e.g., vehicular, agricultural, biomass burning, power plant, airports, shipping) and are different methods of attributing source emissions to ambient concentrations more suitable for different types of health studies?

Do different methods of attributing components to sources reveal differences in source characteristics that are of importance to health?

Group B — Discussion Leaders: Michelle Bell/Hopkins and Phil Hopke/Rochester

How does the interaction between source emissions and/or atmospheric chemistry potentially affect the toxicity of particles? For example, do acidic materials catalyze the formation of peroxides and other particle-bound ROS or does the deposition of acidic components onto the surface of the particles make other components such as metals more toxic?

Group C — Discussion Leaders: Rick Phipps/Rochester and Tony Wexler/UC Davis

Do emissions from different sources that contain the same component lead to different levels or types of health effects?

What source signatures are being used for which sources and why? Have we made any progress in attributing source signatures to toxic effects?

Group D — Discussion Leaders: Jon Samet/Hopkins and Ian Kennedy/UC Davis

What is the contribution of co-pollutants to PM observed health effects?

What are relevant co-pollutants (both gas and particulate), how can they be identified, and what is known about their interaction with PM?

How can one characterize dose-response relationships when co-pollutants are involved?

Group E — Discussion Leaders: Mark Utell/Rochester and Jamie Schauer/SCPC

Do current approaches to attributing sources to ambient concentrations adequately capture the variation in exposure to different particle sizes?

What are sizes of relevance or how can size lead to different health effects or different pathways, and is it feasible to generate laboratory surrogates that mimic sources or size ranges?

Group F — Discussion Leaders: Lucas Neas/EPA and Helen Suh/Harvard

In-vehicle and near-roadway studies: How can these studies evaluate the relative toxicity of components derived from specific sources (emissions, brakes, tires, etc.)?

San Joaquin Valley Aerosol Health Effects Research Center

Anthony Wexler, Director
Kent Pinkerton, Co-Director
University of California, Davis, CA

EPA Grant Number: R832414-010

Center Overview:

Objectives: This proposal will investigate the mechanistic links between ambient particles and the health effects that they elicit. This objective entails two goals: (1) understanding the metabolic response of tissue and organs when they are exposed to particulate pollutants; and (2) understanding the characteristics of the particulate pollutants and their gaseous co-pollutants that elicit these responses.

Experimental Approach: The proposed Center is composed of five projects supported by five cores. The projects will investigate the metabolic response to pollutant exposure in pulmonary and cardiovascular tissues, whole animal effects of exposure, transport of particles from the airways to other tissues, and the effects of particles and gases on lung development in juveniles. The projects will take both top-down approaches, identifying the characteristics of particles that elicit health responses, and bottom-up approaches, examining the metabolic responses that these particles elicit. We will perform both field and laboratory studies. The field studies will take place in the San Joaquin Valley of California, one of the worst violators of the National Ambient Air Quality Standards for particulate matter. The laboratory studies will examine the effects of particles from sources or laboratory-generated particles with carefully controlled properties that model ambient ones or those from dominant sources. The research team, composed of physiologists, toxicologists, chemists, and engineers who already collaborate on air pollution studies in the University of California Davis Air Quality Research Center, will use their complementary skills to address the Center's objectives.

Expected Results: Epidemiological studies show a correlation between elevated particulate matter concentrations and increased rates of morbidity and mortality, but these studies do not suggest the mechanisms or the particle properties that cause this correlation. The Center's scientists will investigate the properties of the particles that are responsible for these health effects, the metabolism that underlies these effects, and the consequences of chronic exposures, especially during childhood, that make individuals more susceptible to adverse effects.

San Joaquin Valley Aerosol Health Effects Research Center Description

The San Joaquin Valley Aerosol Health Effects Research Center will seek to establish a causal link between the physical and chemical characteristics of particulate matter (PM) and their resulting health effects. Elevations in PM concentrations are statistically associated with acute population morbidity and mortality. These associations have been established using a wide range of PM and health indicators in the United States and many other countries. Certain subpopulations may be more susceptible to these challenges, and susceptibility may be due in part to a history of PM exposure, especially during development. The characteristics of the PM that lead to these increases in morbidity and mortality are not well established, confounded by the multitude of particulate sources and ambient characteristics. Also, little is known about the biological mechanisms and pathways whereby PM induces responses at the cellular, tissue, organ, and/or whole body level. These mechanisms may be different during childhood and adulthood.

The San Joaquin Valley Health Effects Research Center will focus on five complementary projects, supported by five cores, to:

- Identify the mechanism of response,
- Define how these responses manifest themselves, and
- Characterize the particle properties that elicit these responses.

The San Joaquin Valley (SJV) provides an ideal location to study the nexus of air pollutants and their health effects because its residents are exposed to some of the worst particulate air pollution in the country. Air quality-related health indicators in the region reflect this; for example, asthma and cardiovascular disease rates are some of the highest in the country. Preliminary studies show a linear correlation between these health effects and PM concentration (Cahill et al., submitted). The SJV routinely experiences some of the highest fine airborne PM concentrations in the United States, with fine and ultrafine particle concentrations that rival those found in Los Angeles. The American Lung Association reports that three of the four most polluted American cities are located in the SJV. Many of the air pollution source categories in the SJV are the same as in the rest of the country: transportation, wood smoke, refineries, and agriculture. The Valley also encompasses sharp gradients—concentrations are higher in the south-central portion of the Valley and significantly lower in the foothills and further north; there is a seasonal variation in the concentrations as well. The SJV's large extent, rapidly expanding population, and severe air pollution make it one of the greatest emerging U.S. public health threats and an ideal location to study PM's acute and chronic health effects.

The University of California at Davis (UC Davis) is ideally situated to study the relationship between PM and adverse health effects in the SJV. UC Davis is at the northern end of the SJV, and many of the project investigators, including the director and co-director, study air pollution and its health effects in the Valley. We will use ambient particles from the SJV and laboratory-generated model particles to investigate a complementary set of hypotheses related to the mechanisms and loci of action that cause acute and chronic responses in normal animal models of children and adults and those made susceptible by exposure to particles and ozone during development.

- These studies aim to identify the mechanistic connection between ambient particles and their observed health effects so that remedies can be found for those chronically and acutely affected.

- In addition, by identifying the particle characteristics (concentration, size, composition, morphology, and sources) that lead to the observed health effects, the National Ambient Air Quality Standards can be more finely tuned and concomitant emissions regulations can be instituted that limit only the pollutants or pollutant mixes that cause increased morbidity and mortality.

This proposal derives from the Air Quality Research Center (AQRC) at UC Davis, which comprises a comprehensive team of biologists, physical and chemical scientists, engineers, and social scientists who work together to study the Nation's air quality problems (see http://airquality.ucdavis.edu). AQRC encompasses over 50 faculty members and scores of their students and post-doctoral fellows. The scientists on the AQRC team who are submitting this proposal investigate pulmonary and cardiovascular biology and aerosol and atmospheric science, working together to examine urban and regional smog and its health effects.

Projects, Hypotheses, and Specific Aims

Three primary questions will be addressed in this proposal:

- What are the physical responses to PM exposure?

- What mechanisms cause decrements in cellular, tissue, and organ function as a result of particle exposure?

- What properties and sources of ambient particles and their co-pollutants elicit these responses?

These questions are at the root of establishing a causal link between particles and physiological responses. Since none of these questions have previously been answered, investigators are faced with a double dilemma: they do not know what elicits a response, or what response to monitor. In addition, children exposed to particles during development may become predisposed to acute responses as adults, which leads to susceptible populations that respond in unique ways. This proposal contains five projects that approach these complementary questions.

The first two projects, Pulmonary Metabolic Response and Cardiovascular Metabolic Response, investigate the two most commonly posited loci of response using a bottom-up approach, starting with cell cultures and then moving into excised tissue, rodent, and finally, primate models. Complementing these, the Transport and Fate of Particles Project investigates the transport of particles through epithelia and endothelia and through the circulatory system to their final disposition. Taking a top-down approach, we will investigate the particle disposition within these organs radiologically and then histologically. While these three projects start from a biological point of view, the Particle Screening and Source Identification project starts from the aerosol point of view. This project's goal is to narrow the range of particle properties that elicit responses on the cellular and whole-animal level. The Architecture Development and Particle Deposition Project explores the differences between neonatal and adult responses to exposure, particularly how exposure alters pulmonary architecture during development and concomitant lung function and particle deposition patterns in the airways.

We believe that children are driven into a susceptible population pool by exposure to pollutants during development, which disrupts normal cell growth, proliferation, and differentiation. Children are thought to be more susceptible to PM because they spend more time outdoors and respire more per unit of body mass than adults. Less well appreciated is that the developing lungs of children are architecturally and morphologically distinct from those of adults. Studies by Peters and coworkers (Gauderman et al., 2000, 2002; Avol et al., 2001) in Southern California demonstrate that children exposed to elevated levels of particulate pollutants exhibit lung function decrements. Recent studies from UC Davis show that lung development is compromised when rats or primates are exposed to ozone during key periods of development (Evans et al., 2003; Fanucchi et al., 2003; Miller et al., 2003), which suggests a causal link between architectural development and lung function and demonstrates the value of animal models in developmental studies related to PM. We will initially focus on rats to further explore our developmental hypotheses, using primates when the hypotheses are sufficiently well supported. During development, we will expose these models to particles with a range of concentrations, sizes, compositions, and morphologies, with and without ozone, to quantify their influence on the developing and adult airway. The populations will include normal and exposed neonates, normal adults, and adults who were exposed during early development. Endpoints will include cell populations, airway architecture, lung function, particle deposition patterns and efficiencies, and rates of particle transport into the bloodstream.

Ultimately, the effects of PM on health occur via expression of genes. The most timely and cost-efficient way to explore the wealth of particle characteristics and mechanisms of action is to begin with studies of cells in culture and excised tissue, both of which have been successfully employed for similar studies at UC Davis. These models enable rapid screening of particle size, composition, and morphology and the mechanisms of response that they elicit. Once these particle classes and mechanisms are clarified, studies on juvenile, adult, and knock-out mice and juvenile and adult rodents will validate the particle characteristics that elicit responses and which responses they elicit. Finally, the hypotheses with the greatest weight of evidence will be explored with monkeys in the inhalation exposure facilities at the California National Primate Research Center. These studies will employ exposure facilities at UC Davis that have been used for years to subject models to air pollutants and assess their response.

Most mechanistic evidence relating particles to health effects has focused on pulmonary and cardiovascular loci, but more recent evidence suggests that other organ systems may also be involved. Our initial hypotheses address cardiopulmonary effects, but we will also examine the translocation of particles from the airways to other organs in the Transport and Fate of Particles Project. We will use state-of-the-art imaging techniques to locate these particles within organs and identify their disposition at the tissue, cellular, and sub-cellular level. By identifying their disposition in neonates and adults, pathologists and physiologists on the team will posit metabolic and cellular mechanisms of response for further pursuit.

Although epidemiological evidence shows that particulate mass correlates with morbidity and mortality statistics, this relationship is primarily because particulate mass is the quantitative measure that is available at the most sites in the United States and worldwide. Increasing evidence suggests that specific characteristics of the particle size distribution, such as particle number or surface area, may be a better correlate of health effects. Atmospheric aerosols are a complex mix (Tolocka et al., 2004; Phares et al., 2003, Rhoads et al., 2003) emitted from a wide range of sources that have been processed photochemically in the atmosphere. Ambient particles of concern to health effects range in diameter from about 10 nm to about 10 microns, which spans three orders of magnitude. This translates for surface area into a range of six orders of magnitude and for particle mass into nine

orders of magnitude. The size of the particle influences the amount and location of deposition in the lung and is likely to also influence the rates of clearance or transport into the bloodstream. Ambient aerosols are composed of inorganic, hygroscopic electrolytes; polar and hydrophobic organic compounds; soot composed of graphitic carbon associated with large PAHs clusters; numerous metals; crustal matter; and more. The surface composition probably influences endocytosis (Wilhelm et al., 2003) and its oxidative stress (e.g., Ball et al., 2000). The morphology (e.g., shape, fractal dimension) can influence the deposition pattern in the airways (and is employed by pharmaceutical companies for targeted drug delivery) and particle clearance, and it probably also affects transport across epithelial and endothelial membranes. Thus, particle composition, size, and morphology are all possible factors contributing to health effects.

The team of aerosol scientists at UC Davis has years of expertise in collecting, measuring, and generating aerosol particles with wide-ranging characteristics for health and atmospheric studies, which will be key to exploring the hypotheses that seek to determine which particle characteristics lead to the observed health effects.

Core Support

Five cores will support these projects. Three cores will produce and characterize particles with a realistic range of sizes, morphologies, and compositions so that projects can investigate health effect endpoints as a function of these variables:

- The SJV Core will expose cells, tissues, and animals to ambient and concentrated particles, characterize the properties of the particles and gases, and identify pollutant sources.

- Because ambient particles have complex chemistry, morphology, and size distributions, we need to construct models of them so that we can control for these variables as we investigate health effect endpoints. The Particle Generation and Characterization Core will develop well-controlled laboratory-generated models of these particles and their atmospheric transformation.

- The Imaging Core will also generate particles with realistic size and surface composition, but will focus on generating models that can be tomographically imaged with 3-D reconstructions to elucidate deposition of particles and their ultimate disposition via a number of imaging modalities.

Two cores will focus on the biological response to the particles produced and characterized by the cores listed above:

- The Animal Exposure Core will supply three animal models to these studies: mouse, rat, and monkey. Primates are expensive, so they will only be used near the end of the projects when we have narrowed our hypotheses and are ready to validate them in an animal model that is more similar to human than rodents. One of the unique features of this proposal is the availability of primate models at UC Davis, which enables us to more realistically explore hypotheses than is possible with lesser animals. Rodents will be used for the majority of whole animal studies, both in the laboratory and field.

- The Bioanalytical Core will support projects that investigate the genetic and metabolic response to particulate insults. Gene arrays, 2-D and Western blots, and other analytical techniques will uncover the cellular mechanisms underlying the elicited responses.

Management Structure and Approach

Dr. Anthony Wexler, director of this study, has been the director of UC Davis's Air Quality Research Center since its inception in 2002. His expertise is modeling and measuring the chemical and physical characteristics of ambient aerosol particles and understanding their dosimetry. Dr. Kent Pinkerton, the study's co-director, has been the director of the Center for Health and the Environment at UC Davis since 2001. His expertise is measuring the toxic effects of ambient aerosol particles using rodent and primate models. Both directors of the SJV Aerosol Health Effects Research Center possess complementary expertise in the physical and chemical characterization of ambient particles and their resulting health effects.

The Executive Committee will be composed of the directors, quality management leader, and project and core principal investigators. It will advise the director on scientific and financial decisions and provide membership to the PM Centers Committee working groups. The Science Advisory Committee, composed of external scientists with expertise in the health effects of PM, will help the directors monitor project and core performance and advise them on redirecting resources where applicable.

Our philosophy is that the core efforts serve to explore our hypotheses via the projects. As a result, we have included budget justifications but not budgets with the cores. All core funding is included in the project budgets.

We are eager to share UC Davis' unique facilities and expertise with other EPA PM Centers and learning from the successes of other centers. An advantage of creating a center is that investigators can work together so that the sum of their efforts and expertise is greater than as individuals. The same applies to the five centers funded by the EPA—each will have its strengths, and by working together, these strengths can complement each other and push the field forward more quickly.

To facilitate inter-center collaboration, we will collect standard reference particulate matter (SRM-PM) from the SJV as part of our inhalation toxicology research, cellular metabolism research, and Quality Management Plan. The SRM-PM will be preserved using standard operating procedures and distributed to other PM Research Centers upon request for chemical, physical, or biological analysis. The goal of the PM Research Center is to understand the properties of ambient PM that elicit adverse health effects, and by working together to complement each other's capabilities, we can make substantial progress towards this goal.

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Project 1: Pulmonary Metabolic Response

Michelle Fanucchi, PI; Charles Plopper, Co-PI; and Alan Buckpitt, Co-PI
University of California, Davis, CA

EPA Grant Number: R832414-010

Project Summary:

Objectives: Experimental studies performed in our laboratories have demonstrated that neonatal animals are more susceptible to pulmonary injury by bioactivated pollutants, such as the poly-aromatic hydrocarbon (PAH) naphthalene and the nitro-PAH 1-nitronaphthalene, than adult animals. This project will determine whether the increased neonatal vulnerability to bioactivated toxicants that has been documented in rodents is exacerbated when the PAH is adsorbed to particulate matter.

Experimental Approach: We will apply transdisciplinary approaches to test these hypotheses, including histological, biochemical, and microarray analyses. Using defined synthetic particles consisting of graphitic carbon, a PAH, and a transitional metal, we will compare responses in intact airways at different sites of susceptibility (branch points and airway wall) in postnatal and adult rats to responses in human airway epithelial cell lines. As the Architecture Development Project defines the sites of deposition, we will adjust the sites that we are evaluating in the lung accordingly.

Expected Results: Humans are exposed to multiple compounds early in life, yet most toxicological studies focus on the effects in adults. In addition, decisions regarding acceptable levels of environmental contaminants are based on adult data, which may not translate to children, the most susceptible portion of the population. Our work has already demonstrated that exposure to bioactivated pollutants produces much higher pulmonary toxicity in neonates than in adults. These studies will further define the role of particles in neonatal pulmonary susceptibility to environmental pollutants. Understanding the changes in lung cells following particle exposure at the gene and protein expression level will provide a basis for the development of biomarkers for assessing exposure and toxicity in young children.

Project 2: Cardiovascular Metabolic Response

John Rutledge, Co-PI, and Dennis Wilson, Co-PI
University of California, Davis, CA

EPA Grant Number: R832414-010

Project Summary:

Objectives: This project will determine the potential relationship between vascular disease and effects of circulating ultrafine particulate matter (PM).

Experimental Approach: We will evaluate the transcriptional responses to environmentally derived PM in human endothelial cell cultures in the context of functional models of inflammation and coagulation system activation. Microarray transcriptional screening of environmental samples collected in the San Joaquin Valley Inhalation Exposure Project will identify characteristic endothelial cell responses. Based on these data, key gene transcripts with potential influence on pro-inflammatory and hemostatic activities will be used to develop a panel of RT-PCR based transcriptional assays. We will study the functional significance of these activities by examining PM-induced alterations in the endothelial cell permeability barrier and inflammatory cell interactions with the endothelium of intact vessels. To determine the significance of endothelial cell reactions *in vivo*, we will extrapolate our understanding of the endothelial cell transcriptional response to determine whether and where similar responses occur in concentrated ambient particles (CAPs) exposed animals using histology, laser capture microdissection, and RT-PCR assays of potential target organs and vessels. We will extend these results to cardiovascular disease using mice as models. We will determine whether PM selectively accumulates in atherosclerotic plaque from isolated arteries of predisposed ApoE-/- mice and determine with similar *in vivo* laser capture studies whether this selectively upregulates proinflammatory reactions in plaque of CAPs-exposed atherosclerotic mice.

Expected Results: Microarray studies should provide significant insight into the specificity of responses to particulate matter. Signal transduction and endothelial cell functional studies will answer similar questions about specificity. These should help distinguish between non-specific oxidant injury and potential interference with specific endothelial cell activation pathways. The combination of gene and protein responses assayed in CAPs-exposed animals should provide significant evidence of PM-induced vascular proinflammatory and coagulant responses in normal tissues. Experiments with atheromatous mice exposed to CAPs should determine whether specific accumulation of PM in altered regions of the vascular wall could contribute to the initiation of plaque rupture. They also will address the possibility that atheromatous plaque might be selectively susceptible to the induction of inflammatory responses to circulating PM.

Project 3: Inhalation Exposure Assessment of San Joaquin Valley Aerosol

Kent Pinkerton, PI; Mike Kleeman, Co-PI; and Ann Bonham, Co-PI
University of California, Davis, CA

EPA Grant Number: R832414-010

Project Summary:

Objectives: Epidemiological evidence suggests that the association between cardiac mortality and PM_{10} concentrations changes between the summer and winter months in the San Joaquin Valley (SJV). This shift is likely caused by seasonal variation in the size and composition distribution of airborne particles. This project will perform inhalation exposure and particle characterization studies at rural and urban locations in the summer and winter months to quantify the features of the airborne particles that are associated with adverse health effects.

Experimental Approach: Inhalation exposure studies will be carried out using mice that are exposed to ambient airborne particles and concentrated ambient airborne particles in the SJV. Exposures will be conducted in the summer and winter in both an urban and rural location to take advantage of the changes that occur in particle chemical composition and size distribution as a function of season and location. Heart rate variability, lung inflammation, and markers for oxidative stress will be monitored. Collocated measurements of $PM_{2.5}$ and $PM_{0.1}$ composition will be made to quantify health effects associated with chemical composition and support source apportionment calculations. Inhalation studies of direct emissions will be conducted for the dominant sources of $PM_{2.5}$ and $PM_{0.1}$ that are identified during ambient studies. Based on our results, we will postulate possible mechanisms for health effects and test these using laboratory studies and simple model particles.

Expected Results: We will identify the composition and size fraction of the airborne particles that cause adverse health effects in the SJV. The source origin of these unhealthy particles will be determined using source apportionment calculations. These findings will support an improved mechanistic understanding of how airborne particles cause negative health effects.

Project 4: Transport and Fate

Dennis Wilson, PI, and Angelique Louie, Co-PI
University of California, Davis, CA

EPA Grant Number: R832414-010

Project Summary:

Objectives: This project will: (1) characterize the time course, tissue distribution, and mechanisms of particulate matter (PM) accumulation in the systemic circulation and target organs; (2) evaluate the effects of size and surface-fixed charge on this process; and (3) determine how altered lung structure affects systemic particle distribution.

Experimental Approach: Ultrafine particulates are transported into the systemic circulation and target organs by mechanisms that have not been characterized. Key questions include whether particulates in the ultrafine range behave differently from larger particles and whether particle composition, especially surface charge, affects systemic absorption. Experiments in this project will address the mechanisms of transport across the pulmonary epithelial barrier, the time course and target tissues for the distribution of particulates, likely means of intravascular transport, mechanisms of particulate interaction with the vascular wall, and the potential for endothelial cell-mediated transport into tissues. We will perform *in vivo* exposures to particles of varied size and surface-fixed charge. These particulates will be composed of materials traceable by microimaging techniques in real time combined; particles also will be histologically quantitated within target tissues. We also propose *in vitro* experiments to evaluate potential routes of passive or facilitated transport across epithelial and endothelial cell monolayers. Finally, we will determine whether animals with lung structure compromised by postnatal oxidant air pollutant exposure have increased systemic circulation of ultrafine particulates and examine the effect of acute oxidant exposure in adult animals on the transport of particles into the systemic circulation.

Expected Results: These experiments use size and surface-fixed charge defined ultrafine particulates to provide baseline information on the time course and extent of their systemic absorption. Understanding the nature of particle transport in blood will be important for recognizing the likelihood and potential mechanisms for interaction with tissues. Combining microimaging of whole animals in real time with quantitative histologic evaluation of tissue distribution should provide insight into the time course and nature of potential biological responses. The high-resolution microscopy and reconstruction techniques to be used in these experiments will not only distinguish whether particles move between or through cells and airway or vessels walls but also, in combination with inhibitor studies, whether this is an active or passive process, thereby providing insight into the responsible biologic processes.

Project 5: Architecture Development and Particle Deposition

Anthony Wexler, PI, and Charles Plopper, Co-PI
University of California, Davis, CA

EPA Grant Number: R832414-010

Project Summary:

Objectives: Epidemiological evidence suggests that children exposed to air pollution develop impaired lungs. We have observed alterations in lung architecture in monkeys exposed to ozone during development. This project will quantify the amount and time course of pollutants that lead to these architectural abnormalities and their functional implications.

Experimental Approach: We will use both experimental and modeling approaches to reach the objectives. Rats will be exposed to ozone, particles, and particles with ozone during different stages of development. We will perform lung function tests and measure airway architecture on normal and exposed adults. Comparing normal and exposed architecture and function will elucidate the significant changes that are due to the pollutants. Particle deposition patterns also will be measured and predicted with mathematical models to understand how alterations in architecture might increase particle deposition or change its location.

Expected Results: Children and the elderly are thought to be the most susceptible to particulate air pollutant exposure. The elderly are more likely to have preexisting impairments that make them more likely to suffer symptoms from inhaling particulates, and children respire much more than adults per unit of body mass due to their higher level of physical activity and greater time spent outdoors. The airways in children grow as their bodies grow, and when exposed to air pollution, the airways appear to grow in ways that lead to diminished lung function. As a result, children who grow up with increased air pollution may be at higher risk when inhaling pollutants as adults. This project will quantify the amounts and kinds of pollutants that lead to airway impairment, determine when the airways are most easily impaired during their development, and identify which functions are impaired due to this exposure.

Novel Exposure Scenarios To Define the Health Effects of Particle Sources

Petros Koutrakis, Director
Harvard University, Boston, MA

EPA Grant Number: R832416-010

Center Overview:

Objectives/Hypothesis: The fundamental objective of the proposed Center is to understand how specific particulate matter (PM) characteristics and sources impact inflammation, autonomic responses, and vascular dysfunction.

Experimental Approach: The Center will investigate the pathophysiological effects produced by exposures to PM and its gaseous co-pollutants and will examine how these effects relate to PM composition, size, and sources. **Project 1** will examine the association between PM exposures and intermediate markers of autonomic dysfunction, systemic inflammation, endothelial activation, and oxidative stress in the Normative Aging Study cohort in Eastern Massachusetts. **Project 2** will use a crossover exposure design to examine the effects of traffic-related PM and gases in a panel of 36 older adults who will be exposed to pollutants during 5-hr long field trips via buses in Boston, MA. This Project will examine whether exposures are associated with autonomic dysfunction, pulmonary and systemic inflammation, and endothelial activation. **Project 3** will examine the cardiovascular effects of fine, coarse, and ultrafine concentrated ambient particles (CAPs) in 50 healthy adults in Toronto. Vascular function and inflammatory examinations will include brachial artery dia- meter, flow-mediated dilatation and nitroglycerin-mediated dilatation, heart rate variability, blood pressure, cardiac output, stroke volume, and systemic vascular resistance. **Project 4** will investigate the relationship between PM composition and vascular response. Normal and spontaneously hypersensitive rats will be exposed to fine CAPs in Boston during either early morning (mostly locally emitted PM rich in elemental and organic carbon) or mid-day periods (mostly transported PM rich in sulfates). Biological outcomes will include pulmonary and systemic inflammation, blood pressure, endothelin-1, endothelial nitric oxide synthase, atrial naturetic peptide, oxidant response in the heart and lung by *in vivo* chemiluminescence, and vascular morphometry of lung and cardiac vessels. **Project 5** will investigate the effects of primary and secondary vehicular emissions from a tunnel in Boston, using the same animal models and biological measurements as in Project 4. The five projects will be supported by three cores: Administration and Research Coordination, Particle Technology and Monitoring, and Biostatistical.

Expected Results: The Center will provide information about the cardiovascular and pulmonary effects of specific PM sources and/or components, which is critical for the development and implementation of Air Quality Standards.

Harvard University PM Center
Overall Goals and Objectives

The fundamental objective of the proposed Center is to understand how specific particulate matter (PM) characteristics and sources impact inflammation, autonomic responses, and vascular dysfunction (Figure 1). To meet this objective, the Center, through its five proposed Projects, will systematically address the following four key scientific questions:

- What types of pathophysiological effects are produced by PM exposures and how do these effects relate to specific particle composition, size, formation processes, and origin (toxic components)?

- What are the effects of gaseous co-pollutants on the observed PM exposure-response relationships?

- What are the biological mechanisms whereby PM exposures can induce inflammation and autonomic responses that lead to pulmonary and/or cardiac dysfunction?

- Are certain individuals more susceptible to PM due to their health condition, age, genetic characteristics and/or nutritional factors?

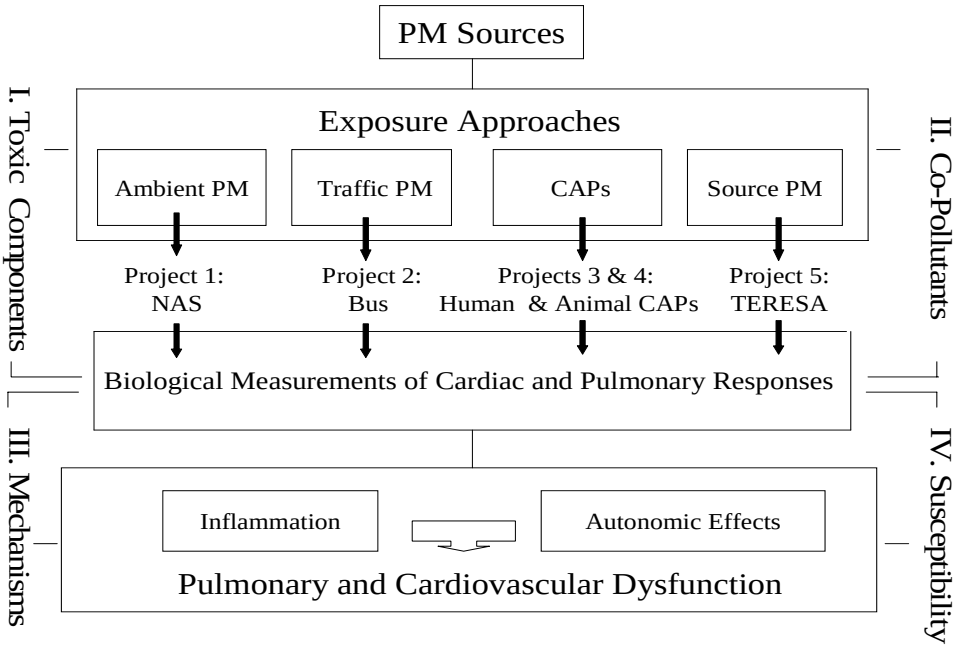

Figure 1. Linking inflammation, autonomic effects and vascular dysfunction to PM sources.

EXISTING EPA PM CENTER

Over the last 5 years, the existing EPA PM Center has supported a large interdisciplinary research group that has collaborated intensively to investigate the health effects of ambient PM, in accordance with the National Research Council's research priorities for ambient particle research. To

date, Center research has produced over 70 peer-reviewed publications.[1-70] Some of our major accomplishments are in the key areas of exposure, susceptibility, biological mechanisms, toxic components, and methodological issues, which are briefly presented below.

Exposure Relationships

A large data set on personal exposures and indoor and outdoor concentrations was collected for panels of susceptible individuals across the United States.[46-48] These investigations suggest that personal exposures to $PM_{2.5}$ of ambient origin are highly correlated with outdoor concentrations. However, the regression slopes of personal exposures on outdoor concentrations, which are usually less than one, vary substantially depending on house characteristics, season, and city climatic conditions. The strong correlations between personal and ambient concentrations were unique to $PM_{2.5}$, as personal exposures to O_3, SO_2 and NO_2 were substantially lower than, and weakly correlated with, corresponding outdoor concentrations.[49]

Susceptible Populations

Our epidemiological studies have provided strong evidence that individuals with congestive heart failure, COPD, and diabetes are at higher risk than healthy individuals.[4, 22, 30, 59, 60, 68-70] In an effort to understand why individuals with certain diseases are at greater risk than others, Center researchers exposed animals with cardiopulmonary diseases such as COPD and myocardial infarction to Concentrated Ambient Particles (CAPs).[2, 10, 24] The findings of these toxicological studies support those of the epidemiological studies and provide insight about possible mechanisms responsible for the observed PM effects.

