# Ultrafine Particles: Characterization, Health Effects and Pathophysiological Mechanisms

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## Description:

### Objective:

The Rochester PM Center brings together a multi-disciplinary team of experienced investigators to test the hypothesis that ultrafine particles occurring in the urban atmosphere cause adverse health effects. Epidemiological studies have consistently found an association between small increases in urban particulates and health effects, including increased morbidity and mortality in people with respiratory and cardiac disease. The observed effects are associated with the fine rather than the coarse particles in the atmosphere. Moreover, animal studies have shown that ultrafine particles have a significantly greater pulmonary inflammatory potency than larger submicronic particles. A recent epidemiological study found that particle number - reflecting ambient ultrafine particles - correlated better than fine particle mass with increased symptoms in asthmatics. These results form the basis for the ultrafine particle hypothesis. The proposed studies are designed to rigorously test the ultrafine particle hypothesis by comparing ultrafine (~10-50 nm) with accumulation mode particles (~100-500 nm) in all phases of this research. Thus, the following objectives will be addressed by 5 interactive research cores in the Rochester PM Center over a 5-year study period: (a) to determine the number, mass concentration, and composition of urban ultrafine and accumulation mode particles of an eastern and western U.S. city; (b) evaluate the association between ambient particle number/mass concentrations and respiratory as well as

cardiovascular effects in a susceptible cohort; (c) compare exposure-dose-response relationships for respiratory and cardiovascular endpoints between ultrafine and accumulation mode particles in controlled clinical and animal studies; (d) assess the significance of factors enhancing ultrafine particle effects: age, disease, co-pollutants, pre-exposure history, exercise; (e) measure total and regional deposition of ultrafine particles in the lower respiratory tract of rodents and total deposition in humans for purposes of extrapolation modeling between species and for in vitro dosing; (f) perform mechanistic studies at a cellular and molecular level to test the hypothesis that ultrafine particle effects are due to oxidative stress induced events; (g) support the development of an ambient ultrafine particle concentrator and test the feasibility for its use in controlled in-vitro, animal, and clinical studies; (h) coordinate research efforts and dissemination of results with other centers.

**Approach:**

The multi-disciplinary team of the Rochester PM Center consists of atmospheric scientists, aerosol physicists, biostatisticians, epidemiologists, physicians specializing in pulmonary, cardiac and vascular medicine, inhalation toxicologists and cell/molecular biologists. These investigators work in close collaboration with each other in 5 research cores dedicated to areas of urban PM characterization, field studies of PM effects, controlled clinical studies, toxicological animal studies and mechanistic in vitro studies. These research cores are supported by 5 facility cores which provide specific expertise for particle generation and characterization, biostatistical input for study design, evaluation, and extrapolation modeling for immunologic analyses, assessment of vascular and coagulation effects, and measurement of subclinical cardiac effects. The activities of the facility and research cores are closely integrated with each other. Interim results from individual research cores will be integrated into the study design of other cores so that there is continuous feedback between the cores to further advance our knowledge of ultrafine PM induced effects.

***Core 1, Ambient Particle Characterization (Cass),*** research on the characterization of urban ultrafine and accumulation mode particles will allow compositional analysis in real time of single particles. The objective of the research proposed here is to greatly expand the understanding of the chemical composition of ultrafine particles, both in the atmosphere and in source emissions. New instrumentation will be assembled that is capable of determining ultrafine particle chemical composition both in bulk samples of ultrafine particles collected by cascade impaction and by a novel ultrafine particle aerosol time of flight mass spectrometer designed to determine the chemical composition of ultrafine particles at the single particle level. This instrumentation will be field tested and then used to characterize ultrafine particles in the atmosphere of one western city and one eastern city during each season of the year. In addition, archived data on ultrafine particle chemical composition measured previously during source tests by the Caltech group will be reprocessed to display the chemical composition of the smallest particles emitted. Comparisons will then be drawn between atmospheric samples and source samples to determine the extent to which the two data sets do or do not resemble each other, which will shed light on the extent to which atmospheric ultrafine particles may be affected by atmospheric chemical transformations or new particle formation. These results will serve to adjust the composition of the laboratory generated PM for the controlled clinical, animal and in vitro studies.

