UNIVERSITY OF ROCHESTER – EPA PM Center

Assessment of Ambient UFP Health Effects: Linking Sources to Exposure and Responses in Extrapulmonary Organs

Günter Oberdörster[1]; Alison Elder[1]; Jack Finkelstein[1]; Mark Frampton[1]; Phil Hopke[2]; Annette Peters[3] Kim Prather[4]; Erich Wichmann[3,5]; Mark Utell[1]

([1]University of Rochester; [2]Clarkson University; [3]GSF, Germany
[4]UC-San Diego, [5]LMU-IBE, Germany)

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Abstract (tentative)

The focus of the Rochester PM Center studies was an assessment of our ultrafine particle (UFP) hypothesis that states that exposure of susceptible members of the population to UF mode particles (<100 nm, $PM_{0.1}$) in the urban atmosphere can cause adverse health effects. Some key findings of our studies from measurements of ambient particles, from conducting epidemiological cohort studies, and using surrogate UFP in controlled clinical studies, toxicological animal studies and *in vitro* studies confirmed that ambient UF/fine particles:

- Contain reactive oxygen species (ROS);
- Can be measured in real-time with an Aerosol Time-of-Flight Mass Spectrometer (ATOFMS) down to particle sizes of 50 nm and in random mode down to 10 nm;
- Show substantial increases in the organic carbon content when concentrated in an UFP concentrator when compared with the ambient particles;
- Are associated with changes in vascular parameters indicative of an acute phase response, increased coagulation activation and adhesion molecule expression in patients with coronary heart disease;
- Are associated with onset of myocardial infarction 1 hr after exposure to traffic, according to time activity diaries;
- Induce changes in vascular parameters (acute phase response) and ECG (parasympathic stimulation) in rats following on-road exposure to highway PM;
- Induce changes in cardiac function represented by HRV, repolarization parameters and supraventricular and ventricular ectopic beats in patients with coronary heart disease as well as changes in HRV in patients with COPD;

and that inhaled laboratory-generated carbon UFP:

- Have a high deposition efficiency in the respiratory tract, which is further increased in asthmatics and during exercise;
- Translocate to extrapulmonary organs *via* the blood circulation, dependent on particle chemistry, and to the central nervous system, *via* olfactory neurons;
- Cause changes in adhesion molecule expression of peripheral blood leukocytes, indicative of pulmonary vascular effects in healthy and asthmatic humans;
- Alter ischemia-induced hyperemic blood flow in healthy subjects, indicative of particle effects on systemic endothelial function;
- Decrease the pulmonary diffusing capacity for carbon monoxide in healthy humans;
- Accelerate venous thrombus formation in rats;
- Induce greater oxidative stress in lungs of aged rats compared to adolescent rats and that ozone co-exposure can increase this response; and
- Demonstrate greater sensitivity to UFP-induced oxidative stress in the aged organism as is evident in *in vitro* primary cell cultures in alveolar macrophages from aged rats.

In summary, our studies show that ambient PM can have significant oxidative capacity, that UFP can induce significant effects not only in the respiratory tract but more importantly affect the vascular and cardiac system and that age and underlying disease (susceptibility factors) are critical modifying factors. Furthermore, our demonstration of their efficient translocation from deposition sites in the respiratory tract to other organs such as heart and CNS provides plausibility for UFP-induced oxidative stress in those organs. Such translocation could be particularly detrimental in susceptible individuals with dysfunctional vascular endothelium as the earliest manifestation of atherosclerotic vascular disease, such as seen in type 2 diabetes. This is presently being tested in ongoing studies.

1. Introduction

Numerous particle sources of anthropogenic origin (*e*.g., internal combustion engines, power plants, incinerators, smelting, airplane jets) and of natural origin (*e.g*., windblown dust, gas to particle conversions, forest fires, volcanoes) contribute to four modes of atmospheric particulate matter (PM) ranging in size from ~2 nm to >10 μm: Coarse, Accumulation, Aitken and Nucleation Mode, the latter two comprising the ultrafine particles (EPA, 2004). National Ambient Air Quality Standards (NAAQS) have been established for particles < 10 μm (PM_{10}) and fine particles ($PM_{2.5}$) based on evidence from epidemiological and toxicological studies showing association with and induction of adverse health effects. Evidence of adverse effects from exposure to the smallest atmospheric particles, ultrafine particles (UFP, <100 nm; $PM_{0.1}$), was very limited in 1999 at the start of the Rochester multidisciplinary PM Center research program. Thus, we focused on testing our hypothesis that UFP occurring in the urban atmosphere cause adverse health effects.