Toxic Components

Many of our CAPs animal toxicology and human panel studies have linked pulmonary and cardiovascular health outcomes to different PM components such as trace metals, elemental carbon, sulfates and silicon.[2, 10, 45] Reanalysis of the Harvard Six-Cities study provided strong evidence of increased toxicity associated with combustion-related PM from traffic and power plants compared to soil dust.[32]

Biological Mechanisms

We have conducted exposure studies designed to elucidate the biological mechanisms whereby PM can induce adverse health effects. Results from a series of human and animal studies showed that exposures were linked to changes in heart rate variability (HRV), arrhythmias, pulmonary inflammation, and vascular dysfunction.[1, 9, 10, 26, 42]

Methodological Issues

New statistical and epidemiological methods were developed to provide the necessary tools to address challenging PM issues such as: harvesting[54, 65, 67]; confounding[58]; dose-exposure relationships[51, 53, 54, 56]; gaseous co-pollutants[52]; and weather confounding.[6, 7, 26, 38, 39, 40, 41] Many new exposure and monitoring particle technologies were also developed under the aegis of our Center and are currently used worldwide. These include the ultrafine particle concentrator, the toxicological samplers, the miniature multi-pollutant sampler, the personal cascade impactor, and the membrane diffusion denuder.[12-21]

PROPOSED EPA PM CENTER: APPROACH

Center Strategy

The cornerstone of the proposed Center is a multifaceted exposure approach encompassing ambient, CAPs, and specific-source exposures. These novel exposure approaches will be used to elicit a large array of biological responses, in humans and animals, focusing primarily on pulmonary and cardiovascular outcomes. This will make it possible to investigate the effects of toxic PM components (size, number and composition—elemental and organic carbon, black carbon, trace elements, organics and sulfates) and gaseous co-pollutants (CO, NO_2, SO_2, O_3, and VOCs) and explore biological mechanisms and susceptibility (Figure 1).

Center Research

As outlined above, the proposed Center includes five highly interdisciplinary and integrated projects (see Table 1) that collectively will address the four scientific questions.

Project 1 [NAS study] will examine the association between subject-specific PM exposures and intermediate markers of autonomic dysfunction, systemic inflammation, endothelial activation, and oxidative stress in participants of the Normative Aging Study, a large, ongoing prospective cohort study in Eastern Massachusetts. Electrocardiograms, blood samples, food frequency questionnaires, medication use, genetic data and 1-week integrated indoor PM samples for each study participant will be collected. These data together with subject-specific data on medical and behavioral characteristics will be used in structural equation models to examine the effects of particle composition (black carbon, sulfate), genetic phenotypes (GSTM1, HO-long), medication use (beta and calcium blockers, statins), and dietary intake (vitamin C and omega fatty acids), on the observed associations between exposures and biological outcomes. [Scientific Questions I, III, and IV]

Project 2 [Bus Study] will use a crossover exposure design to examine the inflammatory and autonomic effects of traffic-related PM and gases in 36 older adults. Subjects will be exposed to either PM plus its gaseous co-pollutants or gases alone. For both exposure scenarios, participants will be exposed to the pollutants during 5-hour long field trips via diesel-powered buses in Boston, MA. This project will examine whether mobile source-related pollution (particles and gases) is associated with autonomic dysfunction, HRV, pulmonary and systemic inflammation (eNO, CRP, IL-6, fibrinogen, WBC) and endothelial activation (ICAM-1). [Scientific Questions I, II, and III]

Project 3 [Human CAPs Study] will examine the cardiovascular effects of fine, coarse, and ultrafine CAPs. This will be a randomized block design, double-blinded study that will expose 50 healthy adults to fine, ultrafine, and coarse CAPs, in Toronto. Each participant will receive four exposures in random sequence with each exposure session separated by at least 2 weeks. Vascular function and inflammatory examinations will afford pre- and post-exposure measurements of brachial artery diameter, flow-mediated dilatation and nitroglycerin-mediated dilatation. In addition, measurements of HRV, BP, cardiac output, stroke volume, and systemic vascular resistance will be performed during exposure sessions. [Scientific Questions I and III]

Table 1. Center Overview: Approach to link particle sources to pulmonary and cardiovascular dysfunction.

Project	NAS	Bus	Human CAPs	Animal CAPs	TERESA
Scientific Questions	I, IV	I, III, II	I, III	I, III, IV	I, III, IV
Exposure Focus	PM Toxic Components	Gaseous Co-pollutants	Fine, Coarse and Ultrafine PM	PM Toxic Components	Primary and Secondary PM
Exposure Type	Ambient Exposures	Traffic Exposures	CAPs Exposures	CAPs Exposures	Source Exposures
Exposure Parameters	Indoors: PM mass, elements, BC, SO_4^{2-} Outdoors: PM mass, size and counts, EC, OC, NO_3^-, SO_4^{2-} CO, NO_2, SO_2, O_3	Bus: PM mass, size and counts metals, BC, EC, OC, SO_4^{2-}, CO, NO_2, O_3, SO_2, VOCs	Chamber: PM mass, size and counts metals, EC, OC, Organics SO_4^{2-}, CO, NO_2, O_3, SO_2,VOCs	Chamber: PM mass, size and counts metals, EC, OC, Organics SO_4^{2-}, CO, NO_2, O_3, SO_2, VOCs	Chamber: PM mass, size and counts metals, EC, OC, Organics SO_4^{2-}, CO, NO_2, O_3, SO_2,VOCs
Location	Eastern Massachusetts	Boston	Toronto	Boston	Boston
Subjects/ Susceptibility	NAS Cohort	Susceptible Older Adults	Healthy Adults	Healthy and Hypertensive Rats	Healthy and Hypertensive Rats
Biological Measurements	HRV, BP, CRP, ICAM1, VCAM1, GSTM1	eNO, HRV, BP, CRP, Fibrinogen ICAM-1, IL-6, WBC	BAD, BP, HRV, BP, Cardiac Output, Stroke Volume, Systemic Vascular Resistance, FMD, NMD, CRP, fibrinogen, IL-6, ETs, Lung Function	Lung Function, BP BAL,WBC, ETs, IL-6, ANP Heart and Lung Oxidants	Lung Function, BP BAL,WBC, ETs, IL-6, ANP Heart and Lung Oxidants
Project Years	1,2,3	3,4,5	1,2,3,4,5	1,2,3	3,4,5
P. I.	Joel Schwartz	Helen Suh	Frances Silverman	John Godleski	Petros Koutrakis

Project 4 [Animal CAPs Study] will investigate the relationship between particle composition and vascular response. Sprague Dawley rats and spontaneously hypersensitive rats will be exposed to fine CAPs in Boston. Exposures will be administrated during two distinct daytime periods to capture different PM source contributions: (1) early morning exposures, which are comprised of mostly local emissions rich in carbonaceous particles; and (2) mid-day exposures, which are comprised of mostly transported particles rich in sulfates. Biological outcomes to be measured will include pulmonary and systemic inflammation, blood pressure by telemetry, endothelin-1, endothelial nitric oxide synthase (eNOS), atrial naturetic peptide (ANP), oxidant response in the heart and lung by *in vivo* chemiluminescence, and vascular morphometry of lung and cardiac vessels. [Scientific Questions I, III, and IV]

Project 5, the Toxicological Evaluation of Realistic Emission Source Aerosol [TERESA study], will investigate the relative toxicity of primary and secondary vehicular emissions. Exhaust emissions from a tunnel in Boston will be introduced into a photochemical reaction chamber, where secondary PM will be formed. Normal and hypertensive rats will be exposed to the primary and secondary PM, and will be evaluated for pulmonary, systemic, and cardiovascular effects. Project 5 will examine whether atmospheric photochemical processes enhance the toxicity of gases and PM emitted from vehicles and will determine whether susceptible animal models will have greater biological responses to PM than the corresponding healthy animal model. [Scientific Questions I, III, and IV]

In addition to the five projects the Center will encompass three Cores: (1) The Administration and Research Coordination Core will be responsible for research coordination and prioritization, data management, human and animal subject research protocols and quality assurance (directed by Koutrakis and Dockery). (2) The Particle Technology and Monitoring Core will be responsible for the development, evaluation, and application of particle collection and exposure devices as well as exposure monitoring (led by Demokritou and Koutrakis). (3) The Biostatistical Core will provide support for statistical analysis for all projects, including substantial design consultation and analytical work (directed by Coull and Schwartz).

Center Participants

The Center will involve researchers from the following institutions: (1) Harvard School of Public Health; (2) St. Michael's Hospital, University of Toronto; (3) University of Michigan; (4) Veterans Administration Boston Hospital; and (5) Brigham and Women's Hospital, Harvard Medical School.

Center Infrastructure

Our proposed research portfolio builds upon the existing Center infrastructure developed over the past 5 years, including an interdisciplinary research group of faculty, research staff and students as well as the already available cohorts, animal models, standard and innovative biological assessments, statistical tools and particle technologies. More specifically, we will use a large existing cohort, NAS, already developed exposure technologies, such as the fine, ultrafine, and coarse concentrators and a mobile photochemical chamber with a mobile exposure toxicology unit as the basis for the projects. As a result, the ambitious research portfolio outlined in this Center proposal will be timely and cost-effective.

Center Expertise

The proposed research program includes projects that span several disciplines in which our investigators have expertise, including: Exposure Assessment (Koutrakis, Suh, JR Brook, Urch, Silverman); PM Physics and Chemistry (Koutrakis, Demokritou, Lawrence, JR Brook and Wolfson); Epidemiology (Dockery, Gold, Schwartz and Speizer); Cardiology (RD Brook, Vokonas and Stone); Pulmonary Health (Gold, Silverman and Speizer); Toxicology (Godleski, Gonzalez-Flecha, Silverman, Urch and Wellenius); and Biostatistics (Coull, Corey, Schwartz and Zanobetti). This group of investigators has been collaborating on particle health effects research for more than a decade. Also, many other faculty members from our Department who are not included in the budget will be available as needed, including: Drs. Christiani (Occupational Medicine), Spengler (Indoor Air), Milton (Aerobiology), Kelsey (Toxicology), Verrier (Cardiology), Smith (Biomarkers), Brain (Physiology) and Levy (Risk).

Center Administration

The Center Director will be Petros Koutrakis, who is the Director of the existing EPA PM Center. He is a Professor of Environmental Sciences and the Director of the Exposure, Epidemiology and Risk (EER) Program at Harvard. He will be assisted by Deputy Center Director Douglas Dockery, Professor of Environmental Epidemiology at EER.

Expected Benefits

The proposed research will address important scientific issues relating to PM health effects. It will generate critical information on the PM toxic components (Projects 1 and 4), the toxicity of primary and secondary traffic PM (Project 5), the role of gaseous co-pollutants (Project 2), and the toxicity of fine, coarse, and ultrafine PM (Project 3). All five Projects will measure a large array of exposure characteristics (physico-chemical particle parameters and gaseous co-pollutants) and biologic outcomes (pathologic, functional, and molecular parameters of cardiovascular response). This combination of detailed exposure and biological assessments will make it possible to examine the effects of specific PM sources or components (Hazardous Components) on specific mechanistic pathways by which PM cause adverse health effects (Biological Mechanisms) on individuals with cardiovascular diseases (Susceptibility). Thus, the proposed research program will provide important information that is critical for the development of Air Quality Standards and their implementation.

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Project 1: Cardiovascular Responses in the Normative Aging Study: Exploring the Pathways of Particle Toxicity

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EPA Grant Number: R832416-010

Project Summary:

Objectives/Hypothesis: Since 1997, epidemiological and animal studies have identified many potential mechanisms by which particles may impact health. However, the relative importance of these potential pathways and the steps along these pathways are not well understood, particularly as to how they relate to specific particle components and sources for which pathways are likely to differ. We propose to examine the importance and relevance of the inflammatory, endothelial, and autonomic pathways to particle toxicity using the cohort of individuals participating in the Normative Aging Study (NAS), a large prospective cohort living in Eastern Massachusetts.

Experimental Approach: We propose to build on our analyses of the NAS cohort performed as part of our original EPA-Harvard Particle Health Effects Center using novel exposure and epi- demiological approaches. This approach uses pharmacological and natural interventions as well as genetics, to highlight specific biological pathways. Specifically, we propose to collect ECG, blood inflammatory marker, medi- cation, genotypic, food frequency, and particle exposure data for each of the 700 current NAS participants. ECG and blood marker samples will be analyzed for a variety of measures (HRV, ST segments, QT intervals, CRP, sICAM-1, sVCAM-1, and homocysteine) that will serve as intermediate markers of the inflammatory, endothelial, and autonomic pathways. These markers will be related to individual-specific $PM_{2.5}$, SO_4^{2-}, BC, and trace element exposures that will be measured inside each participant's home for 1 week prior to his/her clinic visit and to ambient air pollution ($PM_{2.5}$, PM_{10}, $PM_{2.5-10}$, SO_4^{2-}, NO_3^-, BC, EC, OC, NO_3^-, PC, and trace elements) concentrations that will be measured at our stationary ambient monitoring (SAM) site. The long-term (i.e., annual) effects of specific particle component exposures on measured markers of inflammation, endothelial function, and autonomic function also will be examined using a GIS-based exposure model.

The importance and relevance of the inflammatory, endothelial, and autonomic pathways of particle toxicity will be examined using naturally occurring variations in the NAS cohort in: (1) the GSTM1 and HO-1 genotypes; (2) dietary micronutrient intake; (3) hypertensive and cardiac medication use; and (4) methacholine reactivity.

Expected Results: By examining how these variations modify exposure-effect relationships, natural interventions will be created that enhance or diminish the importance of the inflammatory, endothelial, and autonomic pathways. Given these variations, structural equation models, a powerful technique that combines multiple regression and factor analysis methods, will be used to examine relationships among the multiple particulate matter components and health outcomes and to determine whether these relationships are consistent with specific biological pathways.

Project 2: Cardiovascular Effects of Mobile Source Exposures: Effects of Particles and Gaseous Co-Pollutants

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EPA Grant Number: R832416-010

Project Summary:

Objectives/Hypothesis: The independent and joint effects of particles and gases on acute autonomic function and inflammation changes are poorly defined, especially when the particles and gases originate from the same source. To improve our understanding about pollutant mixtures and source-specific effects, we propose to use a crossover study to examine whether particulate and/or gaseous pollutants emitted from motor vehicles are associated with autonomic dysfunction and pulmonary and systemic inflammation.

Experimental Approach: Thirty-six older adults will be exposed repeatedly either to particulate plus gaseous motor vehicle pollution or to only gaseous motor vehicle pollution. For both exposure scenarios, participants will be exposed through 5-hr long field trips via diesel-powered buses. Participants will include older adults who may be more sensitive to particulate pollution and will likely include individuals with coronary artery disease. Selected participants will live in or near retirement facilities located in suburban Boston. Project 2 will be conducted in Years 3 through 5 of the Center.

Field trips will occur in the fall and spring, when regional pollution is low, so that motor vehicle pollution comprises a larger fraction of particulate mass. Twelve subjects, randomly divided into two groups of six, will participate in each field trip, with groups riding on separate buses that will follow the same route. On one bus, the air will be unmodified, thus naturally exposing its riders to elevated concentrations of particles and gases from the bus and surrounding vehicles. Air in the second bus will be filtered to remove all particles. Thus, individuals traveling on the second bus will be exposed only to gaseous pollution, predominately from vehicles. Exposure scenarios for the groups will be alternated in a second field trip, which will occur 1 to 2 weeks later to prevent carryover of any effect from the first trip while minimizing differences in weather and ambient particle composition. Study participants will be randomized to the sequence of exposures to avoid bias. A total of 72 person-trips will be made (six groups and six trips).

Health and exposure monitoring will be identical to methods used in our precursor St. Louis Bus study, which was performed as part of our existing Center. Before, during, and after each trip, participants will be monitored for heart rate variability (HRV) as a measure of autonomic function, exhaled NO (eNO) as a measure of pulmonary inflammation, and blood markers as a measure of systemic inflammation. In addition, oxygen saturation and blood pressure will be measured repeatedly, as potentially inter-related intermediate markers of cardiac morbidity. Personal group-level measures of black carbon (BC) and particle counts (PC) also will be measured before, during, and after each trip to assess exposures to mobile source pollution, along with measures of $PM_{2.5}$ and $PM_{2.5-10}$, ozone (O_3), carbon monoxide (CO), nitrogen oxide (NO), and total nitrogen oxides (NO_x).

Expected Results: Results from this study will provide critical information about the roles of pollution mixtures and motor vehicle pollution in cardiac dysfunction. Through its crossover design

and use of bus trips to elevate motor vehicle exposures, this study provides a novel, efficient and effective means to examine the combined and separate effects of particulate and gaseous motor vehicle pollution. The crossover design not only allows separation of the effects of gases from those of both particles and gases, but also allows these effects to be examined both by group and by individual. Furthermore, the use of bus trips to elevate exposures allows us to study "frail" older adults, who for ethical reasons cannot be examined in controlled human studies.

Project 3: Cardiovascular Toxicity of Concentrated Ambient Fine, Ultrafine, and Coarse Particles in Controlled Human Exposures

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EPA Grant Number: R832416-010

Project Summary:

Objectives/Hypothesis: This proposal aims to further examine the components and sources of particulate matter (PM) responsible for these cardiovascular physiologic responses. A new state-of-the-art ambient PM exposure facility (to be built at the University of Toronto in collaboration with the Harvard School of Public Health) will allow us to examine responses to fine, ultrafine, and coarse concentrated ambient particles (CAPs), in downtown Toronto, Canada. To gain insight into these responses, cardiovascular outcomes in the proposed study will include not only our more established physiologic outcomes (brachial artery diameter and blood pressure), but also complementary measurements, including cardiovascular hemodynamics, autonomic function (e.g., HRV), markers of systemic inflammation (e.g., CBCs, IL-6, CRP), and endothelial dysfunction (endothelins).

Experimental Approach: We propose to expose 50 healthy adults to fine, ultrafine, and coarse CAPs and particle-free (filtered) air. Each participant will receive four exposures in random order, separated by at least 2 weeks. Cardiovascular outcomes will be measured both pre-, post- and 24 hrs post-exposure and will include measures of brachial artery diameter, flow- and nitroglycerin-mediated dilatation by ultrasonography; stroke volume (SV) and cardiac output (CO) by echocardiography; blood pressure (BP); and venous blood CBCs, IL-6, CRP, and endothelins. Also, during exposures, continuous measurements of beat-to-beat arterial BP by Finometer monitor, including calculated determinations of SV, CO and systemic vascular resistance; and HRV using 24-hr Holter monitoring will be performed. PM exposures will be characterized for particle mass, number, diameter, size, and composition (inorganic ions, trace metals, organic and elemental carbon, and black carbon). Gaseous co-pollutants (carbon monoxide, CO_2, NO, NO_2, SO_2, O_3), temperature and humidity will be monitored continuously during the exposure experiments. In addition, on the days before and after exposures, 24-hr measurements will be conducted for each participant using a multipollutant personal sampler. For each of the observed biological effects, repeated measures ANOVA models will be employed to assess differences among treatments. These models will contain a random effect for subject and a categorical variable for the four exposure treatments (fine, ultrafine, coarse, CAPs, and particle-free air). To assess exposure-response relationships between biological outcomes and CAPs mass or individual components concentrations, single pollutant analyses will be conducted in which a separate linear mixed regression model will be used for each exposure parameter. These models will use biologic response as the dependent variable, subject as a random effect, and either particle mass, number, diameter or component as the exposure metric in the model. Hierarchical linear models will be developed to account for the multiple levels of data, including measurements taken at different time points within an exposure, for a subject.

Expected Results: We expect to find physiologic responses consistent with vascular narrowing (in- creased BP, decreased brachial artery diameter) in response to all three CAPs size fractions, as compared to particle-free air. Also, we expect that the cardiovascular responses may vary by CAP treatment (fine, ultrafine, and coarse).

Project 4: Assessing Toxicity of Local and Transported Particles Using Animal Models Exposed to CAPs

John J. Godleski, Beatriz Gonzalez-Flecha, and Joy E. Lawrence
Harvard School of Public Health, Boston, MA

EPA Grant Number: R832416-010

Project Summary:

Objectives/Hypotheses: Our laboratory has pioneered the development of the ambient particle con- centrator as a means to carry out inhalation toxicological assessments of responses to ambient particles. Normal and compromised animal exposures to concentrated air particles (CAPs) in Boston have produced consistent and reproducible findings of biologic importance. The specific objectives of this project are to: (1) differentiate the cardiovascular effects of locally emitted particles from those of transported particles using normal animals; and (2) determine whether spontaneously hyper- tensive rats, a genetically susceptible population, have enhanced vascular responses to exposures of different particle sources as compared to normal animals

Experimental Approach: To differentiate the toxicological effects of locally emitted and transported particles on important cardiovascular outcomes, short-term animal exposures to CAPs will be conducted during the time periods of 6:00-10:00 a.m. and 11:00 a.m.-3:00 p.m. Starting inhalation exposures at 6:00 a.m. before significant vertical mixing takes place will allow us to capture particles mostly from local sources. In contrast, exposures starting at 11:00 a.m. will be relatively more enriched in transported particles. All outcomes will be assessed in relation to those of filtered air (sham) exposures as well as those of positive controls using particles of known toxicity at both time periods to control for circadian variations. Animal exposures will be characterized using continuous measurements of particle mass, size, number, and black carbon, as well as integrated measurements of particle mass, sulfate, elements, and organics. Specific outcome measurements will include: indicators of pulmonary and systemic inflammation, blood pressure, endothelin-1, endo- thelial nitric oxide synthase, atrial naturetic peptide, *in vivo* oxidant responses in the heart and lung, and quantitative morphology of lung and cardiac vessels. Statistical analyses will use multi-way ANOVA to assess differences among exposure groups and interactions of exposure and potential effect modifiers. Regression techniques will be used to examine dose-response relationships between measured biological outcomes and particle source contributions as reflected by particle composition. Multiple linear regression using tracer elements will be used to assess the independent effects of multiple pollution sources.

Expected Results: This proposal offers the unique application of novel techniques to improve under- standing of the effects of specific sources of particles and mechanisms of health effects. This combination of exposure scenarios and pulmonary and cardiovascular outcomes will provide new data to assess the effect of specific particle sources on specific mechanistic pathways by which ambient air particles produce adverse health effects.

Project 5: Toxicological Evaluation of Realistic Emission Source Aerosol (TERESA): Investigation of Vehicular Emissions

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Harvard School of Public Health, Boston, MA

EPA Grant Number: R832416-010

Project Summary:

Objectives/Hypotheses: This project will investigate the relative toxicity of primary and secondary particulate emissions from motor vehicles. The specific objectives of this project are to: (1) determine whether exposures to fresh and to photochemically aged emissions induce cardiovascular responses in normal and spontaneously hypertensive rats; (2) determine whether atmospheric photochemical processes enhance the toxicity of vehicular emissions; and (3) provide toxicological results to compare with those found in previous TERESA studies of coal power plants, as well as current and previous studies of concentrated ambient par- ticles.

Experimental Approach: To provide the best representation of fleet emissions, the ventilation stack from an urban highway tunnel within the metropolitan area of Boston, MA, will be used as the source of primary vehicular emissions. The mixture of primary particles and gases will undergo photochemical oxidation to form secondary particle matter. Simulations of different types of atmospheres will be conducted, including oxidizing, and oxidizing followed by neutralizing. Five different animal exposure scenarios will be used, including filtered air, primary gas and particle emissions, primary plus secondary particles, primary plus neutralized secondary particles, and secondary particles formed in the absence of primary particles. Normal laboratory rats exposed using each of the five scenarios will be evaluated for pulmonary, systemic, and cardiovascular effects using *in vivo* organ chemiluminescence, histopathology, bronchoalveolar lavage, blood cytology, and continuous measurements of cardiac and pulmonary function. The scenarios generating the largest and the least biological response will be further investigated using spontaneously hypertensive rats using the same biological outcomes studied in normal rats. Exposure atmospheres will be chemically and physically characterized for CO, NO_x, CH_4, NMHC, O_3, particle count, mass and size distribution, black carbon, elemental and organic carbon, trace elements, inorganic ions, NH_3, HCHO, and VOCs.

For the biological effects observed during each exposure scenario, inter-group differences will be assessed using multi-way analysis of variance. To determine the effect of particulate matter composition on biological response, linear regression models containing exposure concentrations as predictors will be fitted to each response outcome measure. Multiple pollutant linear regressions will be used to assess the independent effects of multiple pollution components on biological response.

Expected Results: The proposed study will provide information on the relative toxicity of primary and secondary particles from mobile source emissions, and whether susceptible populations have greater responses to exposure to these mobile source particles. The findings of this study will complement the findings of previous TERESA studies of primary and secondary particles from coal power plants, and studies of Concentrated Ambient Particles.

Southern California Particle Center

John R. Froines, Director
University of California, Los Angeles, CA

EPA Grant Number: R832413-010

Center Overview:

Objective: The overall objective of the Southern California Particle Center (SCPC) is to investigate the underlying mechanisms that produce the health effects associated with exposure to particulate matter (PM), and to understand how toxic mechanisms and resulting health effects vary with the source, chemical composition and physical characteristics of PM.

Experimental Approach: We have assembled a team of highly respected researchers committed to developing a strong multidisciplinary program to address the challenging public health issues posed by PM pollution, with a focus on the unique urban setting of the Los Angeles air basin. Our research projects are united by a hypothesis that chemical composition and physical characteristics related to PM sources determine the toxicity and exposure-response of PM. Variations in exposure according to source, season, and location influence the resulting human health responses. The principal mechanistic hypothesis of the SCPC is that many health effects associated with PM exposure, including adverse respiratory and cardiovascular outcomes, derive from the induction of oxidative stress by reactive chemical species (organic or inorganic) in PM. Oxidative stress and inflammatory responses related to asthma and atherosclerosis are a particular focus. Integral to our toxicological hypothesis is the role of antioxidant defense pathways that protect against the pro-inflammatory effects of PM.

State-of-the-art portable particle concentrator technologies, deployed and refined over the previous 5 years of the SCPC, will be used for concentration and collection of ambient PM at sites selected to reflect the influence of major PM sources. Particle size distribution, chemical composition and airborne concentrations of resulting samples will be analyzed and coupled with source apportionment studies using chemical mass balance methods. Collected particles will be characterized in an extensive array of *in vitro* toxicology studies, including chemical reactivity studies, to investigate redox potential and early biological responses associated with the chemical constituents of PM. Our concentrators will be used to carry out dose-response studies of ambient PM toxicity in normal, sensitized and genetically susceptible animal models of asthma and atherosclerosis, to rigorously investigate biological mechanisms of PM-induced airway and vascular inflammation and disease exacerbation. In a susceptible human population (elderly persons with coronary heart disease), we will examine circulating biomarkers of exposure, oxidative stress, systemic inflammatory responses, and cardiovascular parameters to investigate the hypothesis that oxidative stress is a factor in cardiovascular outcomes associated with PM exposure. Potential modifying factors including genetic polymorphisms will be examined. Data from all projects will be pooled for evaluation of inter-relationships between particle data, chemical and toxicological endpoints, and human biomarkers. Joint analysis will produce conclusions from the Center's research that reach beyond the findings of individual projects.

Expected Benefits: The research proposed by the SCPC will provide extensive characterization of chemical composition, activity, and toxicological potential of a wide variety of ambient PM, including samples collected at freeways, air and sea ports, indoors and outdoors under varying climatological conditions. Results from the toxicological studies will provide a basis for identifying the important chemical and physical characteristics of PM sources that raise the greatest concern for public health in terms of the potential to induce oxidative stress and related health effects. Our work will examine specific mechanisms involved in induction and exacerbation of asthma and atherosclerosis, leading to a greater understanding of causal pathways along the continuum from particulate source through exposure to response. From a policy-setting perspective, the exposure-response relationships that emerge from the toxicological and human studies, in relation to a variety of exposure measures, including size, chemical composition, and chemical reactivity of PM, may be especially relevant. Our panel study of CHD patients and the work with genetically altered murine models will generate insights on the factors controlling susceptibility to PM-induced adverse effects that will prove useful in the interpretation of other studies, protection of public health, and the design of future research. The human study provides information on particle sources and characteristics that affect intermediate endpoints in the progression of atherosclerosis and in acute changes in CV function and thrombosis. We also anticipate the development of new biomarkers for use in future studies. Overall, knowledge of the relative reactivity and resulting toxicity of atmospherically processed PM from key sources, and identification of exposure levels associated with specific toxic endpoints and health effects will provide critical input to the development of effective emission reduction strategies, especially in targeting those strategies to result in the greatest benefit to public health.

Southern California Particle Center
Overall Objectives

Overview of the Southern California Particle Center: The overall objective of the Southern California Particle Center (SCPC) is to bring together outstanding scientists to conduct high-priority research to elucidate the underlying basis for health effects associated with exposure to ambient particulate matter (PM). The SCPC makes use of an integrated approach to address the issues of exposure, dosimetry, toxicology, and epidemiology identified in the EPA's RFA and the Reports of the National Research Council on Particulate Matter. The strengths of the investigators in this Center and our demonstrated record of progress, the powerful assortment of equipment available and the unique characteristics of the Los Angeles Basin airshed (LAB) taken together are key factors in why Southern California provides a particularly attractive environment and opportunity for PM research studies.

We have assembled a team of highly respected researchers committed to developing strong multidisciplinary programs to address the challenging public health issues posed by PM pollution. Principal investigators in this application include Drs. Constantinos Sioutas, Andre Nel, and Arthur Cho who were research leaders in the Center during the past 5 years. Several investigators recognized as being leaders in their disciplines have been added to the Center, including Dr. Jack Harkema, Dr. James Schauer, and Dr. Ralph Delfino. Dr. Harkema has recently collaborated with Center investigators (EPA STAR grant), and brings his state-of-the-art trailer for *in vivo* animal studies. Dr. Schauer is well known for his work on source apportionment and replaces his mentor, Dr. Glen Cass, whose untimely death left a gaping hole in air pollution research. Our collaboration with Dr. Delfino was initiated with recent funding for a panel study of elderly cardiovascular disease patients. Each investigator brings a wealth of talent and diverse resources to the Center.

An extraordinary array of resources is available to the SCPC, including two particle instrumentation trailers for PM characterization in the field, two animal trailers and a van for *in vivo* studies throughout Southern California, and an enormous range of equipment. These resources are available as a result of funding from the U.S. EPA Particle Center grant (1999-2005), an EPA Supersite contract (2000-2005), a major research contract with the California Air Resources Board (CARB) for the design, construction, and implementation of research using mobile airborne particulate concentrators (coarse, fine and ultrafine), and funding from the South Coast Air Quality Management District (SCAQMD) for research and development relevant to asthma and air pollution. We believe that the Center's available resources for PM research, described in detail in the Administrative Core document, are unparalleled.