***Core 2, Field Studies (Wichmann),*** studies will be conducted in Germany by a group with an established program for epidemiologic analyses of ultrafine particle effects on subjects with COPD and angina. The inclusion of this group in a consortia arrangement allows access to a large-scale ongoing European study evaluating ultrafine particle effects. As part of the

epidemiological project the effects of PM-oxidant (e.g., ozone) mixtures will be assessed. The objective of the study is to characterize the association between ambient particle exposures and changes in biomarkers of inflammation in the airways and the blood of patients with stable coronary artery disease as well as of patients with COPD. Monitoring of the autonomic function of the heart will investigate how these changes in the inflammatory state relate to alterations in autonomic control. The following hypotheses will be tested in patients with chronic diseases of the lung and the heart: (1) Concentration of ambient accumulation mode (AP) and ultrafine particles (UP) is associated with inflammation of the airways, as well as increases in plasma viscosity, fibrinogen and other acute phase proteins in the blood, (2) increases in the coagulability of the blood are associated with changes in the autonomic control of the heart, and (3) exposure to UP is more closely related to the health effects than exposure to AP mass. The result of this Core will be a valuable input for the clinical, animal and in vitro studies with respect to planned mixed PM-oxidant exposures.

***Core 3, Clinical Studies (Frampton, Utell),*** will make use of results from the field studies, starting initially with controlled exposures in healthy subjects and asthmatics, and then of elderly subjects with COPD and coronary artery disease, similar to the field study cohorts. These studies will utilize controlled human exposures to examine, in healthy and potentially susceptible subjects, the deposition and fate of inhaled ultrafine carbon particles (UP), and the role of UP and ultrafine carbon particles containing trace metals (UM) in inducing health effects. The proposed pathophysiology for pollutant-induced lung inflammation involves the following sequence of events: (i) Injury to epithelial cells by reactive oxygen species, possibly enhanced in the presence of metals via Haber-Weiss and Fenton chemistry, accompanied by activation of nuclear regulatory factors, leading to elaboration of proinflammatory cytokines, including interleukins-8 (IL-8) and -6 (IL-6), and increased expression of nitric oxide synthase (NOS), with increased nitric oxide (NO) in exhaled air. (ii) Activation of vascular endothelium and circulating leukocytes. Emigration of inflammatory cells from blood to tissue sites involves up-regulation of adhesion molecules and other markers on vascular endothelium and on circulating leukocytes. The events in the process of leukocyte-endothelial binding include a) increased expression of adhesion molecules followed by shedding of adhesion molecules as cells "tether and roll", b) leukocyte activation, c) stable adhesion, and d) transmigration through the epithelium. Platelets become activated and adhere to endothelium and leukocytes. Endothelial activation may further contribute to the increase in exhaled NO concentrations seen with airway inflammation. (iii) Increased release of IL-6 and tissue factor by activated blood mononuclear cells. Interleukin-6 initiates hepatic synthesis of acute phase proteins, including serum amyloid A (SAA), fibrinogen, and plasminogen activator inhibitor-1 (PAI-1). Monocyte tissue factor and endothelial cell activation initiate the coagulation cascade, as reflected by the presence of D-dimer, soluble fibrin, and prothrombin1+2. (iv) Possible adverse cardiac events in patients with critical coronary lesions, as a consequence of increased blood coagulability, platelet activation, and endothelial dysfunction.

***Core 4, Animal Studies (Oberdorster),*** will extend the clinical studies by using rat models of human conditions including hypertension, sensitization and old age as well as transgenic mice to evaluate mechanistic hypothesis of PM induced pulmonary oxidative stress and its ramifications for cardiac and vascular events. The animal studies are designed to be complementary to the field and controlled clinical studies and to form a link to the mechanistic in vitro studies. We will analyze pulmonary and systemic responses to inhaled environmentally-relevant ultrafine (UP) and accumulation mode (AP) particles in rodent models of human disease to test our central hypothesis that the increased morbidity of

susceptible people in association with small increases in urban particles is caused by UP. Thus, the overall objective of the animal studies is to identify factors which are causally associated with adverse pulmonary and cardiovascular health effects after low-level exposures to environmentally-relevant particles. These factors are related to particle characteristics (UP vs. AP; transition metals); dosimetric aspects (lung deposition and disposition); host susceptibility (advanced age; cardiovascular disorders; respiratory tract sensitization); cellular mechanisms (role of Clara cells); and pollutant co-exposure (ozone). Our mechanistic hypothesis is that inhaled ultrafine particles activate resident inflammatory cells leading to oxidative stress and production of cytokines, and that this primary response is further amplified through inflammatory cell recruitment and elaboration of both inflammatory and epithelial cell-derived cytokines. Based upon our preliminary data we further hypothesize that the primary response is greater to UP than AP, and which is further enhanced by endotoxin exposure. Amplification of the pulmonary response leads to a parallel amplification of the systemic acute phase response with associated changes in blood coagulability and cardiac events.