Ambient UFP, $PM_{0.1}$, represent by far the largest mode with respect to particle numbers, yet they may generally contribute very little mass to the total ambient PM concentration (Finlayson and Pitts, 2000). Thus, it would be difficult to control $PM_{0.1}$ by a mass-based standard. Because there are many sources of $PM_{0.1}$, their physico-chemical characteristics can vary widely, and thus, their toxicological potential is likely to be source dependent. Differences in $PM_{0.1}$ concentration, chemical composition, agglomeration and aggregation state and water and lipid solubility are likely to impact on the interaction of UFP with the living organism when the respiratory tract is the portal-of-entry. For example, although predictive particle lung deposition models indicate high size dependent deposition efficiencies of inhaled UFP in all

three regions (naso-pharyngeal, tracheobronchial, alveolar) of the respiratory tract (ICRP, 1994), soluble UFP may instantly lose their particulate state after deposition before entering cells or translocating to extrapulmonary sites. The small dissolved mass burden may be less likely to cause effects.

The Rochester PM Center team designed collectively studies that integrated multiple aspects of UFP physico-chemical characterization, epidemiological field, and controlled clinical studies and animal and in vitro toxicological studies in order to determine the overall health impact of exposure to ambient UFP. Influence on susceptible subgroups was of particular concern. Key studies and results of this effort are summarized in the following report.

2. Physico-chemical Characterization of $PM_{0.1}$

Particle Size Distributions

Ultrafine particles are formed through the nucleation of low vapor pressure compounds. They can be formed in the effluent streams from combustion processes or through atmospheric chemical reactions that convert gaseous compounds into lower vapor pressure products. In Rochester, monitoring of particle size distributions has been conducted since November 2001 (Jeong et al., 2004; 2006; Ogulei et al., 2007). Strong seasonal dependency in particle number concentration was observed. The average number concentration of ambient particles was 9,670 ± 6,960 cm^{-3}. The particle number concentrations were higher in winter months than in summer months by a factor of 1.5. There were also distinct diurnal variations of aerosol number concentrations. The highest weekdays/weekends ratio of number concentration was typically observed during the rush hour period in winter months with a ratio of 2.1. Strong morning particle formation events were frequently observed during colder winter months. Good correlations between particle number and CO as well as temperature suggested that motor vehicle emission lead to the formation of new particles as the exhaust mixes with the cold air. Regional nucleation and growth events frequently occurred in April. Local plume SO_2-related particle formation events most frequently occurred in August. SO_2 and UV-B were highly correlated with particle concentration suggesting a high association of photochemical processes with the local events. The diurnal patterns of the particles in the size range of 11 to 50 nm (N_{11-50}) averaged over the winter and summer periods show a high directionality to the north for particle number and SO_2, indicating the influence of point sources located northwest of downtown Rochester.

The particle size distributions can be used to provide source identification and apportionment (Ogulei et al., 2007). A total of ten different sources were identified, including

two traffic factors, two nucleation factors, industrial emissions, residential/commercial heating, secondary nitrate, secondary sulfate, ozone-rich secondary aerosol, and regionally transported aerosol. The resolved sources were generally characterized by similar number modes for winter, spring, summer and fall. The size distributions for nucleation were dominated by the smallest particles (<10 - 30 nm) that gradually grew to larger sizes as could be seen by observing the volume profiles. In addition, the nucleation factors were closely linked to traffic rush hours suggesting that cooling of tail-pipe emissions may have induced nucleation activity in the vicinity of the highways. Although the diurnal pattern of each of the two traffic factors closely followed traffic rush hour for Rochester, their size modes were different suggesting that these factors might represent local and remote emissions. Industrial emissions were dominated by emissions from coal-fired power plants that were located to the northwest of the sampling site. These facilities represent the largest point emission sources of SO_2, and probably ultrafine (<0.1μm) or submicron particles, in Rochester. Regionally transported material was characterized by accumulation mode particles. Air parcel back-trajectories showed transport of air masses from the Ohio River Valley.