The LAB has been described as the most polluted airshed in the Nation, with complex, persistent, and unique PM. Covering approximately 12,000 square miles, the population is projected to reach 19 million by the year 2020, a 1.5-fold increase from 1990 levels. Vehicle miles traveled are increasing at an even greater rate and the LAB continues to exhibit the most severe ozone and PM air quality problems in the United States. PM is emitted from numerous sources in the basin, including millions of motor vehicles, nearly 300,000 diesel trucks, the Nation's largest marine port, expanding airports, and tens of thousands of factories all putting into the atmosphere primary PM and reactive gases that act as particle precursors for significant secondary aerosol formation. In contrast to other regions of the United States, PM pollution in the LAB is less linked to emissions of sulfur compounds, due to low sulfur fuels and the absence of coal-fired power plants.

The topography and climate of the LAB contribute to the area's high air pollution potential. Several studies, including those of our Southern California Supersite, have shown that the LAB comprises microclimates of distinct meteorological conditions, which result in substantial spatial variations of PM including components of toxicological interest and co-pollutants. In contrast, $PM_{2.5}$ are more uniformly distributed in metropolitan areas in the eastern United States, where a large fraction is regional. Los Angeles' climate also results in a predictable and well-defined meteorology and is suitable for "controlled" laboratory-style experiments using PM as the test aerosol. Afternoon sunlight, persistence of fog and low clouds trigger atmospheric reactions that form secondary particles. The LAB is therefore a unique environment in terms of the composition and sources of PM, local meteorology, the potential health issues that may arise from continued population growth, and the opportunity to make use of existing data sources collected from earlier studies, including those conducted by our Center and the local air district. PM generated in the LAB contrasts with other regions in the United States and provides an opportunity to conduct comparative studies with other PM Centers located elsewhere with differing airsheds, industrial makeup, and meteorology.

The first 5 years of the SCPC were marked by considerable progress, and we plan to build upon our accomplishments. When the SCPC was created, it brought together investigators with distinguished histories in air pollution research who had not previously worked together in an integrated, multidisciplinary research effort. Five years later, participants have established a history of successful research interactions. Selected important accomplishments include:

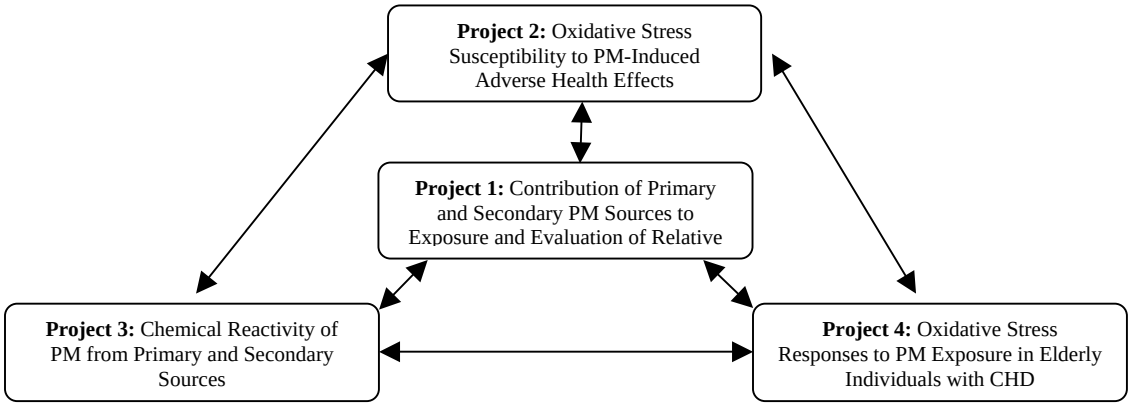
- The most extensive chemical characterization of PM and intensive monitoring studies in the LAB ever conducted.
- Development of continuous monitors for size-fractionated particle mass and chemical composition, and mobile, size-selective PM concentrators capable of collecting large amounts of ambient samples for *in vitro*, *in vivo* and human clinical studies.
- Characterization of the formation and dynamics of PM near freeways.
- Development of chemical assays of redox and electrophilic activity and demonstration of the significant redox activity of PM, particularly ultrafine particles.
- Demonstration of mitochondrial uptake of ultrafine particles and related toxicity.
- Demonstration that pro-oxidative chemicals induce hierarchical oxidative stress effects and development of murine *in vivo* models for induction of acute airway inflammation.
- Demonstration of *in vivo* allergic airway responses, neurological and cardiovascular effects in close proximity to a freeway.
- Exposure of healthy and impaired human volunteers to concentrated PM; first CAP exposure studies of human volunteers to coarse and ultrafine particles.
- Demonstration of a linkage between traffic density and human developmental toxicity.
- Demonstration of the relationship between traffic density (at schools and homes) and primary respiratory outcomes in the CHS cohort.

- Publication of over 70 refereed articles describing the above studies and their findings.

- Based on the success of the SCPC, the SCAQMD awarded significant funding to establish the Southern California Consortium on Asthma and Outdoor Air Quality.

SCPC Research: The EPA RFA defines four specific priority research topics: source linkages, susceptibility to PM**,** biological mechanisms for PM**,** and exposure-response relationships. The studies to be undertaken in the SCPC address these four research priorities, within the context of a primary Center theme, and build upon the successes of the first 5 years. The proposed research will be integrated across disciplines, including aerosol formation and characterization, advanced analytical chemistry, exposure assessment, chemical toxicology, genetic toxicology and immunology, animal toxicology, epidemiology and biostatistics. During our first 5 years, linking the diverse research efforts into a coordinated whole was an overarching theme and we once again stress a fundamentally integrated approach to the research effort. Below is a chart that outlines the theme and Projects of the Center.

Overall Theme of the SCPC: Establishing the linkage between PM source emissions, underlying mechanisms, and resulting health effects.



Project 1: While all four projects proposed to be undertaken by the SCPC are inter-related, Project 1 is central, based on its hypotheses that chemical composition and physical characteristics related to sources have crucial relevance to the toxicity and exposure-response characteristics of PM and that variations in exposure according to source, season, and location influence the eventual human health response. The other Projects build upon these hypotheses, and Project 1 is an integral part of Projects 2, 3 and 4, by serving as the field operations for concentrating ambient PM for toxicity testing and animal exposure studies. The extensive PM characterization at sites relevant to the Project 4 human population study will have direct relevance to the interpretation of the toxicological and health outcomes in that study.

To achieve their primary objective of examining the relationships between PM sources, exposure and toxicity, Drs. Sioutas and his colleagues will conduct extensive sampling at major PM sources in the LAB with a range of temporal and geographical characteristics, using the mobile, size-selective particle concentrators developed by Dr. Sioutas. Several locations have been selected to overlap with the Children's Health Study (CHS). Indoor sources are included as a study of non-volatile vs. semi-

volatile PM. The PM collected from a range of sources will be extensively characterized for chemical composition, size, and airborne concentration. Source apportionment by chemical mass balance methods will involve analysis of defined source tracers. A particular focus of this research will be the determination of how the toxicity, composition, and exposure to the multiple sources varies from source to receptor areas as the aerosol ages and undergoes atmospheric chemistry to learn more about the impact of atmospheric processes in relation to toxicological outcomes. In addition to mobile vehicle sources and wood combustion, we will expand our understanding of the potential conesquences of exposure to PM from major sources in the LAB, which have received inadequate attention including air and marine ports.

Projects 2 and 3: Projects 2 and 3 propose toxicological research that seeks to: (1) identify the important chemical/physical characteristics of PM in relation to a wide array of toxicological endpoints; (2) identify physiological, cellular, biochemical, and molecular mechanisms that explain specific health effects associated with exposure to PM and/or specific PM components with particular reference to the mechanism(s) of PM-induced asthma and atherosclerosis exacerbation; (3) investigate whether a failure of appropriate antioxidant defense could constitute the basis of PM susceptibility; (4) assess the dose-dependent exacerbation of allergic airway inflammation and aortic atherosclerotic lesions by concentrated PM; and (5) study the intracellular disposition and toxicokinetics of PM and its chemical components.

Our principal mechanistic hypothesis for the toxicological research is that PM-induced oxidative stress initiates airway and arterial wall inflammation due to reactive chemical species. These chemical species can be organic or inorganic and act through several possible chemical reactions with biological substrates; we are focusing on redox and electrophilic reactions. We propose that the biological response to oxidative stress is a hierarchical event, in which the induction of antioxidant defense at the lowest tier (Tier 1) of oxidative stress protects against the pro-inflammatory (Tier 2) and cytotoxic effects (Tier 3) of higher levels of oxidative stress. Integral to this hypothesis is that a weakened antioxidant defense may define disease susceptibility. This hypothesis has relevance to the study of both acute and chronic health endpoints.

Chemical assays developed during the first 5 years of the Center will be employed to study the quantitative formation of catalytically generated reactive oxygen species (ROS) and to determine the magnitude of electrophilic activity (Project 3). *In vitro* studies will examine the intracellular activity of PM in relation to ROS generation and oxidative stress, using a variety of assays. The importance of the PM matrix in relation to the adsorbed components will be analyzed with extraction techniques as well as the use of synthetic particles. A series of *in vivo* studies will rigorously investigate the mechanistic details of the health effects of PM linked to oxidative stress. We will use normal and genetically susceptible murine models to study PM-induced exacerbation of asthma and atherosclerosis. These experiments will seek to demonstrate that exposure to ambient PM, especially fine and ultrafine particles: (1) lead to asthma and atherosclerosis exacerbation in susceptible murine models; (2) lead to airway and arterial wall inflammation as a result of oxidative stress; (3) act in a dose-dependent manner; and (4) differ in their relative potency due to differences in their content of redox cycling organic chemicals.

The experiments to be conducted in Projects 2 and 3 will couple *in vitro* assays with *in vivo* experiments, carried out at a range of exposure concentrations. The findings will enable us to determine the strongest statistical predictors of *in vivo* outcomes in murine models, using *in vitro* and chemical assay results as exposure metrics of biological activity.

Project 4: The overall goal of Project 4 is to advance knowledge on the importance of particle size and composition to the induction of oxidative stress and systemic inflammatory responses that may be responsible for observed cardiovascular outcomes in epidemiological time series studies of PM exposure. The design is a repeated measures panel study of high-risk elderly people with coronary heart disease (CHD). Aspects of this panel study have been previously funded; SCPC activities will add critical data to the exposure assessment, measure biomarkers of oxidative damage, and develop a genetic susceptibility component for the study.

Intensive exposure assessments are planned that include indoor and outdoor home PM mass, number concentration, and particle composition. Accumulation and ultrafine mode PM will be extracted to measure concentrations of transition metals and tracer compounds for use in apportioning PM exposures to specific sources, including vehicular emissions, photochemical activity, cooking, and wood smoke.

Circulating biomarkers indicating systemic oxidative stress responses will be measured. Changes in these biomarkers are expected to be associated with cardiovascular outcomes and with the inflammatory biomarkers measured in the parent National Institute of Environmental Health Sciences study. The panel study will be coupled with Project 2 through the study of PM-induced oxidative modification of LDL and HDL, leading to altered pro-inflammatory and anti-inflammatory properties, respectively. The association between biomarkers of oxidative stress and exposure to a variety of PM measures, including elemental and organic carbon, and specific metals and organic components used as source tracers will be ascertained. Statistical analysis of biomarker associations with ROS and electrophilic activity provides a direct linkage to Project 3. In addition, genetic susceptibility to oxidative damage will be explored, by genotyping each study subject for polymorphisms in genes likely to be involved in oxidative stress responses.

Coordination and Feedback: The research results developed in each project will be pooled to enable evaluation of the interrelationship between chemical and toxicological endpoints, including cardiovascular biomarkers. A general statistical approach to the overall Center data has been developed to assure consistent data analysis, and is described in the administrative core section of the proposal. The pooled results will be used to develop conclusions from the overall Center efforts and will enable design of studies to extend and clarify the initial studies. This feedback mechanism will be a central feature of the SCPC.

During the first 5 years of the SCPC, we worked closely with investigators from the CHS, a large population-based cohort study.

Expected Benefits: The research proposed will provide extensive data on the chemical and biological activity of a wide variety of PM types, including PM from freeways, air and seaports, indoor and outdoor PM, aged PM, and semi-volatile PM under varying conditions. PM samples will be subjected to extensive state-of-the-art chemical analysis, and evaluated for oxidative potential in a number of assays, enabling characterization of PM well beyond traditional mass based measurements and limited compositional analysis. The extensive database generated from the Projects together will enable analysis of the relationships among source, chemical composition, and size of PM in comparison with chemical assay reactivity and toxicological activity *in vitro* and *in vivo*. Research of this scope and magnitude is possible because of resources available to the Center from its first 5 years of activity and potential new support.

At the conclusion of our research we will have: (1) characterized the physical/chemical characteristics of the wide range of sources and conditions in the LAB; (2) determined the toxicological potential of PM from the respective sources and the importance of varying physical/chemical characteristics, with special attention to fine and ultrafine particles; (3) developed insights into the underlying mechanism of PM toxicity in relation to its chemical components and physical makeup; (4) identified exposure-response relationships for toxicity in relation to a range of parameters; (5) identified the importance of the PM matrix relative to the toxicity of individual components; and (6) determined the role of susceptibility through *in vivo* studies using genetically altered murine models and a panel study of elderly persons with CHD. We will determine the efficacy of the chemical assays for rapid, quantitative characterization of the oxidant activity of a range of sources to supplement to other dose metrics for determining exposure-toxicity relationships. Knowing the relative toxicity of atmospherically processed PM emissions from key sources will support targeted and effective regulatory strategies.

Project 1: Contribution of Primary and Secondary PM Sources to Exposure and Evaluation of Their Relative Toxicity

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EPA Grant Number: R832413-010

Project Summary:

Objectives: The primary objective of Project 1 is to examine the relationships between particulate matter (PM) sources, exposure, and toxicity within the constraints of the urban atmosphere. This project is an integral part of Projects 2, 3, and 4, by serving as the field operations to collect PM samples for toxicity testing and for providing elevated levels of ambient PM for animal exposure models described in these projects.

Experimental Approach: State-of-the-art portable particle concentrator technologies, deployed and refined over the previous 5 years of the Center, allow for concentration and collection of ambient PM at multiple sites throughout the Los Angeles Basin. These sites will be purposely chosen to reflect areas impacted by the different major outdoor and indoor sources in Southern California. As will be discussed later, most of the study locations also will be sampling sites that have served the University of Southern California Children's Health Study (CHS), providing continued linkage to that study. Advanced chemical analysis and source apportionment techniques will provide a quantitative characterization of the PM as well as the source contributions at each sampling site. In addition, state-of-the-art technologies will provide a method to measure the toxicity of PM components, including chemistry and volatility. The central hypothesis of Project 1 is that particle characteristics, which can be related to sources in terms of size and composition, determine the toxicology of PM, and variations in exposure to these characteristics according to source, season, and location influence the eventual human health response. The project consists of the following specific aims to: (1) determine the physical and chemical properties of atmospherically processed PM emissions from real-world sources, including secondary formation, to evaluate how exposure to PM and the toxicity of PM from these sources vary with respect to location, season, and particle size, and in conjunction with Projects 2, 3, and 4 to assess their relative toxicity; (2) assess the contributions of these outdoor sources to indoor exposure and toxicity; (3) determine the physical, chemical, and toxicological characteristics of the volatile and non-volatile particle components that originate from mobile sources; and (4) measure the exposure gradients and intra-community variability of PM in complex urban areas, affected by a multitude of sources, including unstudied sources such as airports and port activities.

Expected Results: This project will provide valuable insight into the source-exposure-response con- tinuum outlined by the National Research Council (NRC, 2004) by developing fundamental understanding on the links between specific sources and adverse toxicological outcomes associated with exposure to PM. The information generated by this project will serve as the basis for linking emissions to local air quality and ultimately to health effects. By providing a wide range of exposure parameters for the toxicological studies described in Projects 2, 3, and 4, the toxicity of different sources and exposure scenarios can be assessed. Knowing the relative toxicity of atmospherically processed PM emissions from real-world sources will allow for more targeted and effective regu-

latory strategies. These data on which PM sources are the most toxic, combined with detailed chemical and physical characterization of PM from these sources will allow for a narrower, more focused effort in identifying the biological mechanisms of PM health effects (as described in Projects 2, 3, and 4).

Project 2: The Role of Oxidative Stress in the Susceptibility to PM-Induced Adverse Health Effects

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EPA Grant Number: R832413-010

Project Summary:

Objectives: The primary objective is to elucidate the mechanisms of PM-induced asthma and atherosclerosis exacerbation. Our principal hypothesis is that PM-induced oxidative stress initiates airway and arterial wall inflammation. We propose that the biological response to oxidative stress is a hierarchical event, in which the induction of antioxidant defense at the lowest tier (Tier 1) of oxidative stress protects against the pro-inflammatory (Tier 2) and cytotoxic effects (Tier 3) of higher levels of oxidative stress. Integral to this hypothesis is the proposal that a weakened antioxidant defense may define disease susceptibility.

Experimental Approach: In Aim 1, we will use normal and genetically susceptible murine models to study the role of oxidative stress in PM-induced exacerbation of asthma and atherosclerosis. We will use low grade OVA sensitization to study the effects of fine and ultrafine particles (UFP) on allergic airway inflammation, oxidative stress, IgE production, mucus hypersecretion, and airway hyperreactivity (AHR) in a BALB/c model. We will use Nrf2 knockout mice, with a weakened antioxidant response, to determine whether this will enhance airway inflammation. A third component of this Aim will be to use atherosclerosis-prone apoE knockout mice to assess dose-dependent atherogenesis and oxidative modification of LDL and HDL during CAPS exposure. In Aim 2, we will use *in vitro* toxicology approaches to assess the effects of various PM sources, with unique and varying chemical composition, on the induction of oxidative stress and inflammatory responses in tissue culture macrophages, epithelial and endothelial cells. This study will use coarse, fine, and UFP, collected at different sites and during different seasons (Project 1), to determine their effects on: (1) phase II enzyme expression by Western blotting and real-time PCR (Tier 1); (2) cytokine and chemokine expression as determined by ELISA assays and protein arrays (Tier 2); and (3) perturbation of mitochondrial function and induction of apoptosis as determined by flow cytometry and functional studies on isolated mitochondria (Tier 3). These biological responses will be compared to the chemical composition of the particles (Project 1), their activity in the chemical reactivity assays (Project 3), and their ability to promote asthma and atherosclerosis in animal models. In Aim 3, we will use serum samples, collected from indoor-exposed elderly human subjects with ischemic heart disease (Project 4), to determine how oxidative modification of HDL affects its anti-inflammatory and atheroprotective effects. We will assess how the increase in oxidized phospholipids in LDL affects its pro-inflammatory effects in an endothelial co-culture assay. We will determine whether oxidative modification of HDL-associated paraoxonase activity modifies its anti-inflammatory effects in this assay.

Expected Results: We expect that due to the presence of redox cycling chemicals, ambient PM induce a series of pro-oxidative and pro-inflammatory effects, which enhance asthma and atherosclerosis. We expect that these effects will be related to particle dose, size, source, composition, and season, and will be exaggerated in individuals and animals with a weakened antioxidant defense. This study will yield important biomarkers that will be linked to specific toxicological components that could be monitored to prevent adverse health effects.

Project 3: Chemical Reactivity of Particulate Matter From Primary and Secondary Sources, and Modification of Chemical and Cellular Properties of PM-Associated Components by the Particle Matrix

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[3]University of Tsukuba, Tsukuba, Japan

EPA Grant Number: R832413-010

Project Summary:

Objectives: The overall objectives of Project 3 are to: characterize ambient particulate matter (PM) samples from a variety of PM sources collected in Project 1 according to their potential to induce redox chemistry and oxidative stress in biological tissues; apply new measures of PM exposure that determine redox activity and thiol depletion; study particle matrix and size effects on the cellular uptake, intracellular disposition, and biotransformation of particles and selected adsorbed components; and characterize chemical interactions in PM with respect to reactivity and cellular effects. The central hypothesis of this project is that many adverse health effects associated with PM exposure derive from the induction of oxidative stress, caused by reactive oxygen species generation or the depletion of thiols. Our previous studies have focused on the chemical basis for these actions (i.e., the chemical reactivities of PM that could cause these processes). We have developed a redox assay that determines the catalytic capacity of PM to generate superoxide and have used it to characterize PM from different sources throughout the Los Angeles Basin (LAB).

Experimental Approach: In the next phase of this research, we plan to apply the redox assay and two other chemical assays, one of which determines the reaction with thiols, to assess differences in chemical reactivity among major source types, season, and size fraction in PM samples from the LAB. These differences will be analyzed in terms of the chemical constituents found in Project 1 and used to interpret the toxicological findings from Projects 2 and 4. We shall investigate the quantitative relationship between ROS chemistry and intracellular measures of oxidative stress and cellular toxicity. In a second component of the Project, we will study the interaction between carbon black particles and various adsorbed compounds to determine the effect of the particle matrix on chemical and biological activity. These studies are based on our observations that demonstrate residual redox activity in diesel exhaust particles after extractions and those of others showing differences in cellular toxicity of organic compounds when they are adsorbed onto particles. We will establish an experimental system of carbon black particles of varying dimensions to which selected organic and inorganic species are adsorbed. The chemical and biological properties of the particles will be determined, and the effects of the matrix on the actions of the adsorbed species will be assessed.

Expected Results: This project will characterize ambient PM samples from key sources according to their reactivity in redox and electrophilicity assays, and in association with toxicological findings, will provide a basis for identifying PM of the greatest concern for public health in terms of potential to induce oxidative stress and related health effects.

Project 4: Oxidative Stress Responses to PM Exposure in Elderly Individuals With Coronary Heart Disease

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University of California, Irvine, CA

EPA Grant Number: R832413-010

Project Summary:

Objectives: The overall goal of this study is to advance knowledge on the importance of particle size and composition to the induction of oxidative stress responses in a high-risk population of elderly people with coronary heart disease (CHD). We hypothesize that biomarkers of oxidative stress responses will be associated with indoor and outdoor home particulate matter (PM) mass and total particle number concentration, which will support the view that PM leads to systemic inflammatory responses. We further hypothesize that biomarkers will be more strongly associated with predicted indoor exposure to PM of outdoor origin (from source tracer analyses). We also will evaluate effects of exposure to specific metals, elemental and organic carbon, and specific organic components used as source tracers. We further hypothesize that biomarker associations with ultrafine and fine PM will be better explained by chemical assays that measure reactive oxygen species (ROS) and electrophilic activity.

Experimental Approach: We will conduct a study of repeated measures to evaluate the relationship between circulating biomarkers of oxidative stress responses and exposure to PM in elderly subjects with CHD. Biomarkers will include: oxidized glutathione (GSSG), reduced glutathione (GSH), an F_2-isoprostane biomarker of lipid peroxidation (8-iso-PGF2α), extracellular superoxide dismutase (SOD) activity, and erythrocyte SOD and glutathione peroxidase 1 activities. The balance of capacity and stress will be represented by the ratio GSH/GSSG, which is expected to decrease with higher PM exposures, while 8-iso-PGF2α, and SOD and GPx-1 activities will increase. Changes in these biomarkers are expected to be associated with cardiovascular outcomes and inflammatory biomarkers measured in the parent study funded by National Institute of Environmental Health Sciences. Subjects will include 72 nonsmokers age 65 and older living in retirement homes in areas of the Los Angeles air basin with high concentrations of both freshly emitted and aged PM. Each subject will be followed with blood draws for biomarkers at the end of each of 12 weeks (864 person-days of observation). This intensive followup will be spread across 240 monitored days over 2 years, and include in each year a period of high photochemical activity and a period of high air stagnation to enhance contrasts in PM composition, number, and size distribution. Intensive exposure assessments will be made at indoor and outdoor home sites, including methods described under Projects 1 and 3. Data will be analyzed with the general linear mixed model controlling for temporal trends, study site, weather variables, as-needed or inconsistent medication use, respiratory infections and key clinical and subject characteristics. We also will evaluate whether individual characteristics that may increase susceptibility predict associations between oxidative stress biomarkers and PM exposure.

Expected Results: We expect to clarify findings in the epidemiologic literature of associations between ambient PM and cardiovascular mortality and hospital admissions. Results of this study will advance knowledge on the acute effects of ultrafine and fine particles on biomarkers of oxidative stress responses that are relevant to acute and chronic cardiovascular outcomes. Results are expected to inform policy-makers on the sources, particle components, size fractions, and concentrations that

affect key intermediate endpoints in the progression of atherosclerosis and in acute changes in cardiovascular function and thrombosis. We will advance understanding of the adverse effects of particulate air pollutants on the cardiovascular health of high-risk individuals living in ethnically diverse neighborhoods with high exposures to airborne pollutants.

Source-Specific Health Effects of Ultrafine/Fine Particles

Günter Oberdörster, Director
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EPA Grant Number: R832415-010

Center Overview:

Objective/Hypothesis: Studies are proposed to identify health hazards of source-specific physicochemical components of fine particulate matter (PM) (e.g., UFP; organics) in epidemiological, controlled clinical, animal, and *in vitro* studies; a main focus will be on characterizing fine PM and determining pathophysiological mechanisms of effects to test the hypothesis that ambient ultrafine (UF)/fine PM cause oxidative stress and trigger cardiovascular adverse health effects, with specific emphasis on events leading to endothelial dysfunction.

Experimental Approach: The Center is comprised of five Research Cores, representing a highly integrated approach linking measurement and physicochemical characterization of ambient ultrafine and fine PM (Core 1) with: (1) epidemiological findings (Core 2) from panel studies in susceptible populations, including diabetics, MI patients and patients with genetic susceptibility; (2) cardiovascular responses seen in controlled clinical exposures (Core 3) of healthy and diabetic subjects exposed to concentrated ambient UF/fine PM; (3) cardiovascular effects observed in acute and subchronic studies in diabetic rats and CNS effects in a mouse model of neurodegeneration (Core 4) following exposures to concentrated ambient UF/fine PM and on-road highway aerosols; and (4) findings from *in vitro* studies (Core 5) to evaluate underlying oxidative stress related mechanisms of injury that will explain *in vivo* findings. The Research Cores are supported by four Facility Cores: Aerosol Generation and Analysis; Vascular and Inflammation; Cardiac; and Biostatistics, which are essential elements in our coordinated research approach.

Expected Results: We anticipate to uncover mechanisms of adverse vascular and cardiac events, directly or indirectly induced by PM. These involve activation of endothelial cells and blood platelets, resulting in thrombus formation with potential fatal consequences in susceptible subjects with predisposing cardiovascular disease. We also expect to see oxidative stress responses in the CNS at sensitive target sites. Our multidisciplinary team approach will result in novel findings to be used in regulatory decision-making for protecting public health.

Rochester PM Center: Source-Specific Health Effects of Ultrafine/Fine Particles

Overall Goals: Relevance to Research and Regulation

The EPA is required to set National Ambient Air Quality Standards (NAAQS) for Particulate Matter (PM) to protect public health with an adequate margin of safety. We envision the atmospheric aerosol as a mixture of mixtures where sources (anthropogenic and biogenic) produce a complex combination of particles and gases. Some of those gaseous emissions can also be converted into particles. Thus, identification of the nature of those source mixtures that have higher toxicity could ultimately provide a basis for a new targeted approach to regulations that reduce the amounts of PM that most directly affects health rather than the current mass-based standard that is the best we can do at this time, but it likely to be only a crude indicator of the toxic particles in the air.

Our proposed Center on "*Source-Specific Health Effects of Ultrafine/Fine Particles"* will focus on the fine ($PM_{2.5}$) fraction, which includes the ultrafine particle (UFP) fraction. Our new studies will continue to identify health hazards of source-specific physicochemical components of fine PM (e.g., UFP; organics) in epidemiological, controlled clinical, animal, and *in vitro* studies; however, a new main focus will be on sources and on pathophysiological mechanisms by which ambient ultrafine (UF)/fine PM trigger cardiovascular adverse health effects, with specific emphasis on events leading to endothelial dysfunction (see Objectives/Hypothesis). Results will be crucial for risk assessment and will strengthen risk management decisions.

In Figure 1.1, we outline several steps involved in and necessary for the risk assessment and risk management process as described by NRC (1983). Our PM Center's goal is to provide the required scientific input for this regulatory process for PM by supporting all five components. We will continue to provide human and animal data for the three research steps: Hazard Identification (that is, controlled human and laboratory animal exposure studies to characterize the adverse effects of UFP at pulmonary, cardiovascular, and other extrapulmonary sites); Dose-Response assessments (*in vivo* exposure-response studies in humans and animals as well as *in vitro* dose response studies in target cells); and Exposure Assessment (evaluation of actual human exposures in our epidemiological studies and development of biological markers of exposure and effects). These three steps are a necessary input for Risk Characterization (that is, calculation of a specific risk for humans based on human clinical and epidemiological data and extrapolation from mechanistic animal and *in vitro* data) and Risk Management (establishing regulations and NAAQS, considering public health and other implications) (see Figure 1.1). This will be accomplished by a coordinated multidisciplinary team approach, which is detailed in the individual project descriptions in this application. Using real-world UF and fine PM in all phases of our studies, we will provide data that can be used to answer questions related to the fine PM standard such as: Is there a need for a $PM_{0.1}$ standard, in addition to the fine ($PM_{2.5}$) standard? If so, should the standard be expressed as particle number concentration? Does the $PM_{2.5}$ 24-hour standard need to be changed to a shorter time (e.g., 8 hr.) standard? Also, questions related to regulating emissions from specific sources are: Which sources can be identified as emitters of toxicologically important UF/fine particles? Can specific engineering devices or modifications of technical processes (step 5 in Figure 1) be designed to lower toxicologically important components of UF/fine PM from respective sources? For example, will the new 2007 particle filter trap technology for diesel engines be effective in preventing adverse health effects?

Therefore, our studies are designed to integrate detailed physicochemical analyses of ambient PM (including hourly size distribution of particle number concentrations for volatile and non-volatile fractions of ambient aerosols, particle mass for volatile and non-volatile fractions of ambient aerosols, particle bound polycyclic aromatic hydrocarbons (PAH), black smoke, particulate nitrate, sulfate, organic and elemental carbon, total oxidative capacity) with our epidemiological, clinical and toxicological studies when evaluating effects and mechanisms. While most of these studies will be performed using ambient UF/fine PM, we will also use model particles in *in vitro* and animal studies—to address specific mechanistic questions. The composition of the model particles will be based on our findings with real-world ambient UF/fine PM and on the results of source-apportionment analyses.