***Core 5, In vitro Mechanisms (Finkelstein),*** studies will further expand the testing of oxidative stress hypotheses to a cellular/molecular level, making use of a novel in vitro exposure system which allows for realistic airborne exposures of pulmonary target cells. The in vitro and ex vivo experiments proposed within this project are designed to address specific mechanistic hypotheses regarding the interactions between inhaled ultrafine particles and specific pulmonary cell populations. We will use cell lines and primary cells derived from rats and humans to test the overall PM Center hypothesis that increased morbidity and mortality in susceptible populations is due to the unique characteristics of ultrafine particles in comparison to accumulation mode particles of similar composition. The proposed experiments are intended to provide a link between the whole animal and controlled clinical (human) exposures, described in the other programs of this PM Center, by defining mechanisms that follow particle cell contact and to test the specific hypothesis that many of the subsequent physiologic effects are the consequences of cellular oxidative stress. We further plan to examine host and environmental factors, including age, the influence of co-exposure to gaseous oxidants or prior priming or activation by pre-exposure to other inflammatory stimuli. A key component of the proposed studies is our plan to examine these particle cell interactions in individual cell populations to begin to assess the role of epithelial, inflammatory and interstitial cells in the systemic response to UP. We suggest that production of both inflammatory and fibrotic mediators following particle interaction is not limited to classic inflammatory cells, and that pulmonary parenchymal elements including epithelial cells (type II, Clara cells) and fibroblasts may also contribute to the milieu.

The ***Facilities Cores*** consist of Particle Generation **(Morrow)** , Biostatistics **(Cox)** , Immunology **(Looney)** , Vascular **(Marder)** , and Cardiac **(Zareba)** facilities which serve to link the projects. A number of the same endpoints related to pulmonary inflammatory responses and cardiac events as well as measurements related to the blood coagulation cascade will be measured by the respective facility cores in samples received from the epidemiological field studies, the controlled clinical studies and the toxicological animal studies. The controlled clinical, animal and in vitro studies will use the same freshly generated carbon particles of ultrafine (~25 nm) and accumulation mode (~250 nm) size at concentrations down to 10 ï¿½g/m3. Subsequently, the impact of controlled additions of transition metals to the carbon particles ? as determined from ambient PM measurements by the Exposure Research Core ? on respiratory and systemic endpoints will be assessed. This coordinated approach ? from particles ? target cells ? animals ? humans ? allows the

extrapolation of results and mechanisms from in vitro studies to the human target, supported further by extensive dosimetric measurements in the animal and clinical studies.

As a ***Pilot Project*** an ultrafine particle concentrator will be developed, which allows exposures with concentrated urban ultrafine particles. The objective of this study is to build a prototype particle concentrator that will be capable of concentrating urban ultrafine particles (UP, <0.1 ï¿½m) for use in toxicological studies at ambient pressure.

Currently available urban particle concentrators are based on the principle of virtual impaction and are effective for particle sizes down to about 0.2 ï¿½m. They operate at lower than ambient pressure. An ultrafine particle concentrator would allow controlled exposures of test animals, cell systems in vitro and eventually human subjects to real world urban UP in addition to using surrogate UP. The heads of the five Research Cores constitute the Executive Committee that monitors progress and recommends utilization of resources and coordination and integration of the Center's research to the Center director and co-director. They will also be in contact with members of the external Scientific Advisory Committee (SAC) to request advice in specific areas of expertise. The two directors will provide overall oversight, coordination and integration of the Center's activities and coordinate contact with other PM Centers. They are assisted by the Administrative Core which is responsible for the day-to-day logistical needs including communication with the consortia. New projects in the form of pilot studies to pursue specific questions in one of the Research Cores are reviewed annually by the SAC members who provide recommendations for funding to the Center Director. The development of an ultrafine particle concentrator for use in in-vitro and animal/clinical studies is the first in this series of pilot projects. Additional Enrichment Programs will further enhance the activities of the Rochester PM Center , including a dedicated seminar series, educational programs and visiting scientists.

**Expected Results:**

The integrated multi-disciplinary approach of the Rochester PM Center will answer the key question of causality of ultrafine particle involvement for effects observed in previous epidemiological studies. The expectation is that the controlled laboratory studies will reveal that ultrafine particles, but not larger accumulation mode particles, at environmentally relevant concentrations will show pulmonary inflammatory and secondary cardio-vascular effects in the compromised organism; and that the field studies will show that ultrafine particles are better correlated with health effects than larger particles.

**Improvements in Risk Assessment or Risk Management:** In support of Improvements in Risk Assessment or Risk Management, it is expected that the Center will uncover specific cellular and molecular mechanisms which can explain the progression of UP-induced pulmonary responses to vascular and cardiac events. Confirmation of the ultrafine particle hypotheses will have significant consequences for public health and regulatory PM standards and lead to important improvements in risk assessment and risk management. Not only will the new PM2.5 standard have to be re-evaluated, but an additional standard defined by particle number needs to be considered. Furthermore, sources of ultrafine particles - e.g. combustion engines, residential natural gas heating - may require further regulation.