Ultrafine Particle Composition

There have been very few studies that have investigated the chemical constituents within the ultrafine range. Twenty-four hour size-segregated ($0.056 < D_p$ (particle diameter) < 1.8 μm) particulate matter samples collected during five days in August 2000 at sites in Houston, TX were analyzed by ICP/MS to quantify 32 elements (Dillner et al., 2005). One site was surrounded by refineries, chemical plants and vehicular and commercial shipping traffic, while the other site that was 25 miles inland, was surrounded by residences, light industrial facilities and vehicular traffic. Concentrations of particulate matter mass, sulfate and organic carbon at the two sites were often not significantly different from each other and had smooth unimodal size distributions indicating the regional nature of these species. Elemental concentrations varied widely across events and sites and often showed sharp peaks at particle diameters between 0.1 and 0.3 μm and in the ultrafine mode ($D_p < 0.1$ μm) that suggested that the sources of these elements were local, high temperature processes.

Sardar et al. (2005) explored the size-fractionated ultrafine (10-180 nm) chemical composition at urban source sites (USC and Long Beach) and inland receptor sites (Riverside and Upland) in the Los Angeles basin over three different seasons. Size-fractionated ultrafine particles were collected over a two-week period at each site. Measurements of ultrafine mass concentrations varied from 0.86 to 3.5 $\mu g/m^3$ with the highest concentrations observed in the fall.

The composition of the ultrafine particles ranged from 32 to 69% for organic carbon (OC), 1-34% for elemental carbon (EC), 0-24% for sulfate, and 0-4% for nitrate. A distinct OC mode was observed between 18 and 56 nm in the summer, possibly indicating production of secondary organic aerosol. The EC levels are higher in winter at the sites in source areas because of the lower mixing heights as also seen in the particle size distribution data discussed above. The values are higher in summer at the downwind receptor sites because of more effective transport from source areas. Nitrate and sulfate were measurable only in the larger particle size ranges of ultrafine PM.

Characterization of Individual Particles

Aerosol time-of-flight mass spectrometry (ATOFMS) has been used extensively for on-line measurements of the size and chemical composition of individual atmospheric particles in real-time (Gard et al., 1997). For the EPA Center project, we developed an ultrafine version of the ATOFMS instrument (UF-ATOFMS) which can measure small ultrafine aerosol particles with sizes down to 50 nm (Su et al., 2004). This instrument has been used in a number of lab and field studies of ultrafine particle chemistry; initially, it was used to characterize the purity of individual model UF aerosols used in exposure studies (Su et al., 2005). The UF-ATOFMS was also used to characterize ultrafine aerosols in the output of a UF particle concentrator. The UF particle concentrator requires the addition of water before enriching the number of particles using a virtual impactor. The water is then removed before using the UF particles in exposure studies. In the concentrator study, it was shown that the chemistry of the ultrafine particles changes after the particles exit the concentrator; most notably nominally "pure" ultrafine EC particles become coated with organic carbon and nitrate (Su et al., 2006). It is likely the addition of water and additional species changes the shape of the particles.

Ambient studies have been conducted using the UF-ATOFMS in a number of locations, focusing on charactering the chemistry and sources of ambient ultrafine particles. A number of studies have been conducted in major cities including Raleigh-Durham (NC), Rochester (NY), Riverside (CA), Boston (MA), Atlanta (GA), and San Diego (CA). One study focused on differences in particle chemistry in a rural city in Tuxedo Park (NY). Regardless of location, in the 50-100 nm size range, most of the particles are carbonaceous and composed of elemental and organic carbon mixtures. As far as other non-carbonaceous compounds, the most common species associated with the UF particles is sulfate followed by ammonium and nitrate. An analysis of the percentage of particles containing sulfate, nitrate, and ammonium for 50-300 nm particles sampled in Atlanta, GA shows that sulfate is associated with the majority of the