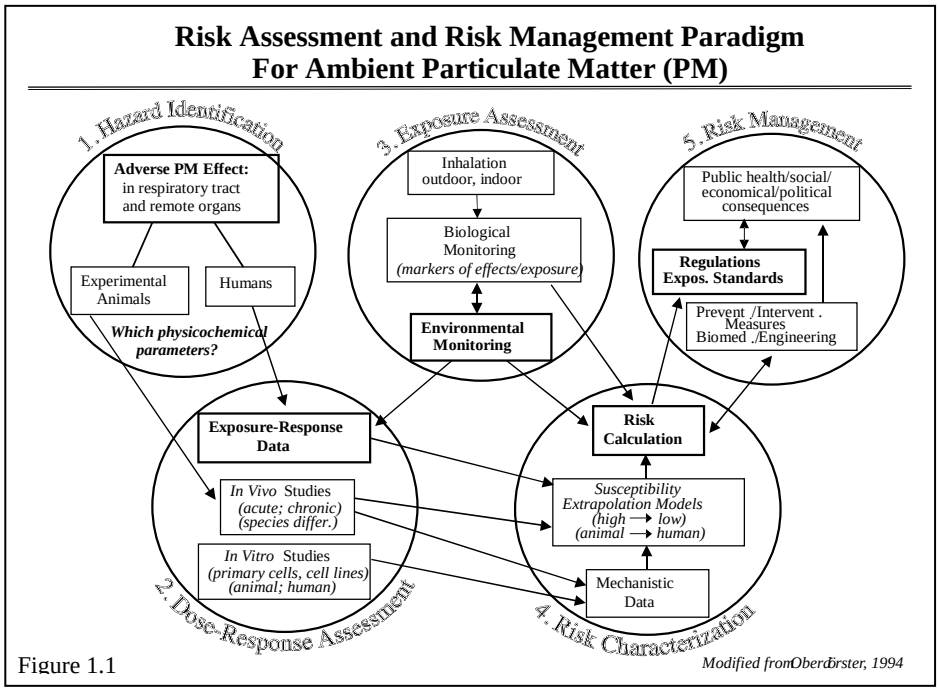

Figure 1.1

Objectives/Hypotheses

The objective of our Center's proposed multidisciplinary research is to conduct well-coordinated studies covering several aspects of the Source—Exposure—Dose—Response paradigm. Our main focus regarding health effects will not only be the respiratory tract but also extrapulmonary organ systems, such as the vascular system, the heart, and also the CNS. This focus is based on epidemiological and experimental findings, including our own, of PM effects and awareness of newer results related to the pathophysiology of endothelial dysfunction and thrombus formation associated with cardiac events in susceptible parts of the population.

We propose to study PM-induced thrombogenic mechanisms, with blood platelets and endothelial cells as key cellular elements. Their role in clot formation and their emerging role in chronic inflammation involving release of pro- and anti-inflammatory mediators such as CD40 ligand (CD154), IL-1, cyclooxygenase products like thromboxane A_2 and prostacyclin, endothelins, nitric oxide is well known; when activated, these cells release microparticles whose role in cardiovascular disease is becoming an area of intense research (Van Wijk et al., 2003; Horstman et al., 2004). Chronic inflammation is a known element in the genesis of cardiovascular disease including

diabetes. People with Type II diabetes have been identified as a susceptible group for PM effects (Zanobetti et al., 2000; Bateson and Schwartz, 2004). Most diabetics now die of cardiovascular complications. We suggest, therefore, that a susceptible population, such as diabetics, will experience increased activation of platelets and endothelial cells resulting in acceleration of the cardiovascular disease state and acute events like MI or stroke. The following paragraphs and the scheme depicted in Figure 1.2 outline hypotheses, which will be tested in our proposed PM Center studies.

Fine particles emitted from combustion processes consist of the accumulation mode (AMP) and nuclei mode particles, the latter being defined as UFP (<100 nm in diam.). Condensation of vapor phase compounds on both modes provides coating of organics on a carbon or inorganic core, which results in a time-dependent change in PM surface chemistry. Accumulation mode particles act as a sink for UFP through heterogeneous coagulation of the UF onto the larger mode particles. Atmospheric aging of fine PM may further change surface chemistry and particle-to-particle binding. Inhalation of fine PM—consisting of UFP and AMP + coagulated UFP—will result in significant size-dependent deposition of UFP and AMP in the upper and lower respiratory tract. UFP in the nasopharyngeal and tracheobronchial region can translocate along sensory neurons to the CNS and to ganglia of the autonomic nervous system (ANS) (Oberdörster et al., 2004). Dependent on the chemistry and surface binding, we hypothesize that following deposition of the accumulation mode particles by inhalation in the alveolar regions, deaggregation of some UFP occurs facilitated by the surfactant action and thereby adding to the pool of inhaled UFP which were deposited as singlets. A second conceivable scenario is that the large soluble fraction of AMP are solubilized, leaving behind coagulated poorly soluble cores of UFP; or a combination of deaggregation and dissolution processes takes place. Our planned studies will test this hypothesis in a cellular *in vitro* assay using simulated lung fluids with lung surfactant.

There is mounting evidence that UFP, once deposited in the lung, "escape" alveolar macrophage surveillance and gain access to the interstitium and the blood circulation (Oberdörster, 2004). There is further evidence that nano-sized particles, after transcytosis into pulmonary capillaries via endothelial cells, are taken up by blood platelets, the most numerous white blood cells (Berry et al., 1977). In addition, UFP can be transferred through the circulation to the liver, spleen, and bone marrow (Terashima et al., 1997; Ballou et al., 2004; Gibaud et al., 1994, 1996, 1998; Cagle et al., 1999; Bazile et al., 1992).

We suggest that the sequence of events following UFP deposition and translocation is based on particle-induced oxidative stress, cell activation, and inflammatory processes. We hypothesize that platelets become activated resulting in the release of the potent cytokine CD154 (CD40L), which in turn activates endothelial cells to express adhesion molecules and chemokines (Phipps et al., 2000). Endothelial cells may also be activated directly by translocating UFP. Activation of endothelial cells and of blood platelets results in the release of microparticles, small (<100 nm) vesicles containing cell-specific proteins, which are increased in cardiovascular diseases including angina, hypertension, diabetes, and others (Van Wijk et al., 2003; Horstman et al., 2004). Endothelial cell-derived microparticles (EMP) and platelet-derived microparticles (PMP) appear to play causal roles in these states. In fact, soluble CD40L, released by activated platelets (Phipps et al., 2000; Phipps et al., 2001) may be part of the microparticles. Activated platelets are preferentially associated with atherosclerotic plaques; it has been established that the interactions of CD40 on endothelial cells with CD154—on platelets or released by platelets—are involved in the pathogenesis of atherosclerosis (Phipps, 2000). The activated platelets also release thromboxane A_2 (TXA_2), which has thrombus-forming and vasoconstrictive properties. The balance between TXA_2 and prostacyclin (PGI_2, released by endothelial cells and inducing blood vessel dilatation and inhibiting platelet aggregation) is

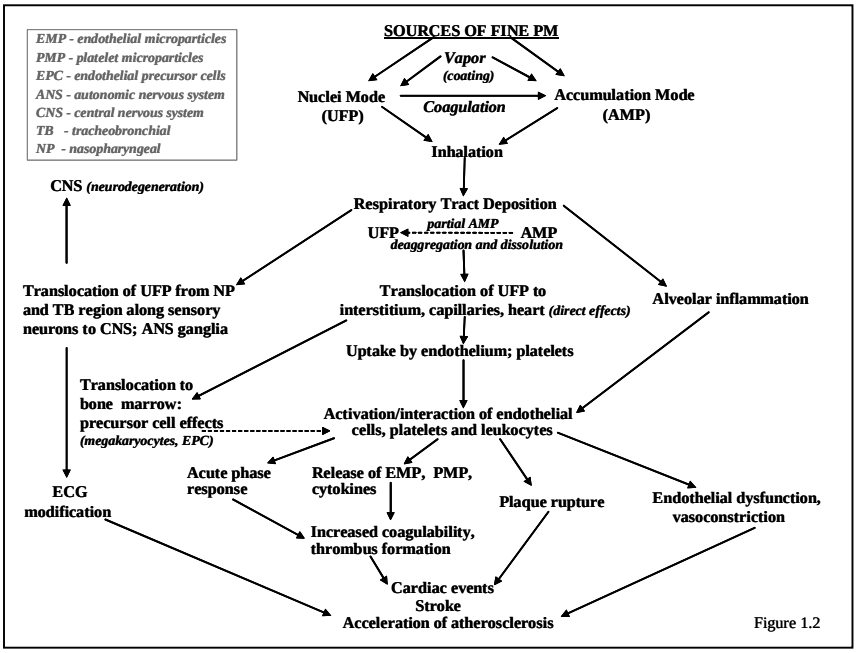

Figure 1.2

disturbed in atherosclerosis and in MI patients. Patients with a compromised cardiovascular system, such as Type II diabetics, are at increased risk as well. Indeed, diabetics have a dysregulated coagulation system and platelets that are activated.

An important part of our hypothesis is the interaction of the activated platelets with injured endothelium and formation of microthrombi in susceptible parts of the population, such as diabetics. The role of EMP and of PMP as a cause or as a consequence of thrombus formation is still debated in this new field of cardiovascular disease mechanisms. Our data suggest that carbon UFP exposure alters endothelial function in humans, and that exposure to traffic PM alters heart rate, blood endothelin levels, and cardiac gene expression in compromised rats (Elder et al., 2004). However, virtually nothing is known about the role of UFP in activation of platelets and their subsequent interactions with EMP and PMP. Our proposed epidemiological, clinical, animal and *in vitro* studies represent a coordinated approach to test our hypothesis that EMP and PMP are increased following exposure to fine PM and are part of a mechanism in PM-induced cardiovascular events. As shown in studies by Nemmar et al. (2002) and in our own studies (Silva et al., 2004), intravenous, intra-tracheal, or inhalation exposure with UF polystyrene or carbon particles causes significant acceleration of thrombus formation in veins, even at doses as low as 0.2 µg UF carbon administered to the lungs of rats.

Approach

Our proposed studies represent a highly integrated approach among our five Research Cores to test these hypotheses in order to answer questions of the correlation between the physicochemical makeup of ambient fine, including UF, particles and effects on blood coagulation, thrombus formation and cardiac events, with the activation of blood platelets and endothelial cells playing a central mechanistic role. Our approach involves several research cores performing specific yet highly integrated experiments by a multidisciplinary team of atmospheric scientists, chemists, epidemiologists, pulmonary, vascular and cardiac physicians and scientists, inhalation-, neuro-, cellular- and molecular-toxicologists, diabetologists, and immunologists.

Research Core 1 (Characterization and Source Apportionment) performs the measurements and charac- terization of UF and fine PM to understand the link between physicochemical properties (EC/OC; inorganics, surface reactive oxygen species, EPR), sources, and health effects. This will test our general hypothesis that PM-induced oxidative stress may initiate platelet activation as well as endothelial activation. Research Core 2 (Epidemiological Studies) uses real-world PM exposure of susceptible populations, including diabetics, MI patients, and patients with genetic susceptibility, to understand the mechanisms and genetic determinants of susceptibility and to identify the PM components responsible for these effects. Research Core 3 (Clinical Studies) uses controlled exposures of healthy and diabetic subjects with concentrated UF/fine PM to understand the mechanisms of vascular function including platelet activation, platelet function, and cardiac effects. In addition, the role of cyclooxygenase products in platelet activation, using pre-treatment with aspirin will be examined. Research Core 4 (Animal Models) is using a diabetic rat model exposed to concentrated ambient UF/fine particles and on-road highway UF/fine particles. Endpoints parallel those of the epidemiological and clinical studies; mechanisms of *in vivo* thrombus formation will be studied as well. Using a mouse model of neurodegeneration, CNS effects will also be evaluated. Research Core 5 (*In Vitro* Studies) uses exposures of epithelial and endothelial cells, macrophages, neurons, and platelets to study underlying mechanisms of injury that will explain the *in vivo* findings. *In vitro* toxicity ranking of PM samples from different sources will be verified in *in vivo* animal studies. Four Facility Cores provide essential support to the 5 Research Cores with respect to Aerosol Generation and Analysis, measurements of Vascular and Inflammation parameters, analysis of Cardiac events, and Biostatistics for study design and data analysis.

Figure 1.3 depicts how the different Research Cores interact with each other with respect to: (1) parallel epidemiological, clinical, animal and *in vitro* studies with a common focus on endothelial dysfunction and underlying mechanisms; and (2) sequential studies starting with *in vitro* investigations of the biological/toxicological activity of fine PM from different sources followed by selection of the most potent PM for subsequent *in vivo* studies when administered by oropharyngeal aspiration to rats. Results of the *in vivo* and *in vitro* studies combined with results of detailed physicochemical analysis of the UF/fine PM from different sources will be used for correlating source specific and/or chemical specific adverse effects and toxicity. Our integrated multidisciplinary approach is designed to explore the Source—Exposure—Dose—Response paradigm with the ultimate goal to correlate adverse pulmonary, vascular, and cardiac effects of airborne fine PM and to uncover the mechanisms, which cause these effects.

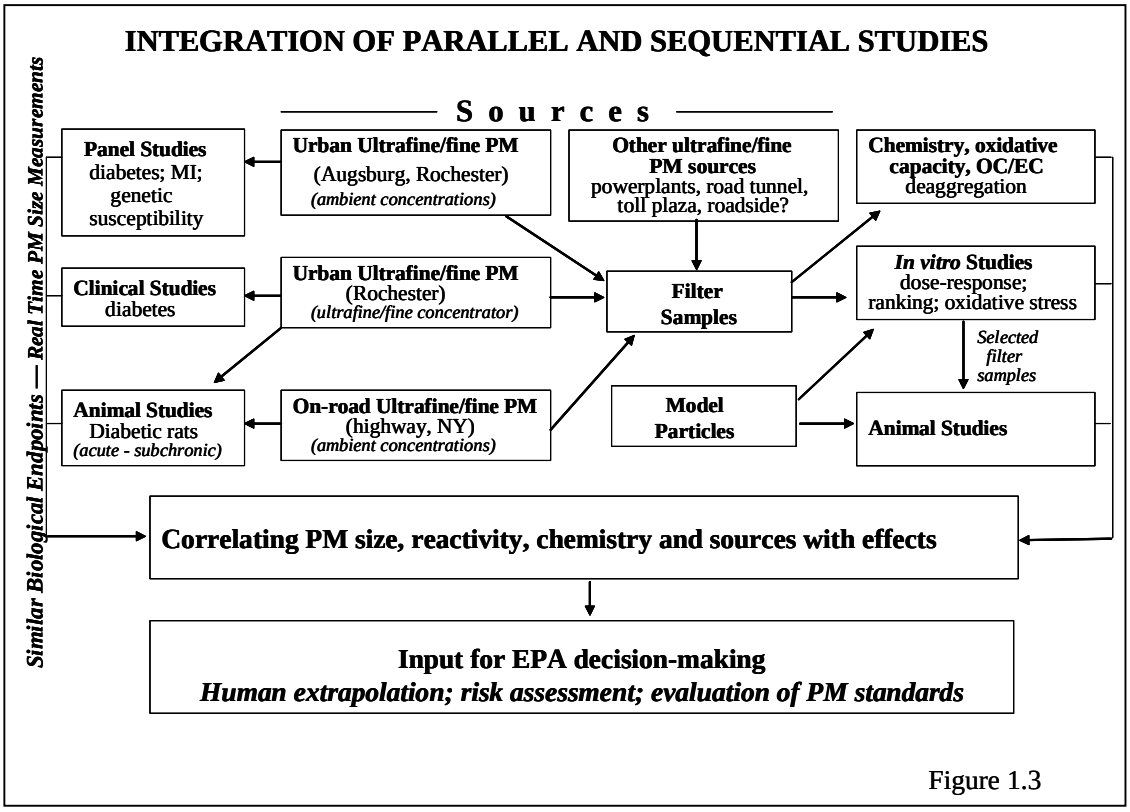

Figure 1.3

Project 1: Characterization and Source Apportionment

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EPA Grant Number: R832415-010

Project Summary:

Objectives: This core will provide critical information on the physical and chemical properties of ultrafine and fine aerosols to be used by health effect researchers in the other research cores. We will link state-of-the-art measurements with data analysis methods that will permit apportionment of the major source types contributing to airborne particulate matter (PM). The core will apportion PM mass apportionments to support epidemiologic, toxicological, and clinical studies. We will develop methods to characterize particulate compositional changes as they are processed in the atmosphere in transit from the source to the receptor site with a focus on the identification of the chemical species contributing to the measured reactive oxygen species. We will use the data characterizing the composition of the ambient aerosol being concentrated for animal and clinical exposures in Rochester and state-of-the-art receptor models to apportion the sources of these particles.

Experimental Approach: A suite of tools will be used to characterize ambient, source, concentrated, and model aerosol size and composition. Particles will be sampled at different locations (urban and remote; eastern and western United States) from various sources (i.e., roadways, power plants) to assess the effects of aging on the oxidative capacity of particles and the changes in their composition during transport. In addition to online size-resolved, chemical characterization, particles will be studied using a variety of offline techniques. We will develop sampling techniques using spin trap agents to stabilize oxidative radicals so that their chemical structures can be identified by chromatographic/MS and EPR analysis. Using ambient measurements of specific organic compounds made at selected STN sites, source apportionments using the CMB model will be made for STN sites at which PMF studies have apportioned the aerosol mass. CMB and PMF results will be compared for the STN sites to ascertain the ability of PMF to resolve source types.

Expected Results: The results from this core's studies will be essential to interpret the results of the other cores. These measurements will permit development of a delivery system to provide exposures of specific radical species on model particles for toxicological studies. Source apportionment of ambient PM will be made using the measured compositions of integrated particle samples, single particle size, and composition data from ATOFMS measurements and particle size distributions to support epidemiological studies. The CMB-PMF results will provide a quantitative test of PMF source apportionments. We will collect ambient aerosol samples, providing them to the Toxicology Core for *in vitro* studies measuring particle toxicity. These results will be used to determine the relative toxicity of various species and apportioned source types.

Project 2: Epidemiological Studies on Extra Pulmonary Effects of Fresh and Aged Urban Aerosols From Different Sources

Annette Peters[1], H. Erich Wichmann[1], Alexandra Henneberger[1], Regina Rückerl[1], S. Breitner[1], M. Stölzel[1], R. Phipps[2], Mark Utell[2], and W. Zareba[2]
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EPA Grant Number: R832415-010

Project Summary:

Objectives/Hypothesis: Our objective is to evaluate the role of key components of urban aerosol on acute phase reaction and prothrombotic states in the blood, endothelial dysfunction, cardiac function, and classical cardiovascular risk factors in susceptible subgroups. We will focus on determining the effects of fresh ultrafine particles from specific sources such as traffic as well as aged aerosols in the accumulation mode, which are mixtures of particles from different sources and secondary aerosols. We hypothesize that the following subgroups are susceptible to effects of ambient fine and ultrafine particles compared to control subjects: (1) patients with coronary artery disease during rehabilitation; (2) myocardial infarction (MI) survivors; (3) diabetics; and (4) individuals with enhanced inflammatory responses due to genetic polymorphisms in inflammation regulating pathways and/or in detoxifying pathways.

Experimental Approach: In Rochester, 80 patients taking part in a cardiac rehabilitation program will be studied. In Augsburg, panel studies enrolling a total of 400 subjects will be conducted, including 100 subjects per group b-d. A random sample of the population of Augsburg will be screened for genetic predispositions for enhanced inflammatory responses and slower detoxification pathways to select participants with genetic polymorphisms as well as healthy controls. In a substudy, data on 24-hour ECG Holter monitoring, a time-activity diary including traffic exposures and stress level, and continuous personal exposure to the total particle number concentrations will be collected in 30 participants of each group. Outcome measures will be determined by the Vascular and Inflammation Core, and ECG parameters will be provided by the Cardiac Core. The number concentration of ultra fine particles, particle size distributions, and continuous particle mass measurements are conducted in Rochester, NY. Ambient air pollution in Augsburg will include continuous particle mass measurements, particle size distributions separated into volatile and nonvolatile fractions, particle surface, elemental and organic carbon, sulphates, and nitrates. Particulate matter associated oxidative stress will be measured in Rochester and Augsburg by the Aerosol Generation and Analysis Core. In collaboration with Core 1, source apportionment analyses will be conducted.

Expected Results: The studies will give evidence on health effects of key characteristics of the urban aerosol such as oxidative stress and on the role of sources in urban areas. In addition, they will assess the potential role of ambient air pollution concentrations on smaller time-scales than 24 hours.

Project 3: Human Clinical Studies of Concentrated Ambient Ultrafine and Fine Particles

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University of Rochester, Rochester, NY

EPA Grant Number: R832415-010

Project Summary:

Objectives/Hypothesis: These studies will use controlled human clinical exposures to test the hypothesis that inhalation of ambient ultrafine and fine PM causes measurable changes in coagulation and cardiovascular function, that these effects are determined by PM-associated reactive oxygen species, and that subjects with Type II diabetes are at increased risk for these effects.

Experimental Approach: Three human exposure protocols will be conducted using the Harvard ultrafine ambient particle concentrator, in collaboration with the Aerosol Generation and Analysis Facility Core. The first protocol will examine effects in healthy subjects, the second protocol will examine effects in age-matched subjects with type 2 diabetes, and the third protocol will assess the role of pretreatment with the cyclooxygenase inhibitor aspirin in preventing the cardiovascular effects of ultrafine/fine particle exposure. In collaboration with the Vascular and Inflammation Facility Core, we will determine particle effects on platelet function and release of endothelial and platelet microparticles into the circulation, and will examine effects on platelet-leukocyte adhesion, bone marrow stimulation, and changes in gene expression in blood mononuclear cells. Continuous ECG monitoring, in collaboration with the Cardiac Facility Core, will detect changes in cardiac repolarization, and noninvasive impedence cardiography will measure changes in cardiac output. Genomic DNA from exposed subjects will be analyzed for candidate gene polymorphisms identified in Research Core #2, Epidemiological Studies. Exposure studies will be designed and conducted in parallel with similar animal exposure studies conducted by Research Core #4, Animal Models. The impact of PM-associated reactive oxygen species, size, composition, and source will be examined in collaboration with Research Core #1, Characterization and Source Apportionment, and with the Biostatistics Facility Core.

Expected Results: Confirmation of our hypothesis that exposure to ambient ultrafine and fine particles promotes coagulation and alters cardiac function will have important implications for air pollution regulatory efforts, and will provide new approaches for the prevention of cardiovascular health effects.

Project 4: Animal Models: Cardiovascular Disease, CNS Injury and Ultrafine Particle Biokinetics

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EPA Grant Number: R832415-010

Project Summary:

Objectives/Hypothesis: The animal studies are designed to test our central hypothesis that ambient ultrafine (UF) particles induce oxidative injury in target cells of the cardiovascular system, resulting in adverse health effects in susceptible parts of the population. The goals of the animal studies are to: (1) identify cellular mechanisms that lead to systemic and thrombogenic responses and associated cardiac events; (2) identify the particle sizes and chemical constituents that induce effects; (3) define pathways of direct UF/fine translocation to extrapulmonary sites; (4) evaluate the neurotoxic potential of UF/fine particulate matter (PM) exposure; and (5) analyze mechanistic pathways linking PM inhalation and deposition in the respiratory tract with effects at the portal of entry and in remote organs.

Experimental Approach: The diabetic JCR rats will be used as a model of cardiovascular susceptibility to identify mechanisms of PM-induced thrombogenic events following acute and subchronic inhalation exposures to concentrated ambient UF/fine particles. Additional exposures of JCR rats to freshly generated Diesel exhaust from a truck equipped with future (2007) filter trap technology also will be performed, using a mobile laboratory while driving on highways. Other animal studies will evaluate potential neurotoxic effects using a mouse model of neurodegeneration and subchronic exposures to ambient concentrated UF/fine particles. Particle size-dependent translocation pathways of inhaled UF/fine particles from the respiratory tract to extrapulmonary target tissues, including endothelial cells, blood platelets, bone marrow, heart, and CNS also will be examined. These studies are designed in close collaboration with the other Research Cores: information provided by the PM Characterization and Source Apportionment Research Core (Core 1) is an integral part of the animal studies, which are complementary to the epidemiological (Core 2) and controlled clinical studies (Core 3), and provide a link to the mechanistic *in vitro* studies (Core 5). In addition, this Core has strong collaborative ties to the Center's four Facility Cores: Biostatistics, Aerosol Generation and Analysis, Vascular and Inflammation, and Cardiac.

Expected Results: The Animal Core studies will help to answer the question of why subpopulations are at increased risk of adverse health outcomes following PM exposure. They will identify the cellular and molecular mechanisms that underlie cardiovascular susceptibility. Exposure-response relationships will be defined and we expect to learn how exposure duration, temporal variability in PM concentration and composition affect responses. Results of the animal studies, integrated with those of the epidemiological, controlled clinical, and *in vitro* studies, along with source-specific PM characterization and source apportionment analyses, will be used for correlating effects with specific PM constituents and sources.

Project 5: Ultrafine Particle Cell Interactions *In Vitro*: Molecular Mechanisms Leading to Altered Gene Expression in Relation to Particle Composition

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EPA Grant Number: R832415-010

Project Summary:

Objectives/Hypothesis: These studies are designed to address specific mechanistic hypotheses regarding the interactions between inhaled ultrafine particles and specific pulmonary and cardiovascular cell populations. We will use cell lines and primary cells derived from rats and humans to test the hypothesis that increased morbidity and mortality in susceptible populations are due to the unique characteristics of ultrafine particles inducing oxidative stress and activation of target cells. It is further suggested that this ability is based on composition that is related to specific sources.

Experimental Approach: Studies will use isolated and cultured cells that have been identified as the principle targets of inhaled PM and carry out hypothesis testing studies that support the *in vivo* studies (Cores 3 and 4). Cell specific endpoints of PM-induced toxicity for inflammatory, epithelial, and vascular endothelial cells will be determined in a dose-responsive manner. These cells will be obtained from both human and animal tissues. Our use of multiple endpoints ensures that we can determine the relationship between a cellular response and the critical variable of a specific particle. We plan studies with both "real world" and laboratory generated PM of known composition that may represent specific sources to establish source-related effects.

Expected Results: The results of these *in vitro* studies will identify specific mechanisms that are trig- gered following particle cell contact in a cell specific context and identify markers of cellular response. We predict that many of the subsequent physiologic effects *in vivo* are the consequences of cellular oxidative stress, cell activation, and apoptosis. The proposed *in vitro* experiments will provide a link between the whole animal and controlled clinical (human) exposures, described in the other programs of this PM Center. By evaluating "source specific" responses *in vitro*, we can determine the relative potency of a given particle type and allow for studies *in vivo* to be more focused and mechanism based. These studies also may provide information regarding strategies to reduce PM effects. As correlations are established between *in vitro* responses and whole organism studies, the *in vitro* studies, which require less material and a shorter timeframe, may be applied as a routine screen for potential PM risk and as support for setting regulatory standards.

Johns Hopkins Particulate Matter Research Center

Jonathan M. Samet, Director
Johns Hopkins University, Baltimore, MD

EPA Grant Number: 832417-010

Center Overview:

Objectives/Hypothesis: The Johns Hopkins PM Research Center brings together a multidisciplinary research team: biostatisticians, epidemiologists, exposure assessors, lung biologists and respiratory toxicologists, pulmonary clinicians, and atmospheric scientists to address the most critical gap in current understanding of health and particulate matter (PM)—the physical and chemical characteristics that determine risk to human health. The Center's conceptual foundation lies in mapping health risks of PM across the United States, based on an analysis of national databases on air pollution, mortality, and hospitalization, and then using the maps to guide detailed monitoring and collection of PM samples for physical, chemical, and biological characterization in assays relevant to pulmonary and cardiovascular outcomes.

Experimental Approach: The Center's three-phase agenda builds progressively from mapping the health risks in the first phase. During this first phase, we also will explore patterns of PM characteristics across the United States using existing data; we will develop protocols for detailed PM monitoring and collection of sufficient materials for bioassays; adapt existing *in vitro* and *in vivo* bioassays for assessing the comparative toxicity of the PM samples; and develop relevant microarray approaches. In the second phase, we will monitor and collect PM from 10 sites, selected from the higher and lower extremes of the effects of PM on human health; we will then assess the comparative toxicity of the PM samples in the bioassay battery. The third phase is likely to involve assessment of risks of more toxic particles in susceptible populations, source-related relative risk characterization, exposure assessment studies, and more focused studies of mechanisms of injury by these particles. The Center will have three discipline-based Research Projects and two scientific cores, the Data Base Core and the PM Collection, Sampling and Analysis Core. The Administrative Core will be the focal point for interdisciplinary interactions.

Expected Results: The Center will map health risks of PM across the United States, based on analysis of national databases on air pollution, mortality, and hospitalization, and then use the maps to guide detailed monitoring and collection of PM samples for physical, chemical, and biological characterization in assays relevant to pulmonary and cardiovascular outcomes.

Johns Hopkins PM Research Center
Overall Description

The Johns Hopkins PM Research Center brings together a multidisciplinary team of investigators, including biostatisticians, epidemiologists, exposure assessors, lung biologists and toxicologists, pulmonary clinicians, and atmospheric scientists to address one of the most critical gaps in current understanding of health and exposure to airborne particles—the physical and chemical characteristics that determine the risk posed to human health by inhalation of airborne particles. The conceptual foundation of the Center lies in our ability to map the risks posed by particulate matter (PM) exposure across the United States. Over the 5 years of funding, the Center's researchers will identify those areas in the United States where PM exposure poses greater and lesser estimated risks to human health, collect and characterize particles in these locations, and assess their toxicity in a battery of assays relevant to pulmonary and cardiovascular outcomes. The design involves a three-phase program over the 5 years of funding that builds progressively from the starting point of mapping health risks across the Nation (Figure 1). The Principal Investigator, Jonathan M. Samet, M.D., M.S., is a pulmonary physician and epidemiologist who has focused on the health effects of inhaled pollutants throughout his career; the three component projects are led by Francesca Dominici, Ph.D., a biostatistician whose career has emphasized the development and application of statistical methods for research in environmental epidemiology; Joe G. N. Garcia, M.D., a pulmonary physician and lung biologist whose laboratory has focused on use of state-of-art methods to characterize mechanisms of lung injury; and Patrick Breysse, Ph.D., whose work has focused on exposure assessment and the measurement of exposures in the community setting. The team also includes Dr. Scott Zeger, Chair of the Department of Biostatistics at the Bloomberg School of Public Health and a leader in the development and application of methods for longitudinal analysis; Drs. Timothy Buckley and Alison Geyh, who work in exposure assessment and pollutant characterization; Dr. Aidan McDermott, with expertise in managing large, complex databases; Dr. John Ondov of the University of Maryland, Principal Investigator for the Baltimore Supersite; Dr. Steven Chillrud of the Lamont-Doherty Earth Observatory; Dr. Saugata Datta of Georgia College and State University; and Dr. Michelle Bell from Yale University, who brings expertise in both air quality modeling and epidemiological data analysis.

The plan for the Johns Hopkins PM Research Center is grounded in the research portfolio of the National Research Council's Committee on Research Priorities for Airborne Particulate Matter (National Research Council 1998). Since 1998, researchers in the United States and other countries have pursued a broad agenda of research on airborne PM that has been guided by the Committee's framework (National Research Council 1998). The Committee's first report in March, set out a 13-year research portfolio based around 10 topics that covered critical uncertainties in the evidence base. Over the subsequent 6 years, progress has been made on some of the 10 topics, as described in the Committee's fourth and last report (National Research Council 2004). The committee's final report specifically highlighted the need for further research directed at characteristics of particles (chemical and physical) associated with greater or lesser toxicity (Topic 5 in the Committee's list). The report also noted a continuing need for studies of mechanisms of injury and of susceptibility. Other unmet needs related to combined effects of particles and gases and, elaboration of the emissions inventory and further development of air quality models. The Committee also called for new integrative and multidisciplinary research approaches.

The Committee viewed Topic 5 as pivotal in advancing understanding of PM and risk to health and in possibly evolving from a NAAQS based on mass concentration alone, to approaches that

targeted those particles and sources contributing to the burden of PM-associated morbidity and mortality. Recognizing the current critical relevance of new information on PM characteristics and health risks, the proposed Johns Hopkins PM Research Center will focus on Topic 5, *Assess Hazardous Particulate Matter Components*. Inherently, the scope of research will extend beyond Topic 5, as the epidemiological analyses will explore combined effects of PM and other pollutants (Topic 7), the bioassays will provide insights into mechanisms of injury (Topic 9), and both the epidemiological and bioassays will consider susceptible populations (Topic 8). The exposure assessment activities will provide information relevant to Topic 2 (*Exposures of Susceptible Subpopulations to Toxic Particulate Matter Components*) and also to Topics 3 and 4, concerned with emissions sources and air quality models. We will also contribute new methods for statistical analysis (Topic 10).

Appropriately, our approach will be integrated and sequential, following three planned phases over the 5 years of funding (Figure 1). The Center will have three discipline-based research projects with agendas that will evolve as the work moves through its three phases; these research projects include: (1) Estimation of the Risks to Human Health of PM and PM Components; (2) PM Characterization and Exposure Assessment; and (3) Biological Assessment of the Toxicity of PM and PM Components. Additionally, the Center includes two scientific cores; the Database Management Core and the PM Collection, Sampling, and Analysis Core, along with the required Administrative Core.

The first phase, setting the foundation for the work to follow, will involve a detailed mapping of the health risks of PM across the United States in order to identify those cities and regions where risks have been estimated to be the highest and the lowest. This approach addresses a critical obstacle in approaching PM characteristics and risks to health—the many potential combinations of characteristics that may be relevant to the diverse health outcomes linked to PM. The NRC Committee noted the scope of the array defined by PM characteristics and health outcomes, and the need for a guiding principle in exploring this matrix. To this point, research on Topic 5 has been complicated by the numerous characteristics of PM potentially relevant to their toxicity and the possibility that different characteristics may be relevant to different health outcomes. Our epidemiologically based approach provides that guiding principle, using the actually occurring variation in risks to health as a basis for exploring the matrix defined by PM characteristics and sources and health out- comes.

The mapping of health risks in this first phase will build on the methods and databases already assembled for the Health Effects Institute funded National Morbidity, Mortality and Air Pollution Study (NMMAPS) and the STAR Grant (RD-83054801: Chronic and Acute Exposure to Ambient Fine Particulate Matter and Other Pollutants: National Cohort Studies of Mortality and Morbidity), which has supported development of a nation-wide cohort of Medicare participants (referred to as the National Medicare Cohort). These health databases have been linked to the monitoring data for PM_{10} and $PM_{2.5}$, as well as other pollutants, along with information on demographic characteristics from the Census Bureau and meteorology from the National Weather Center. In addition, these health databases will be linked by geographical location to the data from the Speciation Trends Network and to the EPA's Supersite Program.

State-of-the-art statistical methods will be developed to analyze these databases for a variety of health outcomes. Methods will build upon the previously developed Bayesian hierarchical regression models for NMMAPS, which will provide regression coefficients that estimate the increment in risk per unit increase in PM concentration for each of these outcomes. To guard against random variation in these coefficients and to gain evidence across locations, the coefficients will be smoothed using

Bayesian methods. We anticipate generating maps for key outcomes: total mortality and cardiovascular mortality, and hospitalizations for cardiovascular outcomes (myocardial infarction and other ischemic events, congestive heart failure, and arrhythmias), for pneumonia and influenza, and for chronic obstructive pulmonary disease (COPD) and asthma. These maps will guide the work of subsequent phases.

During this first phase, we will also develop methods and carry out analyses of the accumulating data from Speciation Trends Network and also of the already collected data from the Supersites Program. These analyses will have the purpose of better understanding the correlations among pollutants, the extent of intra-site and cross-site variation in pollution patterns, and their association with health outcomes. The findings will be useful in guiding monitoring strategies for the second and third phases of the Center's activities.

The first phase will also involve the development and testing of laboratory approaches for assessing particle toxicity and of field and laboratory approaches for sampling and characterizing particles (Figure 1). A battery of *in vitro* and *in vivo* bioassays will be developed, building from the work of Dr. Garcia and colleagues in lung injury and in pulmonary/cardiovascular interactions. The range of assays will include not only widely used indicators of injury, for example, inflammatory cytokines and markers of oxidant activity, but novel approaches based on microarrays for gene expression. Mouse models will be used that include not only wild type C57B6 mice, but murine models of asthma and congestive heart failure (CHF). In this first phase, these assays will be assessed using a set of real-world PM collected from multiple, distinct sites. The selected battery of genes will reflect candidates identified in previous work on lung injury. For the second and third phases, particles will be collected in sufficient quantity for characterization and use in the bioassays. The emphasis in the second phase will be on comparative toxicity of PM from the selected locations, while the activities of the third phase will be more hypothesis-based and directed at mechanisms of injury.

In the first phase, we also extend and further evaluate methods for collection of sufficient mass of particles; a key goal will be to evaluate the extent to which the collection method alters PM characteristics and to measure the stability of the materials collected. The core set of measurements of PM characteristics will be developed, along with the protocol for detailed onsite monitoring by the Johns Hopkins team.

In the second phase, the national maps of the risks to health of PM will be used to select locations for particle collection and monitoring (Figure 1). Maps will be developed that describe the comparative risks of PM across the country for total and cardiovascular mortality and for hospitalization for cardiac and respiratory diagnoses. Guided by these maps, locations will be selected where the toxicity of PM is clearly distinct. Dr. Breysse's team will then travel to these locations and conduct in-depth monitoring using the protocol developed during the first phase and also collect sufficient PM mass for both detailed characterization and the bioassays. The protocol will give consideration to potential seasonal variation in PM characteristics and toxicity. The onsite monitoring will cover particle size and number, metals and metal oxides, polycyclic aromatic hydrocarbons (PAHs), sulfates and nitrates, and elemental and organic carbon (EC and OC).

The collected particles will be tested in the *in vitro* and *in vivo* assay battery developed in the first phase. While we recognize the inherent limitation posed by extrapolation of findings from these assays to human health, we are using the models and assays in a context set by an initial comparative ranking of the human health risks from the PM in the various locations. The results from the Phase 2

laboratory assays will provide information on the comparative toxicity in well-characterized test systems, providing further insights into toxicity and mechanisms of action. By using *in vitro* and *in vivo* models, we will be able to compare toxicity measures across the assay systems, recognizing that responses, even on a comparative basis, may not be the same in different assay systems.

In the three-phase plan for the center, Phase 3 will build from the findings of Phase 2 and details cannot be specified at present. We anticipate generating specific hypotheses for further research related to Topic 5, based on the findings in Phase 2, and returning to some of the originally selected locations for further monitoring and particle collection, personal exposure studies, panel studies of susceptible individuals, and more refined and targeted bioassays. Additionally, we will attempt to link health risks to sources of the more toxic particles as identified in the work in the earlier phases of the Center's program. The directions of this research would be set by the Center's leadership as Phase 2 findings were obtained.

In formulating the Johns Hopkins PM Research Center, we plan three discipline-specific projects as the vehicle for carrying out the Center's research. We view a disciplinary base as the starting point for Phase 1, but the approach becomes increasingly multidisciplinary as the work proceeds through the three phases of the Center's proposed agenda. The tasks of the cores and the hypotheses to be addressed would evolve over the 5 years of the Center, as the work progresses through the three phases. The Administrative Core Unit, through its multidisciplinary Executive Committee, would provide a venue for multidisciplinary assessment of the findings from the three different projects and for guiding the evolution of the program of work. An ongoing working group will serve as the principal venue for discussion of findings and for elaboration and refinement of the Center's research directions. A unified database will facilitate synthesis of findings across the various lines of research; for example, the results related to a particular sample could be traced across the risks to health observed in the site where it was collected, to the information on its characteristics, to the findings in the various bioassays. Guidance will be obtained on an annual basis, at the least, from a multi-disciplinary Scientific Advisory Committee to further assure that research directions are set and findings interpreted within a broad, multidisciplinary framework.

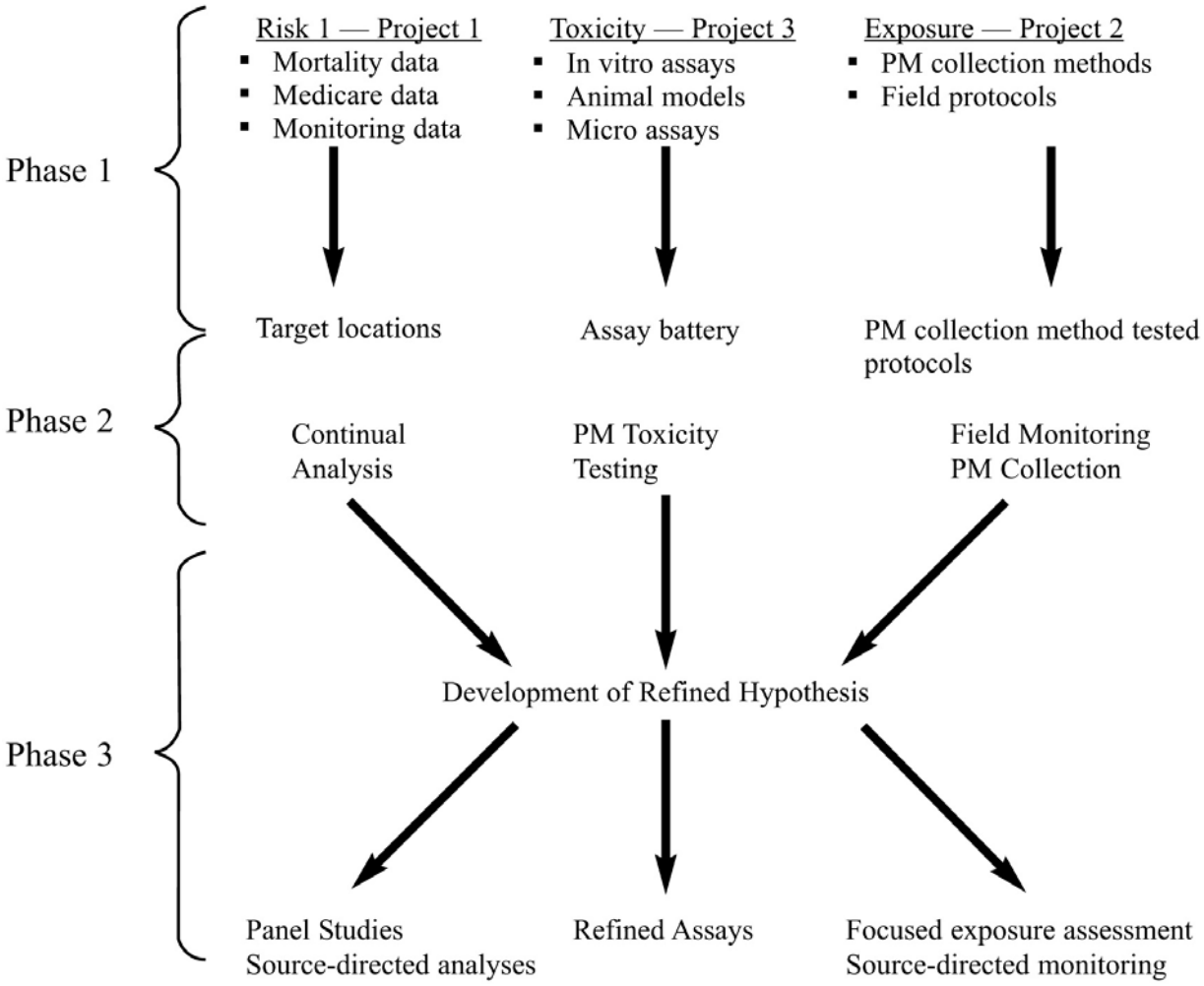

Figure 1. Three Phases of the Johns Hopkins PM Research Centers

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Project 1: Estimation of the Risks to Human Health of PM and PM Components

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EPA Grant Number: 832417-010

Project Summary:

Objectives/Hypothesis: The National Research Council (NRC) Committee on Research Priorities for Airborne Particulate Matter identified knowledge regarding the particulate matter (PM) characteristics associated with toxicity as a crucial research gap. In recent analyses of the National Morbidity Mortality Air Pollution Study, we have found that short-term effects of PM on mortality can vary by region and season, and we postulate that this variation might reflect regional and seasonal differences in PM characteristics.

Experimental Approach: In ongoing studies of air pollution and health, the National Medicare Cohort, comprising individual-level data on diseases, age, gender, and race for virtually the entire population of U.S. elderly, is limited by geographical location to: (1) air pollution concentrations of gaseous pollutants, PM_{10}, $PM_{2.5}$, and $PM_{2.5}$ components; (2) weather; (3) socio-economic data; and (4) smoking and other risk factors for 13,000 Medicare enrollees. In this project, we will develop and apply statistical methods to these national databases to: (1) carry out multisite time series studies for estimating short-term effects of PM and PM components on hospitalization and mortality (Phase I); (2) carry out cohort-studies for estimating long-term effects of PM and PM components on mortality in susceptible populations (Phase II); (3) assess coherence of evidence from bioassays and epidemiological studies on PM toxicity and susceptibility; and (4) explore linkages of sources of harmful PM components to human health risks (Phase III).

Expected Results: This project will address the following objectives of the Center on a national scale: (1) map risks of PM and PM constituents to human health across the United States; (2) use the maps to identify a sampling frame of locations with contrasting higher and lower risks; and (3) carry out more refined epidemiological studies to estimate further the risks of the more toxic particles to susceptible individuals. By providing individual-level health data for the entire U.S. population of elderly, the National Medicare Cohort will allow us to take full advantage of existing and future air quality databases on PM and its characteristics.

Project 2: PM Characterization and Exposure Assessment

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EPA Grant Number: 832417-010

Project Summary:

Objectives/Hypothesis: The focus of Project 2 will be on the measurement of specific chemical components and physical characteristics of particulate matter (PM) from different areas of the country, which will be used to identify those characteristics of PM that determine toxicity.

Experimental Approach: We will collect bulk PM samples for bioassays and bulk and integrated samples for detailed characterization to include mass, inorganic ions, elemental carbon and organic compounds, specifically polycyclic aromatic hydrocarbons, elemental metals and their oxides, and sulfur isotope ratios. We also will evaluate PM characteristics in relation to particle size. We will use this matrix of information to characterize differences in PM composition and biological response by location. The objectives of Project 2 include: (1) developing new methods for collecting bulk PM for use in biological assays; (2) developing a portable system for the characterization of chemical and physical properties of ambient PM; (3) identifying specific regional differences in PM characteristics that may contribute to differential biological responses *in vitro* and *in vivo* bioassay systems; and (4) assessing a relationship between human exposure to $PM_{2.5}$ and biological response during a high $PM_{2.5}$ exposure period and a low $PM_{2.5}$ exposure periods. By successfully completing this project, we anticipate providing a better understanding of how differences in physiochemical composition impact PM health effects.

Expected Results: We anticipate that the results of this project will inform PM air quality regulations, suggesting new PM exposure metrics that can be used to modify existing air monitoring networks. Project investigators are well qualified to conduct this research, and this project leverages support from a wide range of funded projects, including data from existing air quality databases and resources from a previously EPA-funded PM Supersite.

Project 3: Biological Assessment of the Toxicity of PM and PM Components

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EPA Grant Number: 832417-010

Project Summary:

Objectives/Hypothesis: This project will complement the epidemiologic analyses and state-of-the-art characterization of U.S. city-specific particulate matter (PM) and other pollutants by defining the comparative pathobiologic cardiopulmonary responses to inhaled PM exposure.

Experimental Approach: We will utilize standard toxicologic assessment and high throughput toxicogenomic approaches, to gauge the relative toxicity of PM collected in regions where particles have greater and lesser effects on health outcomes. Project 3 will address potential limitations by integrating state-of-the-art genomic technologies with traditional and complementary toxicologic assessment. To exploit comparisons of pathobiologic cardiopulmonary responses to respirable airborne PM, in Phase I, we will optimize a battery of well-developed *in vitro* (human) and *in vivo* (murine) bioassays including multiple cytokines, indicators of reactive oxygen/nitrogen species burden (ROS/RNS), and biomarkers of vascular and cardiac dysfunction for assessing particle toxicity. For this purpose, we will use a set of particles having differing characteristics: standard National Institute of Occupational Safety and Health urban PM, Baltimore tunnel PM, ambient Baltimore air PM, and New York City air PM. We will use oligonucleotide-based microarrays to address the hypothesis that gene expression profiles in human lung epithelium, murine lung and cardiac tissues, induced by respirable PM, are dependent on the fraction of pollutants bound to the respirable PM. In Phase II, we will survey PM fractions from a number of U.S. sites with this battery, selected according to the sampling plan developed in Project 1, and apply the screening methodologies optimized in Phase I to identify sites with the greatest pathobiologic effects and relate these responses to observed adverse human health effects. Phase III will focus on a smaller number of sites showing the greatest PM toxicity and initiate mechanistic studies using transgenic prototypes, *in vitro* molecular dissection of signal transduction pathways, and extensive gene profiling.

Expected Results: The multidisciplinary integration of approaches in Project 3 will lend insight into the interpretation of what constitutes an adverse effect, and accelerate our understanding of the characteristics of PM that determine their toxicity.

Overview of EPA's PM Research Program
EPA's Regulatory Context for PM Research

Under the Clean Air Act (CAA), Particulate Matter (PM) is one of six major air pollutants for which EPA has established National Ambient Air Quality Standards (NAAQS). The CAA mandates periodic reviews of the scientific basis or "criteria" for these standards and requires EPA to prepare a comprehensive scientific assessment of the state-of-the-knowledge for each criteria air pollutant. A regulatory proposal on how, or whether, to revise the existing PM NAAQS is expected in December 2005. The NAAQS deliberations include potential revisions of the $PM_{2.5}$ (fine mode) and $PM_{2.5\text{-}10}$ (coarse mode) as primary health-based standards and possibly secondary welfare standards. Following the promulgation of the NAAQS, states must use analytical tools to assess how to comply with the new standards and continue attaining them over time. Monitoring data coupled with atmospheric models and emissions information are used to develop State Implementation Plans (SIPs) that propose combinations of emissions reductions that are predicted to be most effective in reaching attainment of the standards. There will be varying degrees of challenges associated with NAAQS compliance depending on the air pollution sources and the atmospheric conditions in different regions of the country.

Mission and Organization

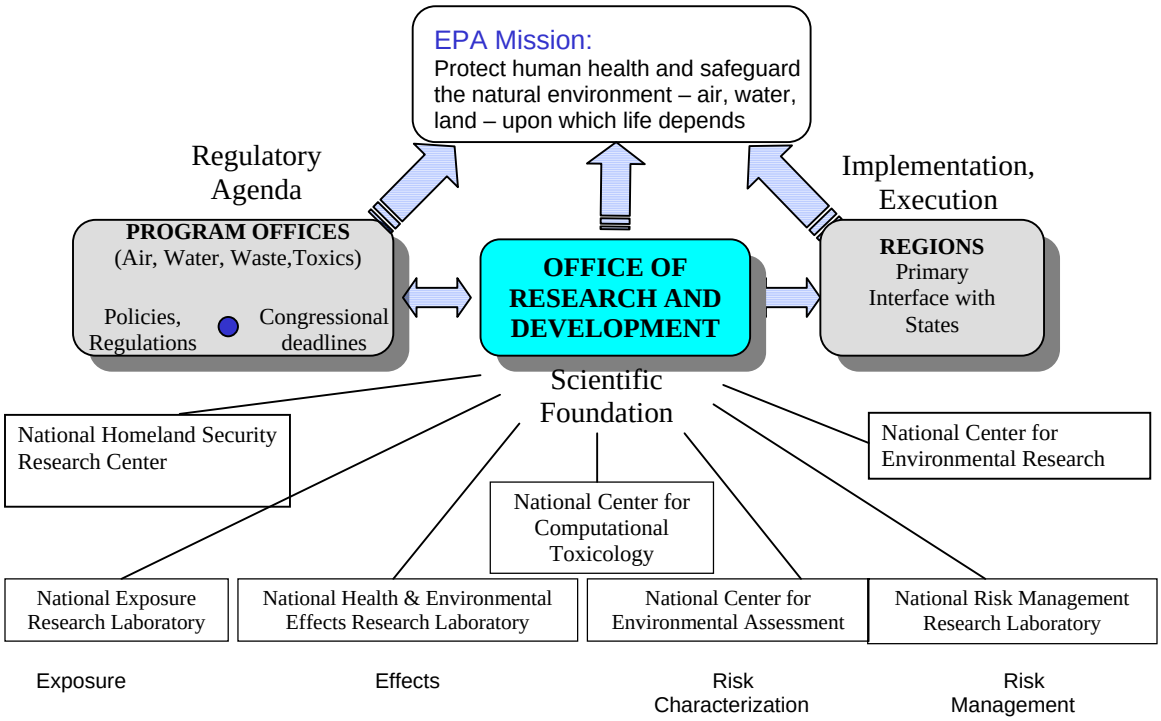
The mission of the Office of Research and Development (ORD) is to conduct and sponsor research to support the Agency's program offices in meeting their current and future regulatory mandates and providing informed guidance to the public. As such, ORD managers and staff develop air research priorities in close cooperation with our primary internal clients: EPA's Office of Air and Radiation (OAR) and EPA's Regional Offices. ORD's role is to provide the critical science for environmental decision-making. Through the development of technical information and scientific tools, ORD's research strengthens EPA's science base, providing the Agency's Program Offices and Regional Offices with sound data for use in developing and implementing tenable environmental policies, regulations, and practices. Comprising three National Laboratories and four National Centers across the country, ORD's broad scope encompasses efforts to assess and manage both human health and ecological risks. Figure 1 provides an overview of that structure.

Overview of EPA's PM Research Program

EPA's PM research program addresses the fundamentally problem-driven question: "How can we reduce health risks associated with exposure to air pollution?"

The National Research Council (NRC) has reviewed EPA's PM research program and issued a series of reports. Report IV of the NRC (*Research Priorities for Airborne Particulate Matter: IV Continuing Progress)* was released in the spring of 2004. The NRC noted that substantial progress has been made in reducing uncertainties, primarily in the areas of exposure, dosimetry, and analytical methodologies for statistical approaches to epidemiology. However, there is still much uncertainty in the area of hazardous components. The NRC saw this area as critical to advancing PM science, not just in health assessment but also in focusing control strategies. A second NRC Committee was convened in 2004 under the Clean Air Act Advisory Committee (CAAAC) to address issues regarding *Air Quality Management*. This Committee looked at air pollution from the implementation and management perspective, and came to similar conclusions. Air quality management cannot be achieved by singular assessments of pollutants or disciplines acting in "silo" fashion. Rather, the NRC concluded that integrated approaches would serve best to improve air quality and that a

properly structured program should be able to self-assess improvements. The EPA program recognizes that further advances in air pollution sciences and air quality management will require the adoption of multi-pollutant approaches, which would bring the strengths and insights of the health and atmospheric sciences into closer alliance. This integrated effort will provide a framework for self-assessments and mid-course corrections and ensure progress—accountability.



While the need to understand the roles of physical and chemical characteristics and constituents remains a key to more fully understanding PM-associated health effects, EPA needs to better understand the impacts of particles from specific sources. Linking specific sources through ambient concentrations and human exposures to health effects is likely to provide data that can be applied to regulatory policy more quickly, and can help support identification of important biologic outcomes and the responsible constituents as well. Source-exposure-effects approaches take into account the complex nature of ambient PM, which is mixed and/or transformed with co-pollutants to form particles with a wide range of possible characteristics and constituents that cannot easily be disentangled.

The EPA is currently in the process of merging its PM research program with its ozone research program. There is little doubt that the Air Research Program in the long run is moving to a "multi-pollutant" or "one atmosphere" concept, which will of necessity consider the NAAQS and the Air Toxic pollutants, the latter of which is currently a separate, but relatively small program. In order to facilitate integration of research across laboratories and centers, ORD has established a National Program Director (NPD) for Air, who is focused on moving the air program towards a more integrated, multidisciplinary approach.

Long-Term Goals and Related Program Activities

The EPA has established two integrated Long Term Research Goals (LTGs) for PM and ozone that encourage interdisciplinary, integrated research targeting measurable outcomes—advances in the understanding of air pollution sciences and protection of public health. These LTGs were developed through meetings and discussions not only within the Agency, but with the science community, the Executive and Congressional branches of the U.S. government, sister Agencies, state and local agencies (through OAR), as well as the public to shape a national air pollution research agenda. This process supports the definition of Program priorities that evolve with advances in the science.

Each LTG has research program activities associated with it that together lead to the achievement of the LTG. Key PM Research activities are described below under the appropriate LTG.

Long-Term Goal 1: Achieve progress toward reducing uncertainty in standard setting and air quality management decisions due to advances in understanding in the air pollution sciences.

Research Is Being Performed in a Number of Areas:

PM size fractions and components—EPA researchers are focusing on the use of concentrated ambient particles (CAPs) in order to conduct controlled experiments investigating the health effects of ambient PM. The goal is to attribute health outcomes to PM components and sources associated with the mixture. Integrated human, animal and *in vitro* studies currently underway or planned include:

- Size-fractionated CAP studies in healthy adults and asthmatics to characterize health effects, and to the extent possible link with epidemiologic, and animal studies.

- PM ultrafine CAP studies in healthy young adults and pre-diabetics to determine if cardiovascular risk factors elevate PM health risks and the relative importance of various PM size fractions.

- Identifying biomarkers of exposure and effect to different size PM or PM components through the use of 'omic' approaches.

- PM animal studies with collected ambient particles at a variety of locations.

- Evaluating the effects of endotoxin on healthy volunteers and asthmatics and comparing its effects with that of ozone.

- PM animal studies with collected source derived particles including several types of ROFA, diesel, coal and coal with controlled contaminants to help identify hierarchy of health effects vs. sources and components.

In July 2005, The Health Effects Institute (HEI) issued a Request for Applications (RFA) for Studies to Compare Components and Characteristics of PM associated with Health Effects. HEI's goal is to develop a comprehensive research program to systematically address questions about the health effects related to different components and characteristics of the ambient PM mixture. Studies in this program also will consider how gaseous pollutants may affect the toxicity of the PM constituents. Preferably, the studies will use a combination of epidemiological and toxicological

approaches. Initially, HEI intends to fund two types of studies: (1) full 4-year studies that begin with a pilot phase and; (2) 9-month planning or demonstration studies, which will entail formation of a multidisciplinary study team to gather and analyze data necessary to design a full study. In early 2007, HEI will issue another RFA to seek additional applications for full studies.

Susceptibility—EPA is conducting cross-cutting research on what makes people susceptible to PM health effects and how exposures to PM component and sources impact different susceptible groups and result in various negative health outcomes. Studies currently underway or planned in this area include:

- Studies to address associations between PM exposure and development of lung injury and inflammation, increases in mucus secretion, and/or increases in airway responsiveness, and mechanisms in exacerbation of allergic asthma.

- Characterizing total and regional lung deposition dose of inhaled ultrafine particles in healthy volunteers and volunteers with respiratory disease.

- Role of neurotrophins in exacerbation of allergic asthma.

- Developing and testing animal models to investigate the role of oxidative stress in susceptibility to cardiovascular and pulmonary effects of PM and PM constituents.

- Characterizing physiological changes in humans with vascular risk factors exposed to PM and asthmatics exposed to PM.

- The role of genetic susceptibility in experimental induction of chronic obstructive pulmonary disease (COPD) and PM health effects.

- Role of carbonaceous and diesel particles on systemic, cardiopulmonary inflammation, and allergic adjuvant effects in experimental animals.

- Pilot study to characterize cardiac biomarkers in a healthy, 55-80 year-old population.

- Panel study to addresses the hypothesis that many of the cardiovascular changes caused by PM are mediated through endothelial cell dysfunction.

- Identification of genetic polymorphisms, which are associated with susceptibility of healthy individuals to PM.

Impact of co-pollutants on health effects—EPA's research program leverages CAP studies and source specific research studies to evaluate the effects of PM and co-pollutant exposures. Current and planned studies in humans and animals include healthy young adults—$PM_{2.5}$ CAPs and NO_x, and $PM_{2.5}$ CAPs and O_3. In addition, to evaluate the relative toxicity and interactions between particles vs. gaseous co-pollutants in diesel, EPA will be assessing the effects of filtered PM or gaseous co-pollutants in animal models.

Physiological mechanisms—EPA is conducting cross-cutting research aimed at determining the physiological mechanisms that underlie observed mortality and morbidity associated with PM ex-

posures. Research is aimed at enabling the extrapolation of effects in *in vitro*, animals, and humans systems. A specific focus aims at determining if there is a common cellular mechanism (e.g., oxidative stress) that can explain how PM, other NAAQS pollutants, and air toxics cause mortality or adverse health effects. Studies currently underway or planned include:

- Characterization of pathophysiological mechanisms which underlie cardiopulmonary toxicity induced by PM and its constituents.
- Investigation of the reversal of PM-induced cardiopulmonary changes in humans.
- Methods for the validation and extrapolation of *in vitro* mechanistic effects of environmental exposures *in vivo.*
- Genomics and proteomic approaches for PM cardiopulmonary toxicity evaluation.
- Characterization of molecular mechanisms responsible for PM toxicity (e.g., oxidative stress, metal transport, signal transduction pathways).
- Cardiac, pulmonary, and thermoregulatory parameters in rats, and developing and validating software and procedures for analyses of electrocardiographic waveform parameters and heart rate variability in rats.
- Characterize the mechanisms of vascular toxicity induced by PM and its constituents.
- The translocation and pathogenesis of particulate matter neurodegeneration at the cellular and molecular levels.
- The development and use of genomic and proteomics techniques to identify biomarkers of exposure, effect, and susceptibility in animals and humans.
- Determining whether interventions (e.g., antioxidants, anti-inflammatory agents) can blunt the response to PM.

Complementing the intramural studies in this area, EPA's extramural research program, together with the National Institute of Environmental Health Sciences, is funding 12 mechanistic studies on the role of air pollutants in cardiovascular disease.