smallest sized particles. In fact, even in source studies, sulfate is most commonly associated with small soot particles, suggesting the sulfate in the particles comes from the sulfur in the fuel (Sodeman et al., 2005; Toner et al., 2006). As the particles grow, they become associated with nitrate and ammonium. By the time the particles reach 100 nm, the majority of them contain additional organic carbon, sulfate, nitrate, and ammonium. Spencer and Prather (2006) have shown that the UF-ATOFMS can be used to determine the fraction of OC associated with EC particles on particles in the 50-300 nm size range. Metals have been shown to be abundant in ultrafine particles (Tolocka et al., 2004; 2005; Bein et al., 2006), including at roadside locations (Ntziachristosa et al., 2007). From mass spectra of some commonly detected UF particles with the UF-ATOFMS, iron is the most commonly detected ambient metal in the UF range. Particles with metal signatures including Pb, V, Ni, Zn, and Cr in the 50-100 nm particle size range have also been observed in the UF-ATOFMS studies across the United States.

The UF-ATOFMS has been used to characterize the UF particles emitted from vehicles (Sodeman, 2006; Toner, 2007). The signatures obtained in source studies have been used to apportion ambient particles in other ambient studies. The major sources of the UF particles at the sampling locations cited above were vehicles. In most locations, diesel UF particles dominated over gasoline emissions. This result is supported by the high abundance of EC particles in the 50-100 nm size range. Previous studies have shown extremely high levels of ultrafine particles emitted from diesel vehicles (Johnson et al., 2005; Kittelson et al., 2006a,b). In addition, many EC particles are associated with lubrication oil species Ca and phosphate (Sodeman et al., 2005; Spencer et al., 2006; Toner et al., 2006). Biomass particles are also detected in the UF mode, but are typically larger in size and thus not as prevalent as vehicle emissions.

Most recently, the UF-ATOFMS has been used to apportion particles from vehicles in a freeway-side study in San Diego. Even though diesel vehicles represented a small fraction of the vehicle fleet (3-5%), their contributions to UF particles were higher than the contributions from cars (Toner et al., 2007). The temporal dependence of UF particles was quite distinct from particles in the larger size ranges. For example, the UF elemental carbon particles peak for several hours each morning during rush hour traffic. There is a strong diurnal trend as the UF particles age and grow to larger particles throughout the day. At mid-day the UF particles have virtually disappeared as they have converted to larger more aged particles due to the addition of secondary organic aerosol, polycyclic aromatic hydrocarbons, amines, ammonium, and nitrate

species. UF soot particles are virtually absent on Sundays due a lower number of diesel vehicles on the road.

By selecting particles of a given size using a scanning mobility particle sizer (SMPS), the effective density of soot particles was determined in the UF-ATOFMS (Spencer et al., 2007). The smallest sized ambient soot particles (50-300 nm) had low effective densities, suggesting they were freshly emitted particles that were highly fractal in nature.

3. Health effects of ultrafine particles – epidemiological evidence

Pulmonary effects

Epidemiological studies reporting on health effects of ultrafine particles on pulmonary function and symptoms in asthmatics date back to the end to the 1990's. In Erfurt, Eastern Germany, decreases in peak expiratory flow were recorded in a study assessing the impact of ambient particles in 27 adults (Peters et al. 1997b), where the effects of ultrafine particles seemed to be the stronger than those of fine particle concentrations. In the same panel, increases in cough and deterioration of self-rated health were observed for both with fine and ultrafine particles. While the immediate effects of particle mass, and especially of particles with a diameter larger than 1 µm were noted, the ultrafine particles were associated with cumulative effects (Peters et al. 1997a). Associations with symptoms and medication intake were also noted in a second panel study including 53 adult asthmatics conducted in the winter 1998/99 (van Klot et al. 2000). Here the cumulative effects of elevated ultrafine particles were recorded over a two-week period manifesting itself in more on-demand asthma medication intake.

Studies conducted in Finland, showed comparable results for adults, indicating decreases in peak expiratory flow rates (Penttinen et al. 1999). Source apportionment analyses further suggested that the effect of the ultrafine particles can be attributed to particles from local combustion sources (Penttinen et al. 2006). A study of patients with chronic obstructive lung disease observed associations between lung function and symptoms for fine but not for ultrafine particles (Osunsanya, Prescott, & Seaton 2001).