Chronic Studies—EPA's research program plans to identify responses that can be measured in acute, short-term, or prolonged exposure studies that are predictive of chronic effects. In addition, studies will evaluate the extent to which acute or short-term responses observed in air pollution develop into chronic adverse effects. Research efforts in this area include: rabbit and Apo E knockout mouse studies to evaluate how particulate matter exposure impacts cardiovascular disease development; extended exposure studies of the SH rats (to 11 weeks) to investigate questions of susceptibility and cardiac effects; and investigating alterations in cardioprotective response mechanisms and vascular function in normal, and diabetic rats.

A major EPA effort in this area is the Detroit Children's Health Study. This study has been developed to evaluate the role of long-term exposures to mobile-source emissions in the develop-

ment, progression, and exacerbation of asthma in a major northern industrial metropolis. This will be the first study to look at intra-urban pollutant gradients (including air toxics) in a northern U.S. city; include newly developed biologic markers of immunologic function; and use monitor-calibrated GIS exposure characterization.

Through its extramural program, EPA is also supporting several epidemiology studies to address the uncertainties regarding the health effects of chronic PM exposure. Five of the studies are developing a retrospective exposure assessment of long-term PM_{10}, $PM_{2.5}$ and other pollutants using currently available information on health and air quality. The investigators are using a permanent documented and traceable dataset from the EPA Air Quality System and are modeling individual exposure to PM based on subject address histories, knowledge of PM_{10} and $PM_{2.5}$ relationships, and additional data. These studies will assess mortality and nonfatal health endpoints in the existing cohorts, which are either regional or nationwide in scope, and will identify susceptible subgroups.

EPA recently awarded a grant to the University of Washington to conduct a longitudinal study of $PM_{2.5}$ exposure, the progression of cardiovascular disease (atherosclerosis), and cause-specific hospital admissions and mortality. The study cohort is being recruited from the NIH-funded Multi-Ethnic Study of Atherosclerosis with field sites in southern California; New York; Chicago, Illinois; Forsyth County in North Carolina; St. Paul, Minnesota; and Baltimore County, Maryland. Long-term individual-level exposure to ambient air pollutants will be modeled using data from existing ambient air monitors, study-specific monitors, questionnaires, and other sources. These studies will provide new and critically important information to understand the relation between long-term exposure to ambient air pollution and morbidity and mortality, and who is most susceptible.

Exposure Studies—Exposure and atmospheric scientists are developing data and models to characterize and predict exposures to PM from different sources relative to that measured at ambient sites, and are conducting field studies such as the Detroit Exposure Aerosol Research Study (DEARS) where elaborate source apportionment models are being used to track ambient PM to indoor and/or personal exposures. This is a 3-year study in Detroit where, over six seasons, PM and air toxics measurements are being taken of ambient air, PM characteristics as well as personal exposure measurements in 120 homes.

Accountability Research—Between 1990 and 2010, the annual cost of air pollution control is estimated to be $20 billion/year. A key research priority for ORD is to demonstrate to policy makers, legislators, industry, and the public how past efforts to reduce air pollution have yielded benefits and whether future efforts will continue to do so. The premise of "accountability research" is that improvements in public and ecological health arising from the implementation of regulations designed to reduce air pollution can be demonstrated with specific measures. ORD has developed a multifaceted approach to this research. Currently, there are pilot efforts to take advantage of advances in proteomic and genomic research to identify biomarkers in human blood or urine that would be indicative of exposure to priority air pollutants. Analogously, EPA is assessing existing databases and developing approaches to assessing ecological improvements associated with air regulatory improvements.

Support of HEI's accountability research program resulted in an initial conceptualization of the problem, methods development, and studies in informative geographic regions. HEI will build on this foundation in the coming 5 years with new studies that measure the health benefits of actions taken to improve air quality and with further development of methods including refining epidemiologic and

statistical approaches to tease out the health impact of long-term changes in air quality resulting from policy actions.

Emissions: Measurements and Modeling

EPA continues to improve source emissions information and models that are used by national, regional, state, or local officials to improve emissions inventories of particulate matter and its precursors (organics, ammonia), as well as ozone precursors (VOCs and NO_x). Near-term efforts will be focused on developing detailed information on emissions from aircraft and burning of various types of biomass, and on understanding sources that contribute to near-roadway exposures. Detailed analysis of adsorbed/absorbed organic particulate for the development of source signatures for receptor models is performed using these samples. Work to improve testing methodologies and techniques that can more accurately characterize emissions from various anthropogenic (mobile, area, stationary, and indoor) and natural (vegetation, trees, fires, open burning) sources will also continue.

Risk Management Research

In the near-term, risk management research is expected to focus on investigating the implications of technology combinations installed on utility boilers to meet PM and ozone standards and to investigate options that can simultaneously control multiple pollutants at reduced costs. As national and regional rules are implemented, the program will begin to shift towards those sources identified as significant contributors in states and localities still unable to meet the NAAQS. Source choices will be informed by the source to effects and source apportionment activities underway within and outside EPA.

Air Quality Monitoring

Federal Reference Methods (FRMs) and Federal Equivalent Methods (FEMs) form the backbone of the EPA's national monitoring strategy. They are the measurement methodologies that define attainment of a National Ambient Air Quality Standard (NAAQS). As knowledge and technology advance, new or modified FRMs and FEMs are developed. The EPA operates the Reference and Equivalency Program to designate FRMs/FEMs and provides support for technical issues that arise. The Reference and Equivalency Program accepts and evaluates applications for new FRMs/FEMs and designates those that meet the criteria. In addition, the Program publishes required guidance and quality assurance (QA) documents in the *Federal Register* and addresses technical or application issues in which the integrity of FRM/FEM monitors is questioned.

As issues arise about the methodologies used to measure the mass concentration of PM coarse, $PM_{2.5}$, and perhaps other PM standards, the EPA employs laboratory and field studies to compare and evaluate existing methods and, as needed, develop improved methods or design specifications. Laboratory studies include the generation of known aerosols, evaluation of method performance on these aerosols, and the application of aerosol science to elucidate performance problems and possible solutions to improve method performance. Emphasis is placed on continuous or semi-continuous methods and semi-automated gravimetric methods such as the dichotomous sampler. Field studies are conducted to compare the performance of various PM methods at a variety of sites and seasons. The field studies sometimes include physical or chemical characterization of the aerosols so that method performance as a function of aerosol properties can be examined.

Air Quality Modeling

Improvements to air quality models significantly reduce the differences between predicted and measured ambient PM concentrations in validation runs using historical data. As these differences are reduced, errors in model inputs (such as emission inventories) have a greater impact on the modeled results than in previous versions of the models. EPA will continue its commitment to improving atmospheric models such as the Community Multi-scale Air Quality Model (CMAQ) to incorporate atmospheric chemistry elements and to improving receptor models for use in developing State Implementation Plans (SIPs).

In recent years, EPA has supported over 30 grants concentrated on atmospheric science research that strengthens our atmospheric models and our ability to measure and predict changes in air quality. These projects focus on better understanding of sources of important components of PM, carbonaceous aerosol, secondary organic aerosol, source apportionment, and continuous measurement. Several of these grants are also relevant to Long-Term Goal 2 below. Results from this research are beginning to appear in journal articles and should increase in 2006 and 2007.

Long-Term Goal 2: Achieve progress in assessing source to health linkages and reducing uncertainties in these linkages.

EPA intends to balance its studies of specific PM components with an increasing emphasis on source-based effects. The goal is to more effectively target reduction of those emissions that drive potential exposures, and, ultimately, reduce public health risk. This new emphasis on linking sources to effects is reflected in the PM Centers program, with many research activities and different approaches described in each PM Center overview and set of abstracts. Source-to-effects research also captures the priorities emphasized in the recent NRC report to implement cross-disciplinary research integrating epidemiological, toxicological, exposure, atmospheric and engineering research. By definition, this research area requires the cooperation of multiple disciplines, an approach the NRC acknowledges has received more discussion than action to date.

Linking health effects to specific sources or components—EPA's research program in this area conducts studies to utilize PM samples, which were collected during controlled generation (combustion) from specific sources. Studies currently underway or planned include:

- Evaluation of different source emission particles on *in vitro* cell and organ systems as related to responses in compromised animal models.

- Evaluation of effects from diesel (truck and generator) emissions on allergy, asthma and infectivity animal models including studies of *in utero* exposures, guinea pigs, and infectivity (influenza and strep models). In addition, to evaluate the relative toxicity of particles vs. gaseous co-pollutants in diesel, filtering of PM or gaseous co-pollutants or retrofit/advanced technology engines may be evaluated. Parallel human studies using the same diesel truck are planned in healthy young adults, asthmatics, and filtering of PM, gaseous co-pollutants or retrofit/new engines.

- Evaluation of combustion emissions from a drop tube furnace (Coal +/- controlled contaminants) in animal models of susceptibility.

- Panel studies to evaluate of the effects of human exposure to sources dominated by single sources wood burning stove emissions.

Multi-Pollutant Multi-City Studies—Over the past several years EPA in partnership with the states has invested in an extensive monitoring network. As data from the STN network matures and sites have operated long enough to accumulate sufficient data to initiate epidemiological studies EPA will examine the links between adverse health effects, specific PM components, and PM sources. Standardized methods are being evaluated to ensure comparability of testing results across investigators and organizations. Improved methods will be identified that are amenable to Computational Toxicology approaches, which will necessitate the development of rapid screening approaches to extend and link the screening results to meaningful health outcomes. Paralleling these epidemiological studies are toxicological assessments (*in vitro* and in animals), which utilize samples from these same or analogous locales in the laboratory in an attempt to tease out component-associated effects and their mechanisms. A better understanding of mechanisms will inform predictions of not only singular component effects but also potential interactions.

Multiple Air Pollutant Program (MAPP)—Both the NRC and the CAAAC reports emphasize the need for EPA to move from a PM program to a multiple air pollutant program, one that is oriented toward PM and the other criteria pollutants, hazardous pollutants, and the other nonclassified components of the atmosphere. The Near-Roadway effort (below) will involve not only the complications of cross-disciplinary research, but also the challenges of multiple air pollutant research. At the same time, EPA is moving its research planning program toward this MAPP approach, such that our future multi-year plan for air research will begin to address the integration of air toxics and criteria air pollutant research. Collaborative efforts by EPA in-house researchers to couple laboratory chambers to biological systems and test realistic atmospheric mixtures to contemporary bioassays are being initiated. This will allow for the study of effects of PM under oxidative conditions that better mimic atmospheric influences and ambient mixtures.

Source Apportionment—EPA will be developing models and methods to identify local and regional source impacts and to identify sources of human exposure.

Several extramural grants supported by EPA are helping to advance the science toward robust source apportionment approaches. These include studies to develop the next generation of receptor models and to develop, improve, and evaluate advanced measurement techniques for source apportionment of organic PM.

Near-Roadway Research—As more state and local governments consider policies related to siting new schools and our federal partners are getting sued for plans to build highways near schools or residential neighborhoods, the impetus to better understand risks from near-roadway exposures has grown dramatically. The EPA research program is developing a focused multi-disciplinary study to examine this major PM source and the variations in components that are associated with toxicity. The details of this work are currently being discussed with the Office of Air and Radiation, and will reflect the input of external stakeholders collected at a meeting held in October 2005. Potential research topics include:

- An assessment of previous epidemiological studies focusing on harmonization of exposure and health end points.

- An evaluation of the validity of existing emissions and dispersion models.

- Guidance for measuring air quality near roads.
- Characterization of air pollution variability near roads.
- Characterization of pollutant infiltration into near-road schools.

Combustion Sources—EPA will continue to work on direct exposure diesel engine experiments examining a variety of engine parameters and animal models. Companion studies are also examining the generation and direct exposure of ultrafine particles from the combustion of pulverized coals. A further effort to connect source emissions to health effects is being conducted under a computational toxicology study designed to build a cross-species computational model describing the relationship between the physicochemical composition of diesel exhaust particles (DEP) and their mutagenic and inflammogenic health effects. Ultimately, this effort seeks to develop methods that will be used in quantitative human health risk assessments.

The National Health and Environmental Effects Research Laboratory Overview

Research Triangle Park, NC

Organizational Structure: NHEERL is the largest research organization in ORD, employing over 630 federal employees at various facilities across the country. The current organizational structure is diagramed in Figure 1. Based in Research Triangle Park, NC, NHEERL has nine divisions that specialize in different facets of human health or ecology research. Our Health Divisions are centrally located in Research Triangle Park and Chapel Hill, NC. Our Ecology Divisions are based in Gulf Breeze, FL; Duluth, MN; Corvallis, OR; and Narragansett, RI, each location representing a significant regional ecosystem (Gulf of Mexico, Great Lakes, Pacific Coast, and Atlantic Seaboard, respectively). Table 1 on the next page lays out the research focus for each NHEERL division. The NHEERL Particulate Matter Research Program is conducted primarily by the Experimental Toxicology Division and Human Studies Division.

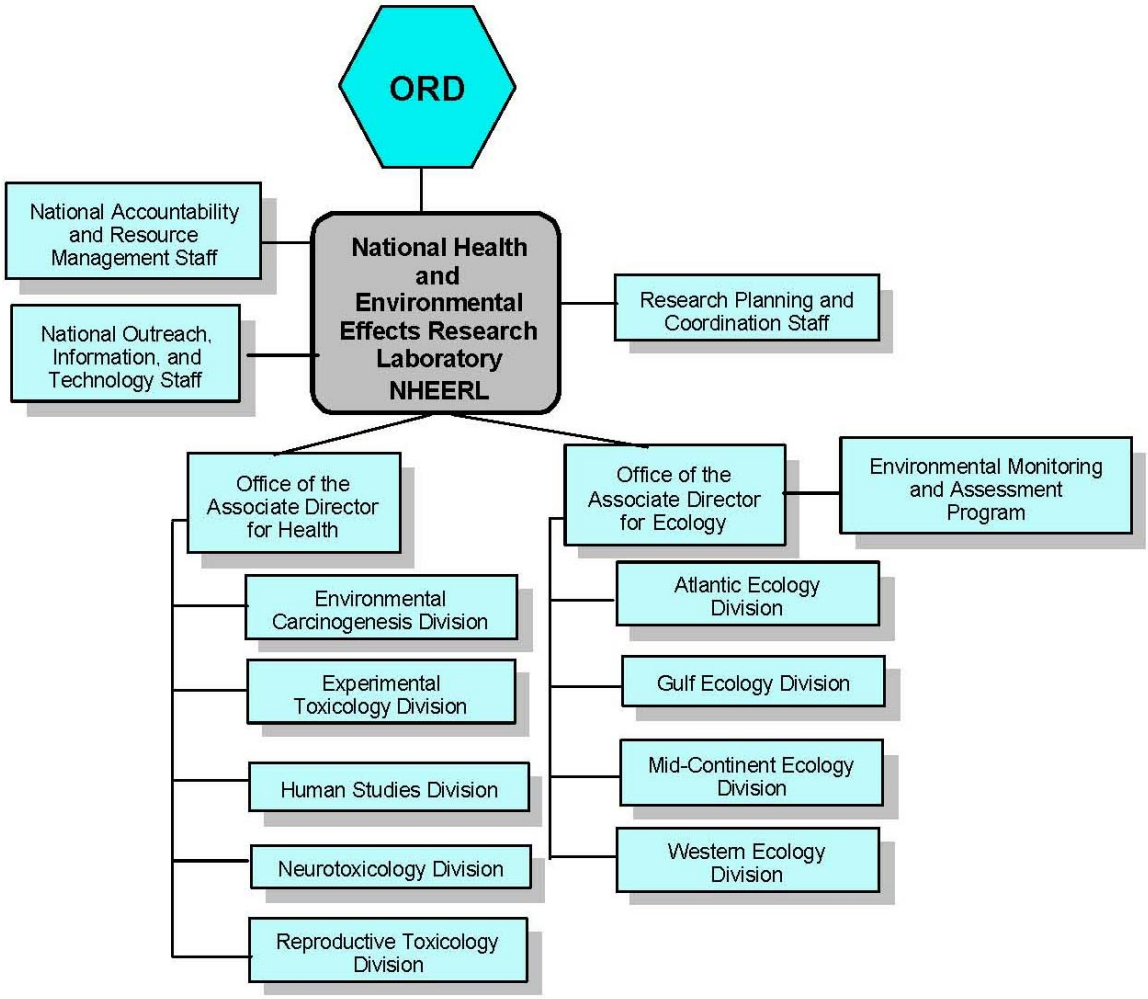


Figure 1. Organizational Structure of NHEERL.

Table 1. Overview of NHEERL's Health and Ecology Divisions.

DIVISION	LOCATION	RESEARCH FOCUS
Atlantic Ecology Division (AED)	Narragansett, RI	Studies the environmental effects of anthropogenic stressors on marine, coastal, and estuarine water quality, with an emphasis on the coastal waters and watersheds of the Atlantic seaboard. Areas of research specialization include modeling cumulative effects of multiple stressors on coastal ecosystems, developing methods for assessing the ecological effects of contaminated marine sediments, analyzing the role of biogeochemical processes on effects, and conducting geographic-based ecological assessments for the Atlantic coast.
Environmental Carcinogenesis Division (ECD)	Research Triangle Park, NC	Performs research to assess potential carcinogenicity of environmental chemicals. The aim is to reduce uncertainty in cancer risk assessment models by developing mechanistic data underlying chemical carcinogenesis for agents of environmental concern (including mixtures). This approach is enhanced by developing and applying biomarkers of response for predicting cancer outcomes and by incorporating information gained from structure-activity and molecular modeling approaches.
Experimental Toxicology Division (ETD)	Research Triangle Park, NC	Performs research to determine the health effects of environmental pollutants and cause-and-effect relationships at pollutant concentrations that mimic those in the environment. Investigations center on the pulmonary and cardiovascular systems; the immune system; and susceptibility to infectious, allergic, and neoplastic disease. Focal point for pharmacokinetic studies to elucidate dose-response relationships for systems susceptible to pollutants.
Gulf Ecology Division (GED)	Gulf Breeze, FL	Assesses the ecological condition of estuaries, coastal wetlands, coral reefs, and other critical habitats of the Gulf of Mexico. Determines cause(s) of affected and declining systems; predicts future risk to populations, communities, and ecosystems from aquatic stressors; and supports the establishment of criteria to protect critical habitats.
Human Studies Division (HSD)	Chapel Hill, NC	Conducts an interdisciplinary program of clinical and epidemiologic research that provides critical data for health risk assessment. Clinical studies determine the pharmacokinetics, dosimetry, and effects of pollutants in controlled exposure studies of healthy and susceptible individuals. Epidemiologic studies evaluate the relationship between real-world exposures and observed health effects in populations of interest. The program focuses on the effects of pollutants in air and water on the pulmonary, cardiovascular, and neurobehavioral systems.

Table 1. Overview of NHEERL's Health and Ecology Divisions. (continued)

Mid-continent Ecology Division (MED)	Duluth, MN Grosse Ile, MI (field station)	Develops methods for predicting and assessing the effects of anthropogenic stressors on freshwater ecological resources, including the Great Lakes and Great Rivers. Conducts cause-and-effect research on the effects of nutrients, clean sediments, climate change, and toxic chemicals on lake, stream, and wetland ecosystems, as well as aquatic life and wildlife communities and populations. Wildlife and aquatic life toxicology research, conducted in collaboration with human health-based research performed in NHEERL's health divisions, establishes advanced animal and dose-response extrapolation models to support integrated risk assessments.
Neurotoxicology Division (NTD)	Research Triangle Park, NC	Performs research to provide the scientific and technological means to predict the neurotoxicity of environmental agents in humans. Human neurotoxic disease is modeled in laboratory animals, and data are collected in animals to make predictions about possible neurotoxic risk. Studies range from the molecular level to the whole organism and include neurobehavioral, neurochemical, neurophysiological, and neuroanatomical approaches. Major emphasis on the study of sensitive subpopulations and developmental neurotoxicity.
Reproductive Toxicology Division (RTD)	Research Triangle Park, NC	Performs research on the effects of environmental pollutants on reproduction and development. Develops biological indices for assessing germ cell maturation, embryonic development, and adult reproductive capacity and endocrine status, integrating information into biologically based dose response models. Major research emphasis on assessing modes of action for endocrine disrupting chemicals and drinking water disinfection by-products in order to reduce uncertainties in the risk assessment of associated adverse reproductive outcomes.
Western Ecology Division (WED)	Corvallis, OR Newport, OR (field station)	Studies estuarine, terrestrial and watershed ecology with a focus on the Pacific Northwest region. Research emphasizes marine, coastal, and inland ecosystem functions and response to stress. Areas of specialization include ecological theory for spatial and temporal analysis of regional environmental data; developing methods for assessing regional-scale condition of ecological resources; and assessing the effects of changes in habitat and land use on terrestrial systems; and modeling of estuarine systems.

NHEERL's Mission: NHEERL is a problem-solving organization. We are EPA's focal point for research on the adverse effects of contaminants and environmental stressors on human health and ecosystem vitality. Our mission is to:

- Perform high-quality ***effects-based research*** to identify, understand, and solve current and future environmental problems;

- Provide ***leadership*** in addressing environmental issues; and

- Provide ***scientific and technical assistance*** at the local, state, federal, and international level.

These three elements, discussed separately below, interface squarely with the missions of EPA and ORD.

Research: Research provides EPA with the necessary information and technologies for detecting, abating, and avoiding environmental problems. NHEERL's approach to research, in accordance with ORD, is founded on principles of risk assessment. Our research is designed, within a risk assessment context, to answer scientific questions and reduce major uncertainties about the effects produced by pollutants and human activities on health and the environment. Our research focuses on two components of the risk assessment paradigm: *problem identification/formulation* (does the contaminant or stressor cause the adverse effect?) and *dose-response/stressor-response analysis* (what are the relationships between the contaminant or stressor and the extent of injury, disease, or damage?).

Rather than characterize our research as basic or applied, we use the terms core and problem-driven. **Core research** seeks to produce a fundamental understanding of the key biological, chemical, and physical processes that underlie environmental systems, thus forging basic scientific capabilities that can be applied to a wide range of environmental problems. **Problem-driven research**, on the other hand, focuses on specific environmental problems. Studies in these areas respond to explicit Agency needs and may be motivated by regulatory requirements or court-ordered deadlines. This type of research is exemplified by the Particulate Matter Research Program, in which the relationships between airborne particles and increases in morbidity and mortality are being studied to address critically important human health questions. Substantial efforts are made by NHEERL to build and maintain research programs that are both relevant to the scientific problem and responsive to Agency needs. The objective is to create an integrated and coherent program, not a collection of disconnected projects. There are three principal types of research product:

- *Methods* for detecting and characterizing hazard (e.g., new bioassays or ecological indicators);

- *Predictive models* for understanding and predicting relationships between stressors and response (e.g., biologically based dose-response models or computer models that predict the effects of climate change); and

- *Data* designed to fill information gaps and address limitations associated with risk assessment (e.g., toxicological or epidemiological data).

Leadership: NHEERL provides vital leadership in the environmental research arena, and its scientists are proactive in the scientific community at many levels. Within the Agency, we help shape the research agenda by contributing to research planning and coordination exercises, and we participate in the development of ORD Research Plans and Strategies. Our scientists represent the Agency on workshops and task forces addressing major risk assessment, public health, and environmental issues. Outside EPA, we influence the direction and priorities of environmental research worldwide. We steer collaborative research efforts at the national and international level, we are members of international planning committees and research review panels, we serve on advisory

boards of other major agencies and organizations, and we serve as adjunct faculty members at major universities across the Nation.

Scientific and Technical Assistance: As part of our mission, NHEERL responds to diverse requests for scientific advice and technical consultation, both within and outside EPA. We provide technical support to the Agency by advising EPA Program Offices and Regional Offices on scientific matters, by participating on Agency workgroups, and by helping to develop testing and risk assessment guidelines. We bring our expertise to bear at the national and international level by organizing scientific workgroups and symposia, and by serving in professional and scientific societies and on publication boards. We provide guidance to local, state, tribal, and international governments and other federal agencies, informing them on issues of environmental importance and enabling them to implement more effective environmental programs. We work to establish partnerships with the corporate, public, private, and educational sectors and assist them in setting and achieving environmental goals. We provide technical training and developmental opportunities for the senior scientist as well as the postdoctoral candidate and the student. By sharing our skills and knowledge, we enhance the ability of other organizations to protect public health and the environment, and we serve as an important catalyst for scientific and technological progress.

NHEERL Research PM Program Capabilities

NHEERL conducts multidisciplinary, problem-driven research to improve the basis of risk assessment in pulmonary, cardiovascular, hepatic, and immunotoxicology to improve the scientific basis for Agency decision-making. In all cases, both mechanistic and dosimetric information are involved at multiple levels of biological organization from molecular and biochemical, to cellular, tissue and organ, and to whole animal models. The work is conducted in an iterative fashion involving linked epidemiological, experimental clinical and animal studies and modeling, both for hypothesis generation and testing and experimental design, and validation of predictions. Below is a brief summary of select capabilities and facilities that support NHEERL's PM program and other programs throughout ORD and collaboration within the larger scientific community.

Animal Inhalation Facility: Inhalation is the primary route of exposure of the cardiopulmonary system to air pollutants. The animal inhalation facility experts provide support for exposures of laboratory rodents to a wide variety of gas/vapor/aerosol air pollutants and combustion sources. Much of this effort is directed toward the engineering support of studies of the health effects of ambient particulate matter (PM), the facility provides support for routine exposures and extensive support of compromised animal models, including:

- *Exposure of rodents to pre-collected PM,*
- *Collection of ambient PM for toxicological studies,*
- *Real–time exposure to concentrated fine and ultrafine ambient air PM, and*
- *Real–time exposure to combustion source derived materials.*

NHEERL scientists study health effects of air pollution generated by fuel oil, natural gas, and coal in combustion emission inhalation exposure facility

Animal exposures capabilities include:

- *Conventional Exposures:* (1) Whole body (O_3, SO_2, ovalbumin) exposures are routinely conducted; (2) Nose-only exposures are provided to meet specific research or test agent requirements;

- *Specialized Exposures:* Since ambient PM available for use in rodent inhalation exposures is very limited, a generator capable of using very small quantities of PM was developed to conduct nose-only exposures;

- *Real-Time PM Exposures:* A Concentrated Ambient Particle system (CAPS), designed by Harvard and NERL–EPA to concentrate and deliver real-time ambient PM to a rodent exposure chamber, was established; and

- *Satellite Combustion Exposure Facility:* In collaboration with NRMRL, rodents exposure to diluted effluent from diesel sources, commercial oil burner sources, coal ash, and other combustion sources are conducted.

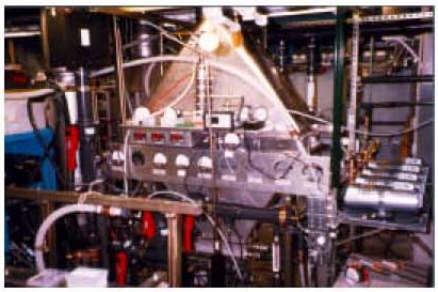
Chemical Analyses: Chemical characterization of PM is performed using inductively coupled plasma–atomic emission spectrophotometry (ICP–AES) and ICP-mass spectrophotometry. Transition metals (e.g., iron, vanadium, nickel, zinc, etc.) are of special interest to the toxicologists. Rodent organs, especially lungs, and body fluids are also analyzed for metals. Additional characterization for organics or semi-volatiles is being conducted in collaboration with NERL–EPA.

Clinical exposure capabilities consist of several exposure chambers, which have the flexibility of producing a wide range of inhaled pollutants as well as associated medical and recruitment facilities. It is made up of the following elements:

- 8 *In Vivo* Exposure Chambers for Human Clinical Studies
- 2 Large (300 sq. ft.) Rochester-style chambers
- 3 Small (64 sq. ft.) Rochester-style chambers
- 2 Neurophysiological testing chambers
- Chambers for studies of concentrated ambient fine ultrafine and coarse aerosols
- *In Vitro* Exposure Chambers for Cell Biology Research
- A Medical Station Staffed and Equipped for the following:
  - physical examinations of subjects
  - routine clinical laboratory work for subjects

- special procedures:
  - Bronchoalveolar and nasal lavages
  - Nasal, brush, and endobronchial biopsies
  - Induced sputum
  - Test rooms for subject training

- Extensive Freezer Capacity for Specimen and Environmental Sample Retention



- An Onsite Contractor To Recruit Subjects for HSD Studies
  - recruitment and payment of subjects
  - conduct preliminary testing and screening

- An Onsite Contractor To Modify, Operate, and Maintain Critical Exposure Systems
  - perform pollutant and processed air delivery
  - perform routine calibration of pollutant delivery and data acquisition equipment
  - update and improve existing exposure systems to meet changing requirements
  - design and build new exposure systems to meet new requirements
  - conduct electrical safety assurance testing on medical instrumentation used with research subjects.

National Exposure Research Laboratory Overview

Research Triangle Park, NC

Who Are We?

The National Exposure Research Laboratory (NERL) is one of three national laboratories that conduct research for EPA's Office of Research and Development. NERL headquarters and two of its research divisions are located in Research Triangle Park, NC. Other research divisions are located in Athens, GA; Cincinnati, OH; and Las Vegas, NV. NERL conducts research and development that leads to improved methods, measurements, and models to assess and predict exposures of humans and ecosystems to harmful pollutants and other conditions in air, water, soil, and food.

What Is Environmental Exposure?

Exposure is the contact of people (or other organisms) with an environmental stressor for a specific duration of time. Stressors can include chemical pollutants, microbes or pathogens, or physical agents, like radiation or even processes such as alteration of wildlife habitat.

Assessing environmental exposure involves a number of elements:

- Characterizing sources of pollution, including mobile sources such as automobiles; point sources such as industrial plants; and non-point sources such as agricultural run-off and land-management practices.

- Understanding and modeling the processes that control the distribution, transport, transformation, and fate of these pollutants–stressors, as they move through the environment, from sources to "receptors" (the humans, plants, animals, or ecosystems that may be exposed).

- Characterizing actual exposure—measuring and modeling how humans and ecosystems come into contact with pollutants–stressors. Since exposure involves both the pollutant–stressor and the "receptor" (e.g., the human or ecosystem) which is exposed, together with duration of exposure, exposure assessment requires a variety of efforts, including:

    - measuring and modeling pollutants–stressors in various media (e.g., measuring pollutant levels in the food people eat, in the water they drink, in the air they breathe, and on the things they touch); and

    - describing the behavior patterns of people or animals that affect their exposure, like the daily activities of people (the what, when, where and how long) that bring them into contact with pollutants or the distribution of sensitive ecosystems within a polluted watershed.

- Assessing the effectiveness of an exposure, including making measurements on receptors that provide evidence that they have been exposed. NERL's research may include: determination and modeling of uptake or transfer efficiencies or describing the dose to target organs; characterizing indicators of exposure, like measuring biological markers of exposure in people or animals; identifying antibodies in humans resulting from exposure to pathogens; characterizing changes in

wetlands or forest cover; or measuring changes induced in the community composition in ecosystems.

Why Is Environmental Exposure Important?

In carrying out its mission to protect the environment and safeguard human health, EPA must understand the risks posed by pollutants and other stressors. Exposure assessment is one critical input used by EPA and others to assess those risks. Chemicals that are quite toxic may pose little actual risk if exposures are low; conversely, relatively nontoxic stressors may pose substantial risks if people or wildlife are highly exposed. As a result, understanding exposure is essential in assessing the risks that may arise from current or new technologies, policies and regulations, and even increased growth in populations, changes in energy use, and fluctuations in the economy. NERL research improves risk assessment through characterizing pollution sources; developing environmental fate and transport computer models that can be used to quantify how risk management options are likely to affect exposures; developing and enhancing measurement methods for pollutants and exposure indicators; and developing exposure models that reflect individual behaviors and microenvironments. Exposure measurements, methods, and models also are important in: (1) determining whether or not a pollutant or stressor represents an unacceptable risk; (2) selecting the most appropriate approaches to reduce that risk; and (3) tracking compliance with environmental regulations and achieving environmental goals.

How Does NERL Conduct Research?

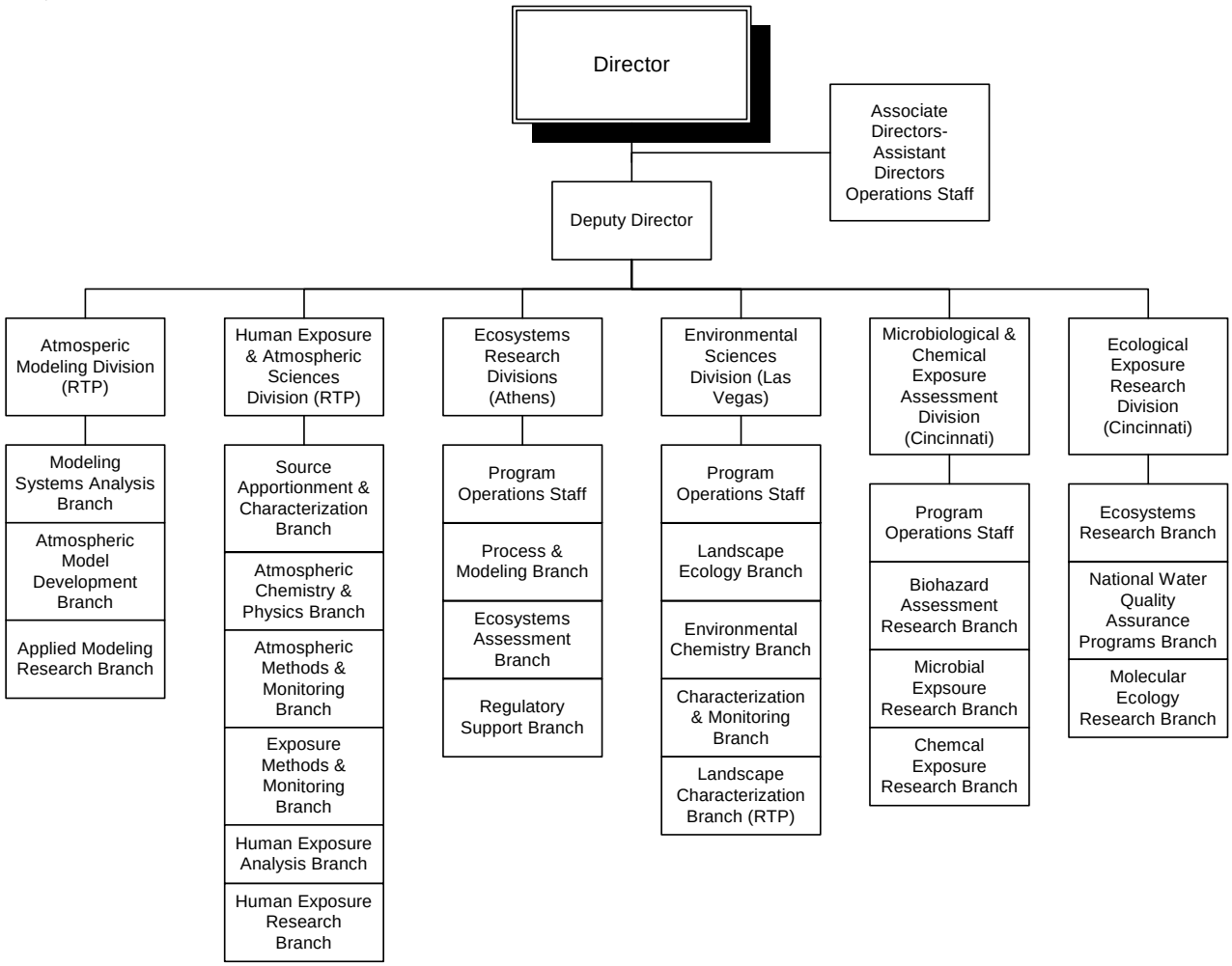
The Laboratory has an in-house workforce of more than 400 scientists, engineers, and other permanent federal employees. In addition, a staff of research meteorologists from the National Oceanographic and Atmospheric Administration work to support NERLs mission under an inter-Agency agreement. NERL also provides temporary training opportunities for undergraduate and graduate students and postdoctoral trainees, and sponsors senior citizens through the Senior Environmental Employment Program. A significant portion of the NERL's research is conducted in collaboration with the other Laboratories and Centers of ORD whose missions are health and ecological effects research, risk assessment, and risk management. This linkage allows ORD to achieve its overall mission of understanding environmental problems and developing tools and technologies to prevent or reduce them. Other NERL projects rely on extensive external collaboration with entities such as other federal agencies, states, industry, utilities, universities, and other nonprofit organizations.

Research and Technical Support for Regulatory Programs

NERL scientists provide a wide range of research and regulatory technical support to EPA program and regional offices, and to the States, and foreign governments. In particular, NERL provides substantial support in regulatory monitoring methods, waste site characterization, computer modeling of pollutant transport and fate, remote sensing, monitoring network design, environmental indicators, and design of exposure assessment studies. NERL uses a variety of mechanisms to communicate its research products to program offices, to the public, and to the international community.

National Exposure Research Laboratory Organizational Structure

The NERL is comprised of six divisions with diversified research specialties. NERL conducts research and development that leads to improved methods, measurements and models to assess and predict exposures of humans and ecosystems to harmful pollutants and other conditions in air, water, soil, and food.



Atmospheric Modeling Division (AMD)

The Division, headquartered in Research Triangle Park, North Carolina, composed of three branches is part of National Oceanic Atmospheric Administration's (NOAA's) Air Resources Laboratory. The AMD conducts intramural and extramural research programs to develop predictive models on local, regional, and global scales for assessing changes in air quality and air pollutant exposures as affected by changes in ecosystem management and regulatory decisions. The AMD research activities are designed to:

- Develop, evaluate, and validate air quality simulation, photochemical, and meteorological/ climatological models that describe and predict air quality and atmospheric processes affecting the ultimate disposition of airborne pollutants on local, urban, and regional scales;

- Perform and direct interagency research necessary to support ecological risk assessment by producing quantitative evaluations of changes in regional climate and air quality attributable to global climate fluctuations;

- Develop and apply fluid modeling techniques that describe atmospheric physical processes affecting buoyant and dense gas pollutant dispersion under unique meteorological situations, terrain features, and source configurations;

- Implement modeling software design, systems analysis, and high-performance computing research within stated Agency requirements of quality control and assurance to support atmospheric dispersion modeling, meteorological/climatological research, and predictive applications;

- Develop and provide to the user community evaluated improvements to existing air quality simulation models, meteorological models, pollutant exposure models, and related model input parameters as well as network access to newly developed air quality models;

- Develop and apply statistical and mathematical theory related to the acquisition, interpretation, and modeling of measurements of human activities, exposures, environmental concentrations, and sources of pollution;

- Provide technical guidance on applying and evaluating air quality simulation models that are used to assess, develop, or revise air pollution control strategies for attainment/maintenance of ambient air quality standards;

- Serve as key point of contact between NOAA and EPA atmospheric research by funneling EPA needs to the NOAA research community and conversely, by transferring and interpreting NOAA research results to the EPA; and

- Maintain communications with the EPA Research, Program, and Regional Offices, and with the national and international scientific community to incorporate and disseminate state-of-the science developments pertaining to meteorological/climatological aspects of environmental quality and exposure assessment.

Human Exposure and Atmospheric Sciences Division (HEASD)

The HEASD, headquartered in Research Triangle Park, North Carolina, is composed of six branches. Five branches are in the Research Triangle Park and one branch is located in Las Vegas, Nevada. The HEASD conducts intramural and extramural research to characterize exposures across the whole of the exposure assessment paradigm from the pollutant source to the exposed person or receptor.

While the principal focus for the Division's research is human exposure and pollutants that are emitted to and move through the atmosphere, much of the Division's process-oriented research is equally applicable to and important for understanding ecosystem exposures and pollutants found in media other than air. The Division's research mission encompasses aspects of all components of the exposure assessment paradigm, including exposure/source research; physical, chemical, and biological processes modeling research; environmental characterization research, including characterizing

microenvironmental concentrations/exposures, and defining the critical routes of exposure; exposure assessment/analysis research; and exposure/dose research.

The scientific insight and understanding that the Division's research provides is critically important to the Agency's risk management efforts. Through identification and characterization of the chemical, physical, or other processes that: (1) affect anthropogenic and biogenic pollutant emissions; (2) control their accumulation, formation, transformation, transport, and fate through the air and into other media; and (3) define the critical routes of exposure and the magnitude of those exposures and the subsequent dose, HEASD provides the Agency with the critical scientific understanding needed to mitigate exposures and risks in a technically sound and cost-effective manner.

Ecosystems Research Division (ERD)

The ERD, located in Athens, Georgia, is composed of three branches and a Program Operations Staff. ERD conducts research on the multimedia fate of organic and inorganic chemicals, greenhouse gas biogeochemical cycles, and land use perturbations that create direct and indirect, chemical and nonchemical, stressor exposures and potential risks to humans and ecosystems. Comprehensive models based on fundamental studies of stressor behavior are developed to predict exposures in multimedia environments, to simulate the interactions of the climate system and the terrestrial biosphere, and to evaluate the aggregate causes of ecological stress, including land use change/ management, within a watershed/regional context. Field and laboratory experiments are conducted to quantify and model greenhouse gas fluxes between the atmosphere and the terrestrial biosphere and to understand abiotic and biotic pollutant fate processes in soils, sediments, and water. ERD develops, tests, applies, and provides technical support for exposure and ecosystem response models used for assessing and managing stressor's risks to humans and ecosystems that are state-of-the-art and produce estimates of known uncertainty. Major modeling emphases are: earth systems models for evaluating and minimizing/managing the global and regional risks from climate and land use changes; ecosystem response models for evaluating and minimizing/managing the exposure and risks from multiple stressors within watersheds; and models for evaluating the multimedia fate of and potential exposures to chemicals.

Environmental Sciences Division (ESD)

The ESD headquartered in Las Vegas, Nevada, is composed of five branches. Four branches are located in Las Vegas, one branch is located in Research Triangle Park, North Carolina, and the Environmental Photographic Interpretation Center (EPIC) is in Reston, Virginia. ESD conducts research, development, and technology transfer programs on environmental exposures to ecological and human receptors. ESD develops methods for characterizing chemical and physical stressors with special emphasis on ecological exposure. The division develops landscape and regional assessment capabilities through the use of remote sensing and advanced spatial analysis techniques. ESD conducts analytical chemistry research and applies advanced monitoring technology to issues involving surface and subsurface contamination. To carry out its mission, the division applies a multidisciplinary, multimedia approach in both laboratory and field settings.

Microbiological and Chemical Exposure Assessment Research Division (MCEARD)

The MCEARD, located in Cincinnati, Ohio, is composed of three branches and a Program Operations Staff. The MCEARD conducts research to measure, characterize, and predict the exposure of humans to chemical and microbial hazards. This research is providing information on environmental pathways over which hazardous contaminants are transported via air, water, food, and soil to populations at risk. Analytical quantitative methods are developed to accurately and specifically measure human risk factors associated with inhalation, ingestion, and dermal pathways. Surveys and monitoring studies are carried out to determine the levels of hazardous chemicals and microbials in environmental matrices, and human populations are studied to determine significant exposure pathways, the levels of exposure, and the sources of exposure factors. State-of the-art tools are used to measure organic and inorganic chemicals, hazardous bacteria, viruses, fungi, and protozoa, and detect evidence of exposure to environmental hazards. The division conducts its multidiscipline research program with a broad skill mix of scientists that includes organic, inorganic, and analytical chemists, bacteriologists, virologists, parasitologists, immunologists, and molecular biologists.

Ecological Exposure Research Division (EERD)

The EERD, located in Cincinnati, Ohio, is composed of three branches. The EERD conducts research to develop diagnostic tools that assist the Agency in identifying stressors and their sources and to quantify the intensity of these stressors in aquatic and terrestrial ecosystems. The division employs laboratory- and field-based study designs to produce research products that enable the Agency to conduct exposure assessments to facilitate the top-down approach to ecological risk assessments. In the pursuit of these broad goals, the EERD:

- Applies molecular, biochemical, and cellular methods to improve detection and quantification of exposures at the cellular, organism, and population levels;

- Utilizes molecular and cellular response measures to determine food web exposure pathways and to integrate cumulative impacts of complex mixtures and multiple stressors for predictive exposure assessment;

- Combines community indices from bio-assessment with biological markers to develop diagnostic source signatures for documentation of causation and for retrospective exposure assessment;

- Applies community-level structural and functional bioassessment metrics as a quantifier of nonchemical stressor (e.g., habitat loss) intensity; and

- To conduct this research, the division engages ecologists, toxicologists, and biochemists to conduct studies on a variety of scales of biological organization ranging from the molecular and organism level to the population and community scale. The division provides biological reference materials to the Regions and states, and assists with performance evaluation studies to validate and standardize Agency biological methods.

National Risk Management Research Laboratory Overview

Cincinnati, OH

Organizational Structure

The National Risk Management Research Laboratory (NRMRL), one of seven Laboratories and Centers within the Environmental Protection Agency's (EPA's) Office of Research and Development (ORD), is staffed by nearly 400 federal employees at various facilities across the country. The current organizational structure is depicted in Figure 1. NRMRL, based in Cincinnati, Ohio, has six divisions that work together to characterize releases to land, water, and air; identify approaches to minimize or eliminate these releases; and transfer the results in forms that can be used by a variety of customers, including other offices within EPA, states, Regions, and other Federal agencies. The Land Remediation and Pollution Control, Water Supply and Water Resources, Sustainable Technology, and Technology Transfer and Support Divisions are located in Cincinnati, Ohio. The Air Pollution Prevention and Control Division (APPCD) is located in Research Triangle Park, North Carolina, and the Ground Water and Ecosystem Restoration Division is located in Ada, Oklahoma. The NRMRL component of the overall Particulate Matter Research Program is conducted through APPCD.

Figure 1. Organizational Structure of NRMRL.

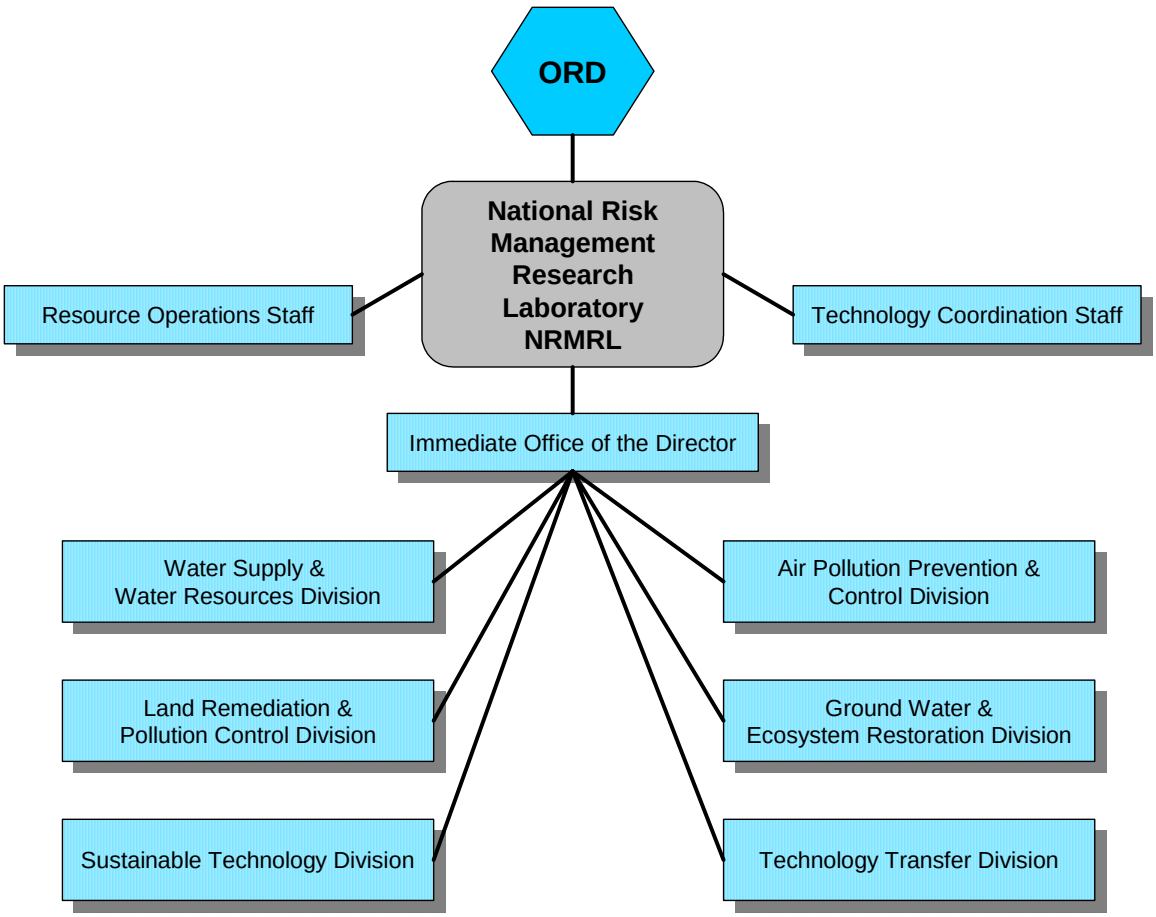


Table 1. Overview of NRMRL's Divisions.

DIVISION	LOCATION	RESEARCH FOCUS
Air Pollution Prevention and Control Division (APPCD)	Research Triangle Park, NC	Characterizes emissions from major air pollution sources (e.g., power plants, incinerators, materials used indoors); develops and demonstrates techniques to prevent or control emissions from these sources; and verifies technology performance. APPCD works closely with trade and professional organizations, industry, and academia in all these areas. The Division concentrates its efforts in five main program areas: Air Toxics, Fine Particles, Indoor Air Quality, Ozone, and Global Climate Change.
Ground Water and Ecosystem Restoration Division (GWERD)	Ada, Oklahoma	Conducts research and provides technical assistance to establish the scientific basis to support the development of strategies and technologies to protect and restore ground water, surface water, and ecosystems impacted by man-made and natural processes.
Land Remediation and Pollution Control Division (LRPCD)	Cincinnati, Ohio	Conducts research to explore innovative solutions to current and future land pollution problems. Its programs include field evaluation and demonstration of innovative technologies; verification of externally acquired data; development, testing of management techniques and disposal practices for municipal waste sites, and restoration of Brownfield sites for beneficial and sustainable uses. LRPCD has a strong technical assistance capability that is applied to both Superfund and non-Superfund contaminated sites. Through these programs, LRPCD encourages the development of reliable and cost-effective alternatives for the domestic, federal, and international markets.
Sustainable Technology Division (STD)	Cincinnati, Ohio	Conducts research to advance clean industrial production methods and processes by developing and applying process design and assessment tools and methods including life-cycle assessments, environmental impact assessment, and property estimation; develops approaches and tools for sustainable environmental management of urban watershed and ecosystems. All of the research is done considering the multimedia implications of risk management approaches.
Technology Transfer and Support Division (TTSD)	Cincinnati, Ohio	Communicates information about EPA scientific advances through technology transfer publications, software, multimedia products, and technical meetings to inform the regulated community, environmental consultants, and local decision-makers.

Water Supply and Water Resources Division (WSWRD)	Cincinnati, Ohio	Conducts research to help prepare the primary and secondary regulations for drinking water and to develop technologies and strategies for controlling waterborne contaminants. The program integrates chemistry, engineering, microbiology, computer modeling, and cost analysis to provide effective, reliable and cost-effective techniques. WSWRD programs include research on urban and non-urban storm water runoff; combined and sanitary sewer overflows; underground and aboveground storage tanks and oil spills; and contaminated sediments.

NRMRL's Mission

NRMRL is a problem-solving organization. We characterize releases of environmental stressors and approaches to prevent and reduce pollution risks associated with them. The laboratory contributes to public health and ecosystem protection through control, prevention, and remediation of pollution of air, land, and water and to restore ecosystems. Our mission is to: (1) understand and develop ways to quantify or estimate pollutant releases from various source types; (2) investigate technologies and other risk management approaches that can reduce or eliminate releases; (3) provide the scientific and engineering information to support regulatory and policy decisions; (4) ensure effective implementation of environmental regulations through technical support and information transfer; and (5) collaborate with partners in the public and private sector to foster technologies that reduce compliance costs.

Research Approach

NRMRL has a flexible research program that can apply a set of core expertise and facilities to a wide spectrum of environmental problems. From research through field evaluation, risk management research activities combine in-house work, extramural activities, and federal/state partnerships. NRMRL's activities range from bench-scale experiments of innovative concepts to full-scale field testing of the most promising technologies. Evaluation and verification of technologies developed by others and all types of engineering and economic modeling are also used to address many of the priority research questions. Laboratory and field studies to quantify pollutant releases are also regularly conducted and are often translated into models that predict these releases at various spatial scales under different operating and environmental conditions. Much of the work is carried out by principal investigators and technicians with varied backgrounds such as chemical engineering, economics, and forestry. The results of the research are transferred to customers in various forms ranging from direct technical assistance to peer-reviewed journal articles. The organization constantly evaluates emerging issues that may impact future environmental risk management and regularly adjusts the balance between problem-driven research and more "core" research to investigate these emerging areas.

Leadership

NRMRL provides vital leadership in the environmental research arena, and its engineers and scientists are proactive in the scientific community at many levels. Within the Agency, we help shape the research agenda by participating on cross-laboratory and EPA committees, and we participate in the development of ORD Research Plans and Strategies. Our engineers and scientists represent the Agency at workshops and on task forces that address major risk management issues. Outside EPA, we influence the direction and priorities of environmental research worldwide. We steer collaborative research efforts at both national and international levels; we are members of national and international planning committees and research review panels, and we serve on advisory boards of other major agencies and organizations.

Scientific and Technical Assistance

As part of our mission, NRMRL responds to diverse requests for scientific advice and technical consultation, both within and outside EPA. We provide technical support to the Agency by advising EPA Program Offices and Regional Offices on scientific matters, by participating on Agency workgroups, and by helping develop testing protocols and risk management guidance. We bring our expertise to bear at the national and international level by organizing scientific workgroups and symposia and by serving in professional and scientific societies and on publication boards. We provide guidance to local, state, tribal, and international governments and other federal agencies, informing them on issues of environmental importance and enabling them to implement more effective environmental management strategies. We work to establish partnerships with the corporate, public, private, and educational sectors and assist them to set and achieve environmental goals. By sharing our skills and knowledge, we enhance the ability of other organizations to protect public health and the environment, and we serve as an important catalyst for scientific and technological progress and innovation.

NRMRL NAAQS Research Capabilities

NRMRL uses its air emissions characterization and control technology facilities and expertise to support the goals and objectives of the Agency's NAAQS program. Examples of these capabilities are listed below:

Emissions Characterization:

Open Burning Test Facility

Subject wastes and biomass are combustion-tested in an enclosed Open Burn Test Facility (OBTF). In-Facility air measurements or stack concentrations are coupled with fuel mass loss and facility gas flow rates to calculate emission factors for PM and other pollutants of concern.

The open burning test facility (OBTF) has been used for several emission characterization studies related to open burning of various materials. The OBTF uses a refractory-lined burn pit on a weighing platform, coupled with a high volume air handling system to simulate in-plume measurements taken during open burning events.

Chassis Dynamometer Facility

NRMRL is now installing a chassis dynamometer for testing of heavy duty diesel trucks. A test cell is being constructed to provide temperature and humidity control and forced air onto the truck radiator to simulate actual speed conditions on the highway. Once finished, the facility will be capable of simulating and testing on-road operation of vehicles as large as 80,000 lbs GVW, as well as long-haul tractor-trailer rigs and pickup trucks and allow for analysis of fine particulate, oxides of nitrogen, oxides of carbon, and air toxic emissions. Different test cycles can be programmed and gaseous emissions can be monitored both as extracted from the tailpipe or after dilution in a standard EPA constant volume sampler. Total particle emissions can be measured in the CVS. Additional analytical equipment includes GC-MS, FTIR, and particle sizing units to enable real-time or near real-time data. This facility can be used as a source of fresh emissions for human and animal testing. A large bay is located next to the facility, which can be used to locate the test subjects.

Emissions Measurement Using Optical Remote Sensing (ORS)

Optical analytical technique can be applied to provide real-time information of air emissions from fugitive sources. Pollutant concentrations are acquired using Fourier Transform Infrared (FTIR) and Tunable Diode Laser (TDL). This information is combined with wind speed and direction to

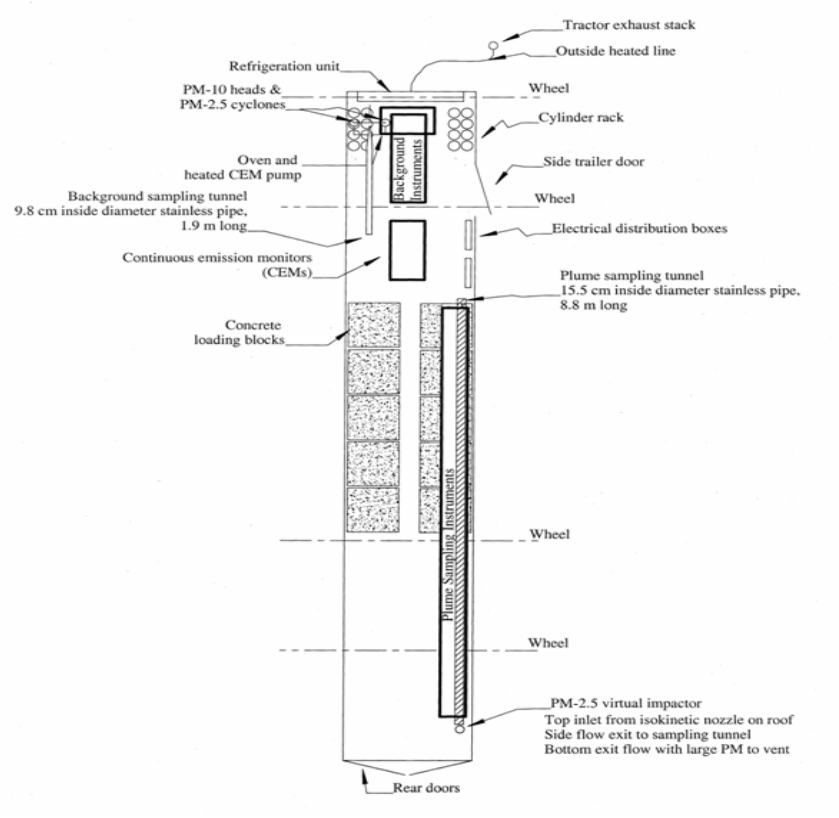
generate a direct measurement of pollutant emission. Data are analyzed to produce emission estimates.

Emission plumes are characterized on both vertical and horizontal planes to determine the amount and/or location of pollutant gases. ORS can be used to characterize emissions from a variety of sources, including roadways, agricultural operations, industrial facilities, and landfills. It can also be used to measure airborne toxic chemicals and warfare agents. This capability includes three FTIRs, Near IR Diode Laser, deep UV-Cerex and UV-OPSIS remote sensing equipment.

Mobile Emissions Real-Time Analysis Laboratory:

NRMRL also has developed a portable laboratory for field testing of fine particulate matter and gaseous emissions. Sampler and analyzers in the laboratory trailer are used to sample emissions directly from the plume of the diesel truck pulling the sampling trailer or from the plume of emis-

sions from other sources. A long sampling probe is used for sampling of jet engines with this laboratory. The trailer may also be loaded on a flat bed car for measurement of emissions from the plume of a locomotive. This Diesel Emissions Aerosol Laboratory (DEAL) can be used to evaluate the effects of natural cooling and dilution on the formation of PM.

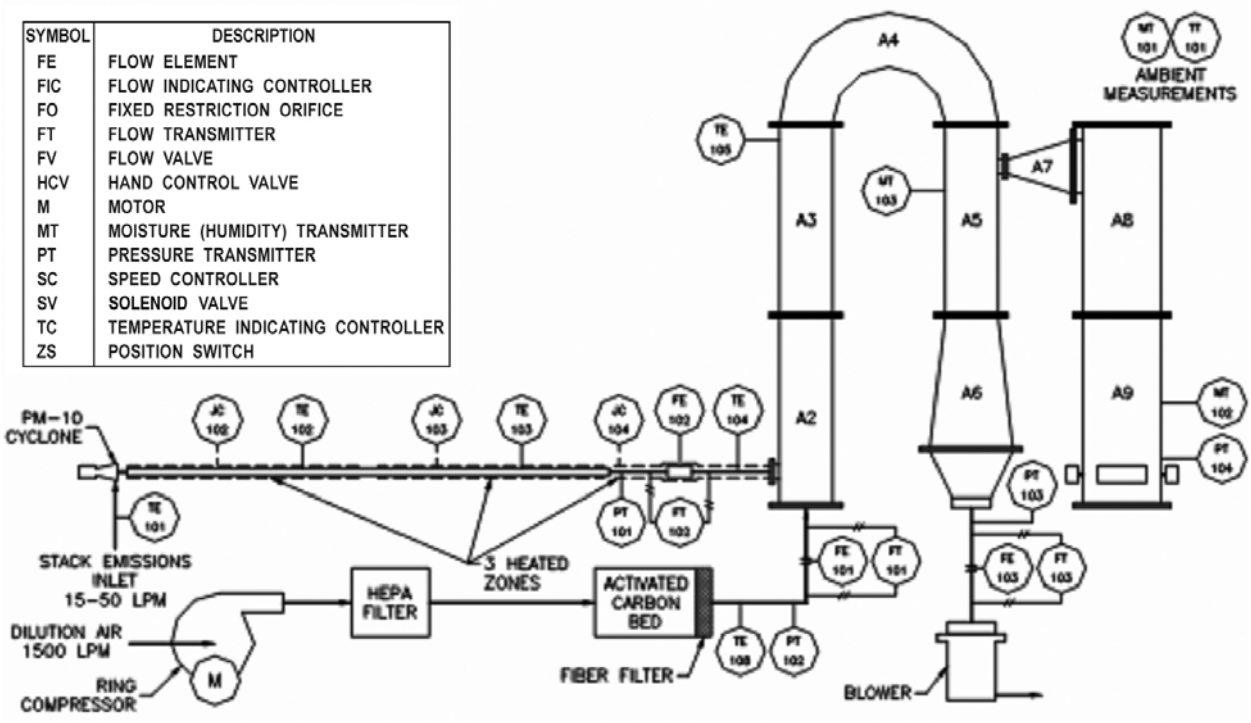


Field Test Stack Sampling Equipment

Field test equipment for sampling emissions from stationary sources includes a dilution sampler stack testing facility. The dilution sampler was developed by the California Institute of Technology for the purpose of collecting samples for receptor modeling analysis. EPA has constructed a similar system to provide the dilution cooling and residence time for near completion of gas to particle partitioning. Multiple sample measurement units can be connected to the sampling ports.