In contrast to the finding in adults, ultrafine, fine and course particles were associated with comparable decreases in peak expiratory flow in children (Pekkanen et al. 1997; Tiittanen et al. 1999).

Mortality

The association between ultrafine particles and daily mortality was first studied in Erfurt, Eastern Germany for the time period 1995 to 1998 (Wichmann et al. 2000) with an updated data analysis including data until 2001 (Stolzel et al. 2006). These analyses indicated that ultrafine particle number concentrations were associated with increased daily mortality 4 days later, with the smallest size-fraction (particles between 10 to 30 nm) exhibiting the strongest effects. These associations were seen both for respiratory as well as cardiovascular disease mortality.

Cardiovascular disease hospital admissions

Daily hospital admissions for ischemic heart disease were associated with ultrafine number concentrations assessed as part of a multi-center European study (Forastiere et al. 2005; Lanki et al. 2006b). More specific data from Rome indicated that the associations were particularly observed between ultrafine number concentrations and fatal, non-hospitalized deaths (Forastiere et al. 2005). During the follow-up of myocardial infarction survivors in Europe, ultrafine particles were associated with readmission to the hospital for cardiovascular diseases (von Klot et al. 2005). In contrast to the associations observed for mortality in Erfurt, studies on myocardial infarctions suggested immediate effects that were observed on the same day. No association between ambient ultrafine particle number concentrations and MI onset was found in a case-crossover analysis (Peters et al. 2005). However, evaluating the times spent in traffic as a potential risk factor indicated that one hour later the risk for an onset of myocardial infarction tripled (Peters et al. 2004) that may be considered as indirect evidence. Analyses of emergency room visits for cardiovascular diseases in the Atlanta metropolitan area in contrast did not support an association between ultrafine particle number concentrations and emergency room visits (Metzger et al. 2004).

Inflammatory responses

Ultrafine particles were associated with inflammatory responses in coronary artery disease patients as indicated by an investigation of 56 coronary artery disease patients in Erfurt, Germany. Elevated concentrations of C-reactive protein, soluble ICAM-1 and von Willebrandt-Factor were observed (Ruckerl et al. 2006). These findings were further substantiated by increases in CD40-ligand, a marker of platelet activation. Evidence was found for immediate as well as cumulative responses indicating that ultrafine particles might exhibit direct as well as indirect effects (Ruckerl et al. 2007). Evidence of effects of ultrafine particle number concentrations within hours were also found by a multi-center study in myocardial infarction

survivors in whom IL-6 increased on days with elevated ultrafine number concentrations (Rückerl et al. 2007).

Cardiac responses

The first evidence for associations between ultrafine particles and cardiac functions was reported in coronary artery disease patients undergoing a mild exercise test (Pekkanen et al. 2002). Elevated concentrations of ultrafine particles were associated with a doubling of the risk to observed ST-segment depressions. The associations were independent of the fine particle mass, but not of their number concentrations indicating the overlap and correlation between number concentrations of particles larger than 100 nm (accumulation mode particles) and particles smaller than 100 nm (ultrafine particles). Further analyses of sources substantiated the finding that local as well as regionally transported particles are associated with ischemia during light exercise (Lanki et al. 2006a). Heart rate variability changed in association with ultrafine particle number concentration in a pooled analysis of coronary artery patients from Amsterdam, Erfurt and Helsinki indicating altered sympothovagal balance during paced breathing (Timonen et al. 2006). Repolarization parameter changed in a study of coronary artery disease patients from Erfurt in association with ultrafine number concentrations, indicating that not only the autonomic control is altered, but that there might be also direct effects at the myocardium (Henneberger et al. 2005). Runs of ventricular and supraventricular ectopic beats were also associated with increased number concentrations of ultrafine particles (Berger et al. 2006) again indicating that particles of different ages and origin jointly contribute to cardiac effects.