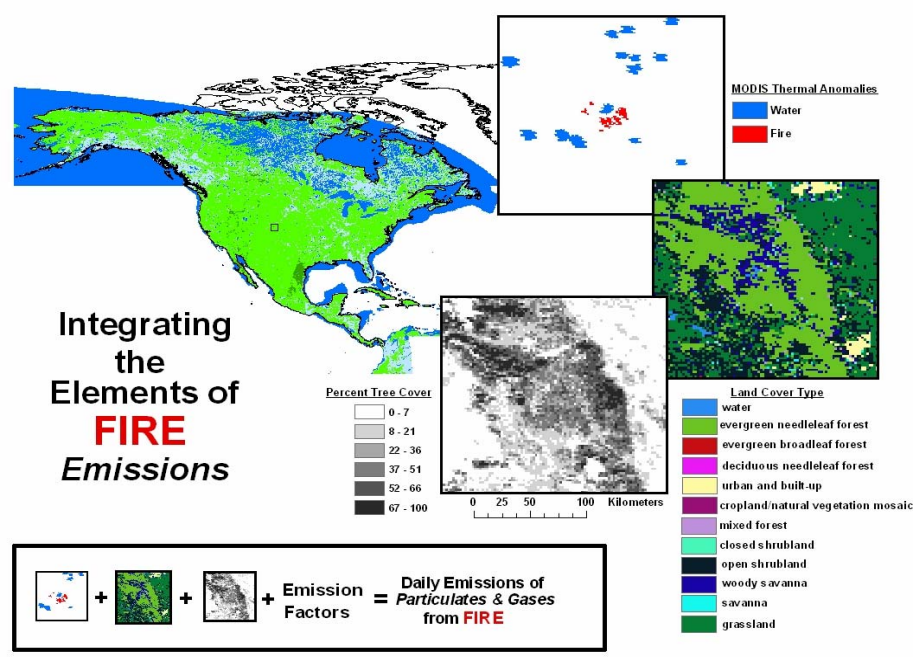
Analytical Chemistry Laboratory

NRMRL has a state-of-the-art facility is available to characterize the organic fraction of the collected particle samples. This facility also works on developing innovative sample analysis techniques. This laboratory contains various state-of-the-art analytical equipment such as Gel Permeation Chromatograph, GC/Mass Spec, Ion Chromatograph Detector, Elemental Carbon/Organic Carbon ratio, and High Pressure Liquid Chromatograph equipment to apportion emissions to the corresponding sources. Source emission profiles for fine PM ($PM_{2.5}$) are needed together with ambient $PM_{2.5}$ composition from local or regional air monitoring stations to enable states to apportion ambient fine PM to the responsible sources. This information in turn is used to construct a strategy for bringing any non-attainment areas into compliance with the $PM_{2.5}$ NAAQS.



Natural and Specialized Ambient Emissions and Modeling:

Emissions from natural sources are important contributors to many environmental problems. Our researchers have the capability to conduct onsite emission testing of natural sources such as forests and other vegetative species at both the ground and canopy levels. These include biogenic VOCs with current emphasis placed on fast reacting species and their role in aerosol formation. In addition, the techniques being developed and utilized address sources of ammonia and its fate in the biosphere. Biomass burning, such as in prescribed burns in forests, are a major activity. In each area, the work includes utilizing the data to enhance databases and to develop and/or improve models. This work has been extended to transboundary pollutants and has involved testing campaigns in other countries (e.g., Brazil and China).



Combustion-Generated Particulate Matter

APPCD's has facilities to combust fuels under conditions that simulate those in full-scale sources, and evaluate the PM emissions for mass concentration, particle size distributions, and particle composition. At the same time, Dr. Linak and his colleagues have developed systems for diluting the exhaust gases to control PM concentrations to levels that are suitable for animal inhalation exposure testing.

This effort provides the health researchers with real-world particles that are well-characterized in terms of their size and chemical composition, and allows a much more complete evaluation of any links between particle attributes and health responses. By connecting the diluted exhaust directly to exposure chambers, the health studies are able to examine the effects of inhalation exposures that do not rely on resuspended particles or particles from sources that are not well characterized. From an engineering perspective, these studies provide guidance concerning what operating conditions may lead to emissions that are relatively more toxic than those generated under other conditions, yielding information about possible risk reduction strategies.

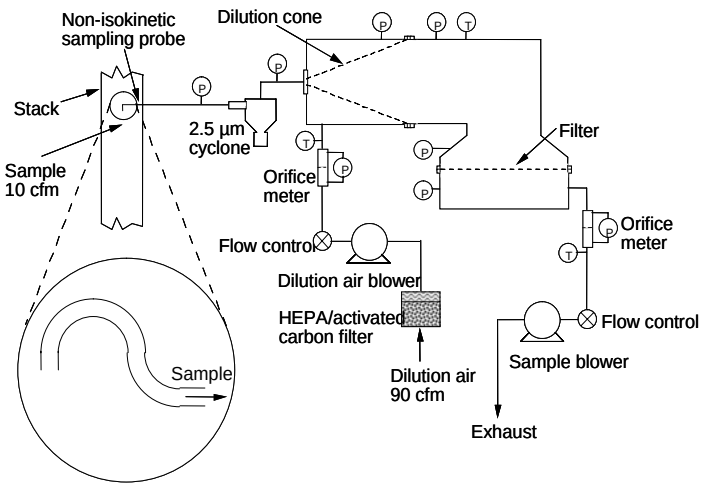


Figure 10. Schematic of PM dilution sampling system.

Two-Compartment Chamber Research Facility

As a result of recent studies indicating significant correlations of increased mortality and morbidity with increases in ambient fine particles, it has become even more important that we better understand personal exposures to fine particles indoors. Most people spend most of their time indoors, and the ones at greatest risk in the studies mentioned above spend nearly all of their time indoors.

One of the major thrusts' of APPCD's fine-particle research efforts is to determine the contribution to personal indoor exposure of fine particles that originate outdoors. The Division's two-compartment research facility is used to study the mechanisms by which fine particles in the outdoors penetrate into the indoor environment.

The facility consists of two, nearly identical, room-sized (30 m^3) compartments. The compartments are separated by a partition in which simulated air entry paths can be mounted. The partition has an open surface area of 9 m^2. This facility is used to verify mathematical models for penetration of fine particles through well-defined entry paths. In the future, it will be used to measure particle penetration through openings created by installation of commercially available building components such as windows and doors.

Control Technology Evaluation:

Pilot-Scale Multi-Pollutant Control Research Facility

NRMRL has a state-of-the-art research facility consisting of 4 million Btu/hr (1.2 MW_t) pulverized coal fired boiler where combinations of technologies can be evaluated to optimize the control of multi-pollutants such as SO_2, NO_x, PM, and mercury. Currently, the facility is equipped to evaluate key technology options such as low NO_x burners, selective catalytic reduction (SCR) for NO_x and mercury oxidation, lime flue gas desulfurization (FGD) for SO_2 and mercury capture, fabric filter for fine PM and mercury capture, and conventional and advanced sorbents for mercury, SO_2, and/or NO_x removal. Future capability will include electrostatic precipitator (ESP) for fine PM and mercury and a circulating fluidized bed for SO_2, mercury, and NO_x.

Multi-Pollutant Sorbent Evaluation System

The NRMRL multipollutant sorbent evaluation system quantifies the capture of multiple pollutants (SO_X, NO_X, Hg) on a fixed sorbent bed. The main components of this experimental system include the gas supply and blending system (tanks, mass flow controllers), the mercury generation system (permeation oven), the gas humidification system, the glass reactor (adsorption vessel), the oven and temperature controller, the moisture removal and reducing furnace, and the gas monitors (for Hg, NO_X, SO_2, and CO_2). The gas adsorption vessel can be operated in a fixed-bed or a fluidized-bed configuration.

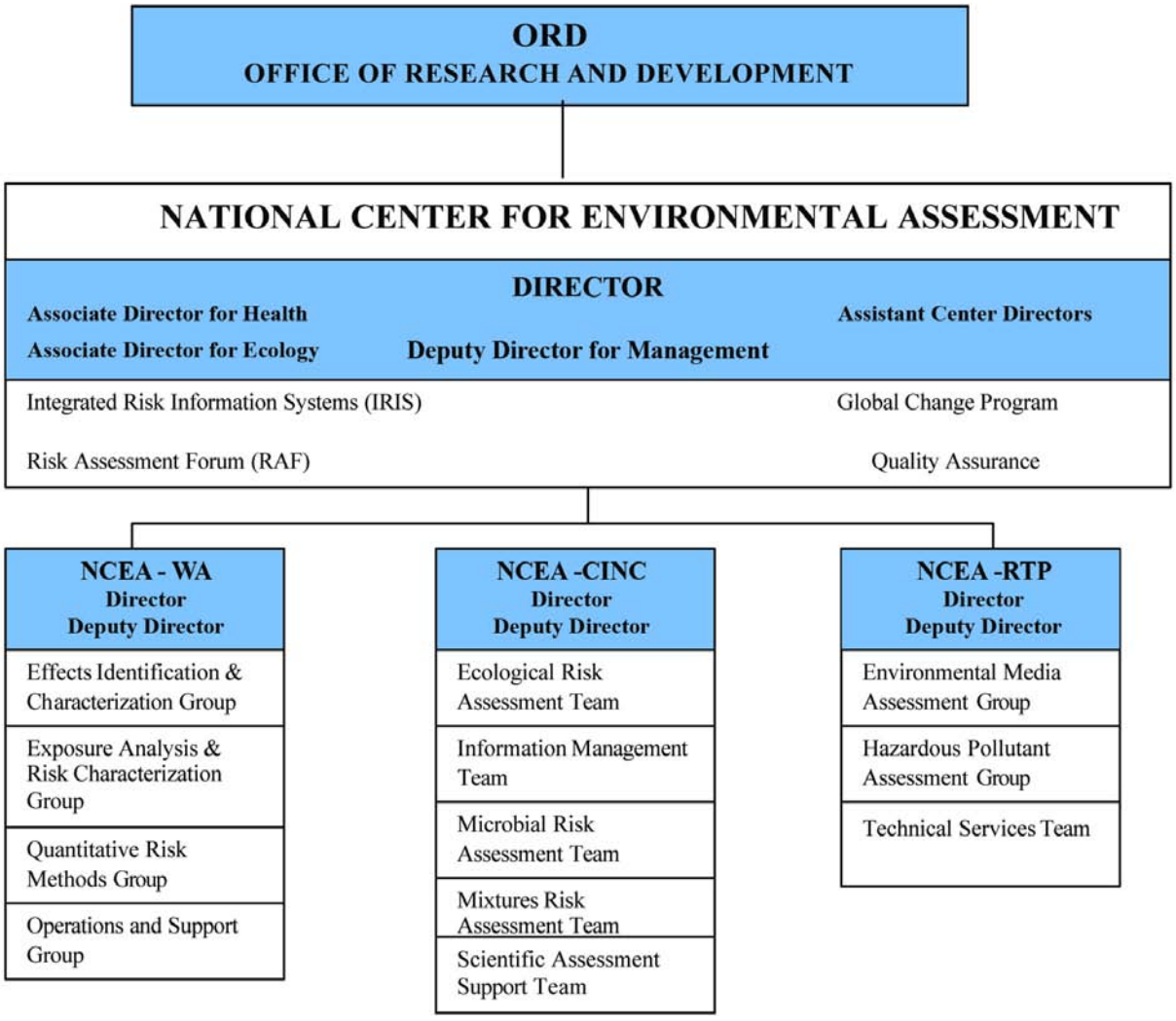
National Center for Environmental Assessment Overview

Washington, DC

Organizational Structure

The National Center for Environmental Assessment (NCEA) is EPA's national resource center for human health and ecological risk assessment. NCEA conducts risk assessments, carries out research to improve the state-of-the-science of risk assessment, and provides guidance and support to risk assessors. NCEA occupies a critical position in ORD between researchers in other ORD laboratories and offices and regulators in the EPA program offices and regions, who make regulatory, enforcement, and remedial action decisions. NCEA is headquartered in Washington, DC, with offices in Washington, DC; Cincinnati, OH; and Research Triangle Park, NC, that specialize in various areas of human health and environmental assessment. The current organizational structure is diagrammed in the figure below.

Organizational Chart



NCEA's Mission

The mission of the U.S. Environmental Protection Agency (EPA) is to protect human health and to safeguard the air, water, and land upon which life depends. EPA's Office of Research and Development (ORD) conducts research to help ensure that efforts to reduce environmental risks are based on the best available scientific information. NCEA conducts risk assessments, carries out research to improve the state-of-the-science of risk assessment, and provides guidance and support to risk assessors in support of the Agency's and ORD's mission to protect human health and safeguard the environment, and to ensure that efforts to reduce risks to humans and the environment are based on the best available scientific information. NCEA focuses its work in three major areas:

- Conduct assessments of contaminants and sites of national significance

- Develop methodologies that reduce uncertainties in current approaches
  - Dose-response models and factors
  - Exposure models and factors
  - Probabilistic models
  - Community-based risk assessment

- Provide guidance and support to risk assessors
  - Databases
  - Risk assessment guidelines
  - Expert tools
  - Expert consultation and program support
  - Risk assessment training.

Also, through the Risk Assessment Forum staff, NCEA is responsible for coordinating and implementing the health and ecological assessment activities of the Forum. These activities include scientific and science policy analysis of selected precedent-setting or controversial risk assessment issues of Agency-wide interest, such as risk assessment guidelines and development of cross-Agency positions on important risk assessment issues.

NCEA-WA

NCEA-Washington is comprised of three groups with focus areas that correspond to the risk assessment paradigm. The Exposure Analysis and Risk Characterization Group (EARCG) works to improve the state-of-the-art of human and ecological exposure assessment science and to promote improved risk characterizations for human health and ecological assessments in NCEA and throughout the Agency. This work involves both methods development and direct program support implementing new methods into site and chemical-specific assessments.

The Quantitative Risk Methods Group (QRMG) primarily works to address critical quantitative risk assessment issues dealing with the dose-response portion of the risk paradigm. The goal is to use more kinds of information in quantitative risk assessments, especially information about human variability, uncertainty, and alternative models and assumptions. Activities span a range from biologically based modeling using pharmacokinetic and mechanistic information to developing credible default procedures that can be applied to the information that is more generally available.

QRMG also develops tools and guidance that enable others to do credible quantitative assessments on their own.

The principal goal of the Effects Identification and Characterization Group (EICG) is to identify and characterize adverse health and ecological effects due to exposure to environmental agents. The EICG works to conduct and promote methods development research and critical evaluation of toxicity data. The EICG also conducts and assists QRMG in addressing dose-response issues involving biologically based dose-response models, pharmacokinetics, and animal models and has a lead in developing and coordinating risk assessment training and guidance for the ORD/EPA.

NCEA-CINC

NCEA-CINC is a scientific center of excellence dedicated to the development and application of methods to assess human health and ecological risks associated with environmental pollutants. NCEA-CINC focuses primarily on supporting the Office of Water and the Office of Solid Waste and Emergency Response. Focal areas of research include mixtures risk assessment, microbial risk assessment, and causal relationships in ecological risk assessment. This research includes the development of methods and guidance, and their application to case study assessments. In addition, assessments of the toxicity of individual chemicals and the pathogenicity of individual microbes are performed for the Integrated Risk Information System (IRIS) database and for specific programmatic and regional needs. Environmental economics is an emerging area of research for the division, which integrates ecological and human health risk assessments with economic analysis to support decisions. Superfund technical support centers for human health and ecology provide guidance in response to requests from regional risk assessors and the program office.

NCEA-RTP

NCEA-RTP conducts programs designed to provide methods and guidelines needed for performing human health and ecological risk assessments, carries out such assessments, provides extensive scientific consultation/technical assistance, and conducts limited research to improve risk assessment methods for inhaled chemicals. Important functions of NCEA-RTP are the development air quality criteria for major air pollutants (PM, SO_x, O_3, NO_x, CO, P_b) in support of NAAQS decisionmaking, providing health and ecological assessments of air toxics in support of decision-making on hazardous air pollutants, and providing assessments and scientific assistance on fuels/fuel additives in support of Agency mobile sources rulemaking actions. NCEA-RTP also provides risk assessment information and assistance on air pollution problems to EPA Regions, other Federal government agencies, state and local authorities, and international agencies. NCEA-RTP provides key scientific assessments and assistance to many EPA Policy offices (OAR, ODW, OSWER, OPPTS) with regard to lead and other metals, and is an internationally recognized center of expertise on metals as well as on air pollution exposure/health effects. NCEA-RTP also provides training and information to Agency researchers, policy-makers, and the public through workshops, public meetings, and publications.

NCEA Particulate Matter Program

In July 1997, EPA revised the National Ambient Air Quality Standard for Particulate Matter (PM NAAQS), recognizing that exposures to both fine and coarse fraction particles are associated with adverse health effects. The NAAQS revisions were based on scientific assessments in the1996 PM Air Quality Criteria Document (AQCD) identifying new epidemiological evidence of increased ill-

ness and premature mortality associated with ambient air PM exposures, Office of Air and Radiation (OAR) exposure/risk analyses, and characterization of policy options. The most recent PM AQCD, published in October 2004 in response to Clean Air Act requirements for periodic review of the PM NAAQS, draws upon newly published research from the ORD PM Research Program and others, which further substantiates the finding of PM exposure-related increases in morbidity and premature mortality. Future ORD Research Program efforts will focus on scientific uncertainties that remain concerning biological mechanisms that cause PM exposure-related premature mortality and morbidity. The next revision of the PM criteria is scheduled to be initiated in 2007.

National Center for Environmental Research ORD'S Extramural Grants Program Science To Achieve Results (STAR) Overview

Washington, DC

The National Center for Environmental Research (NCER) is the primary source of support for extramural research in ORD. The goal of NCER is to enhance the quality of science at EPA by including many of the Nation's best scientists in research to protect human health and the environment.

NCER's extramural research is conducted principally through the Science To Achieve Results (STAR) program. STAR is a competitive, rigorously peer-reviewed program of research grants that solicits proposals from scientists at universities and nonprofit institutions in response to targeted Requests for Applications (RFAs) issued by NCER. These grants support both individual investigator research and multidisciplinary research grants and centers. Approximately 20-25 solicitations have been posted each year, awarding about $90-100 million in grants annually. However, in recent years, awards have totaled closer to $60 million per year. The Air Research program represents approximately $16 million of the NCER budget. Where EPA and other federal agencies' interests coincide, NCER may plan a solicitation with another agency. By partnering with many agencies, NCER leverages its resources by approximately 20 percent, increasing the impact of the program. An attachment contains a description of how RFA topics are selected and applications reviewed.

What Are the Areas of Emphasis in the STAR Air Pollution Research Program?

In the last 5 years, the STAR air pollution research program has had an emphasis on the health effects of air pollution. Almost one-half of the program has been dedicated to funding interdisciplinary PM research centers largely devoted to understanding the health effects associated with PM exposure.

PM Centers Program

In establishing the new National Ambient Air Quality Standards for PM in 1997, EPA and its Clean Air Scientific Advisory Committee agreed on the importance of expanding research programs to address the key issues in the PM criteria and standards review. In 1998, Congress urged EPA to establish as many as five university-based research centers focused on PM. In 1999, EPA funded five centers to address uncertainties in the science associated with health effects of exposure to PM. The PM Centers RFA was designed to solicit research in the following areas: exposure, dosimetry and extrapolation modeling, toxicology and epidemiology. These Centers have been very productive and have worked closely with EPA scientists. A meeting to disseminate their collective findings to interested policy-makers and stakeholders was held September 27, 2004.

Additional research areas include the following:

- Health effects studies focused on the biological mechanisms of PM, including a partnership with the National Institute of Environmental Health Sciences to study the role of air pollutants in cardiovascular disease.

- A prospective epidemiological study to examine the health effects of long-term exposure to PM. The investigators will study the effects of exposure to air pollution on 8,700 people aged 50-89 prospectively for 10 years. This is the largest research grant ever funded by EPA, and it is a joint effort with the National Institutes of Health's National Heart, Lung, and Blood Institute (NHLBI). The majority of the study population and medical examinations are included through the NHLBI Multi-Ethnic Study of Atherosclerosis. The air pollution study, known as MESA-Air, will provide new and critically important information on the role of PM and other air pollutants in cardiovascular disease and mortality.

- Retrospective epidemiological studies (using existing health and exposure data) on the health effects of long-term exposures to PM.

- Atmospheric science studies focused on measurement and modeling methods, with a special emphasis on understanding the sources of carbonaceous particulate matter.

Health Effects Institute

Together with OAR, NCER co-funds the EPA grant with the Health Effects Institute (HEI). A key partner in PM research, HEI is an independent, nonprofit corporation chartered in 1980 to provide high-quality, impartial, and relevant science on the health effects of environmental pollutants. Supported jointly by EPA and industry, HEI has funded over 170 studies and has published more than 100 research reports and several special reports. Particulate air pollution is identified as a priority in the HEI strategic plan, and this public/ private partnership has made significant advances in PM-related research. HEI has worked closely with the epidemiology community to solidify its database and analyses of large urban studies (e.g., the National Morbidity, Mortality, and Air Pollution Study) as well as to provide opportunities for investigations of health (mechanisms), statistics (general additive models used in epidemiology), and effects of changing technology (e.g., diesel engines). An internal EPA coordination committee representing all of the ORD labs and centers, as well as representatives from the Office of Air and Radiation, facilitates communication between EPA and HEI concerning research priorities and direction. The research supported by HEI is highly relevant to the mission of EPA's air quality programs and complements EPA's in-house PM Research program.

What Is the Future of the STAR Air Pollution Research Program?

In 2004, the STAR program issued a new competitive solicitation for PM Centers. As a result of findings in recent years, this RFA called for integrated, multidisciplinary applications to conduct research across the continuum linking sources, PM attributes, and health effects. The focus of these new PM centers is to expand beyond health and exposure research to examine questions related to sources, atmospheric chemistry, and physical and chemical attributes of particles. The intent is for these Centers to be highly integrated to better understand the full spectrum of impact of various sources and attributes of PM on the range of health outcomes in different populations. With this crosscutting theme, the new PM centers RFA called for research in the following areas: susceptibility, biological mechanisms of health effects, exposure-response relationships, and source linkages.

In addition, NCER will continue its commitment to the 10-year epidemiological study of the effects of long-term exposure to PM. In 2005, NCER issued an RFA requesting development of

continuous methods of measuring composition of fine particles. NCER staff will work with EPA's air research planning team to define future RFAs likely to emphasize multidisciplinary approaches to improve understanding of the role of various sources and characteristics of air pollution in contributing to adverse health effects and to improve our ability to measure the impacts of reductions in air pollution.

Abstracts or progress and final reports for any of the STAR grants are available at the NCER Web Site: http://es.epa.gov/ncer/.

What Related Research Is Also Funded Under the STAR Program?

NCER funds a broad range of research through extramural grants (descriptions available on the NCER Web Site above). However, there are several research areas that are closely related to the Air Research Program and as such, NCER staff coordinate in planning RFAs and monitoring research results.

- The Human Health Program funds research related to improving human health risk assessment in areas such as exposure assessment, biomarkers, genetic susceptibility, and asthma. Together with the NIH, the Human Health Program funds several centers of excellence for Children's Environmental Health Research.

- The Global Change Program includes a major focus area exploring the impact of global change on air quality. The projects underway include research linking global models to regional air quality models, forecasting plausible emission scenarios for 50-100 years into the future, and improving models for important emission sources likely to have significant change over the next century.

- The Economics and Decision Sciences Program supports research related to the value of reducing adverse health and ecological effects, market mechanisms, compliance decision-making, and benefits of disclosing information.

- The Nanotechnology Program supports research on the environmental implications of nanotechnology, including toxicity, exposure, transport, and transformation of manufactured nanomaterials.

- Mercury research in NCER includes studies on the atmospheric processes that influence the fate and behavior of mercury.

- The Small Business Innovation Research (SBIR) Program was created to strengthen the role of small businesses in federally funded research and development and develop a stronger national base for technical innovation. The SBIR program has addressed issues related to air pollution measurement and control.

What Have Been the Findings of Previous External Reviews of the STAR Program?

The STAR Program, in general, has been reviewed a number of times (e.g., twice by Subcommittees of the BOSC, the Government Accounting Office, the Agency's Inspector General). In 2002, NCER asked the National Research Council's Board on Environmental Studies and Toxicology to conduct an independent assessment of the STAR program. A committee was formed and

charged with assessing the program's scientific merit, its demonstrated or potential influence on policies and decisions, and other program benefits that are relevant to EPA's mission. The committee was asked specifically to examine the program's research priorities, research solicitations, peer-review process, current research projects, and results and dissemination of completed research in the context of other relevant research conduced or funded by EPA and in comparison with those of other basic and applied research grant programs. In preparing its report, the committee focused on three research programs: Particulate Matter, Ecologic Indicators, and Endocrine Disruptors. They issued a report, "The Measure of STAR," in May 2003 (www.nap.edu/books/0309089387/html/) concluding:

> "The STAR program is a crucial element of EPA's research efforts; and…As the STAR program has evolved, it has developed a grant-award process that in many ways exceeds those in place at other organizations that have extramural research programs."

EPA's Science Advisory Board conducted a specific review of the STAR PM Centers research program midway through the research grants in 2002. The "Interim Review of the Particulate Matter (PM) Research Centers of the USEPA: An SAB Report" (available at http://www.epa.gov/sab/pdf/ec02008.pdf) was favorable with major findings stating that the PM Centers have produced benefits above and beyond what might be expected from individual investigator-initiated grants and that they are likely to continue to produce such benefits through the next several years.

STAR Air Research Program
RFA Topic Areas and Summary of Awarded Grants
1998-2005

RFAYear	RFA Topic Areas and Grant Research Area
1998	Health Effects of PM and Associated Air Pollutants (10 grants-Total) • PM and respiratory effects (7 grants) • PM and cardiovascular effects (2 grants) • PM and morbidity/mortality (1 grant)
1999	Airborne Particulate Matter Health Effects (8 grants-Total) • PM dosimetry (1 grant) • PM cardiopulmonary epidemiology (3 grants) • PM controlled exposure studies (3 grants) • Source evaluation of PM effects (1 grant) Airborne Particulate Matter Centers (5 grants-Total) – Overall Themes: • Exposure, susceptibility, and biological mechanisms • Health risks of PM components • Combustion-derived fine particle composition, exposures and health effects • Mobile source pollution and health effects • Health effects of ultrafine particles
2001	Health Effects of Particulate Matter (4 grants-Total) • Mechanisms of PM respiratory effects (3 grants) • Air pollutants and emergency room visits (1 grant)

2002	Airborne PM Health Effects: Cardiovascular Mechanisms (4 grants-Total) • Diesel exposures (3 grants) • Concentrated airborne particulate and ozone (1 grant) Epidemiologic Research on Health Effects of Long-Term Exposure to Ambient PM and Other Air Pollutants (4 grants-Total) Four Cohorts: • Seventh Day Adventists (California) • Multi-Ethnic Study of Atherosclerosis (MESA) • Medicare Database • U.S. Nurses' Health Study
2003	Measurement, Modeling, and Analysis Methods for Airborne Carbonaceous Fine PM (16 grants-Total) • Emission source estimates of primary organic aerosol and secondary organic aero- sol precursors (3 grants) • Secondary organic aerosol formation mechanisms (4 grants) • Next generation receptor model (1 grant) • Advanced measurement techniques for source apportionment of organic PM (5 grants) • Differences in EC/OC measurement methods (2 grants) • Organic aerosol sampling artifacts (1 grant) Epidemiologic Research on Health Effects of Long-Term Exposure to Ambient PM and Other Air Pollutants (1 grant-Total) • MESA – Air The Role of Air Pollutants in Cardiovascular Disease (6 grants-Total) • Animal models of human disease to evaluate mechanisms (3 grants) • PM effects on regulation of heart rhythm (1 grant) • PM effects on the function of tissue lining blood vessels (2 grants)
2004	Source Apportionment of Particulate Matter (11 grants-Total) • Receptor modeling (3 grants) • Integration of receptor, source-based and inverse modeling (4 grants) • Measurement methods for molecular tracer species and identification of new mo- lecular tracers (4 grants)
2005	Airborne Particulate Matter Centers (5 grants-Total) – Overall Theme • Linking health effects with PM from sources and components Measurement Methods for Particulate Matter Composition

Attachment: Process for Selecting STAR RFA Topics and Reviewing Applications

How Are the Topics for the Science To Achieve Results (STAR) Solicitations Selected?

Research Coordination Teams (RCTs)

- RCTs are composed of representatives from ORD's laboratories and centers and EPA's program and regional offices; they develop a plan for research to be done intramurally in ORD laboratories and extramurally through STAR.

- The research plan is based on the EPA and the ORD Strategic Plans, as well as specific program needs identified through the RCT process.

- A series of criteria are used to decide whether research would best be accomplished internally at ORD or externally through grants, cooperative agreements or contracts. These criteria include:
  - Which organization has the most appropriate expertise?
  - How urgently is the research needed? What is our available in-house capacity?
  - Does the proposed extramural research complement the intramural program?

- NCER staff work with the RCTs to write the Request for Applications (RFAs).

What Is the Review Process That NCER Uses for All Assistance Applications?

Peer review is the cornerstone of high-quality scientific research. Because all NCER applications are subjected to a rigorous, independent peer review, the program funds only the most scientifically meritorious research. The external peer review process is managed entirely by a separate division of NCER, preserving independence from the NCER staff who prepare RFAs and manage grants.

External Peer Review

- NCER staff determines the types of expertise reviewers must possess given the technical requirements of the solicitation.

- Each application is reviewed and critiqued in-depth by at least three expert panelists and discussed by the full review panel.

- For *all* applications, each principal reviewer is required to, and non-principal reviewers may elect to, provide an overall rating of Excellent, Very Good, Good, Fair, or Poor.

- Ratings are tallied and averaged, and the lead principal reviewer prepares a summary evaluation that is consistent with this average rating.

- Applications receiving a Very Good or Excellent are sent to ORD's Programmatic Review Panel.

Programmatic Review Panel

- ORD's Programmatic Review Panel recommends proposals on the basis of relevancy to EPA's mission, balance of research portfolio, and capacity to complement in-house research.

- ORD's Programmatic Review Panel consists of members from ORD, Program and Regional Offices.