4. Extrapulmonary Effects of UFP in Clinical Studies

Carefully controlled, quantitative studies of exposed humans offer a complementary approach to epidemiological investigations and *in vivo* and *in vitro* animal toxicological studies. Human clinical studies utilize laboratory atmospheric conditions, which can be designed to simulate specific ambient pollutant atmospheres. They then document symptoms and health-related physiological effects that result from breathing the atmospheres. Advantage was taken of the highly controlled environment to identify responses to individual pollutants and to characterize exposure-response relationships. In addition, the controlled environment provides the opportunity to examine interactions among pollutants, or between pollutants and other environmental variables such as humidity, temperature, or exercise. In so far as individuals with respiratory or cardiovascular diseases can participate in exposure protocols, potentially susceptible populations can be studied. However, this approach has limitations as well. For

practical and ethical reasons, studies must be limited to small groups, presumably representative of larger populations; to short durations of exposure; and to pollutant concentrations that are expected to produce only mild and transient responses. Furthermore, acute, transient responses seen in clinical studies cannot necessarily be used to predict health effects of chronic or repeated exposure. Endpoint assessment traditionally has included symptoms and pulmonary function, but more recently has been extended using a variety of markers of extrapulmonary effects, most specifically, cardiac and vascular effects.

Dosimetry of Ultrafine Particles

Clinical investigations into pollutant-induced pulmonary and/or extrapulmonary biological responses following inhalation exposures are greatly enhanced by a dosimetric approach. By definition, this provides a quantitative basis for expressing dose-effect and dose-response data. These fundamental relationships are invaluable for examining potency, making species comparisons and interspecies extrapolations, comparing normal and susceptible subjects, elucidating underlying mechanisms and developing exposure guidelines.

Our Rochester Center has examined deposition of ultrafine carbon aerosols, a surrogate for environmental carbonaceous particulate pollution, utilizing a mouthpiece exposure system in both normal subjects and asthmatics. Daigle et al (Daigle et al. 2003) exposed 12 subjects to both 10 and 25 ug/m^3 graphitic carbon (mean CMD 26 nm with a GSD of 1.6) and determined the deposition fraction (DF) for both particle number and mass by analyzing the change in the respective particulate number concentrations and particle sizes in the inspired and expired air. Since the particle sizes of the inspired and expired aerosols were measured in 8 particle size ranges, viz., from 8.7 to 64.9 nm, respectively, that included more than 98% of the particles, a particle size *vs.* DF curve was constructed using the DF values which ranged from 0.80 at 8.7 nm to 0.55 at 64.9 nm. The DFs were then compared to theoretical deposition curves for the same size range. The experimental data generally agreed with predicted data under conditions of rest, but DF was found to be significantly greater than predicted under conditions of exercise. No significant DF differences were seen between sexes or between exposures at 10 and 25 µg/m^3.

Another study in subjects with asthma (Chalupa et al. 2004) allowed for a comparison between healthy and asthmatic subjects. The total DFs for healthy subjects were 0.66 ± 0.11 at rest and 0.83 ± 0.04 during exercise. Asthmatic subjects showed higher respective total DFs, viz., 0.76 ± 0.05 and 0.86 ± 0.04. Thus UFP deposited with greater efficiency in subjects with asthma than in healthy subjects, both at rest and during exercise. Fine particles also deposit with

greater efficiency in patients with obstructive airways disease than in healthy subjects, although the overall deposition efficiency is much lower than for UFP. These findings provide a potential mechanism to explain epidemiological findings suggesting that people with asthma, and people exercising outdoors, are at increased risk for UFP effects.

Markers of Extrapulmonary and Cardiovascular Disease, Findings with Laboratory-generated Ultrafine Carbon Particles

Advances in non-invasive or minimally invasive medical diagnostic procedures have provided new opportunities to investigate the cardiovascular effects of air pollution. Table 1 provides examples of measures of cardiac and vascular function, many of which have been and are being applied in clinical studies of air pollution exposure. However, very few studies have specifically examined clinical effects of UFP exposure.

The "ultrafine hypothesis" relates health effects to the small particle size and high surface area characteristic of UFP. It is therefore essential that the exposure technology used in clinical studies preserves these particle characteristics. This necessitates exposure to fresh UFP aerosols with careful control of particle residence time to avoid agglomeration. Issues of ultrafine particle exposure and generation are covered in more detail elsewhere in this document.

Currently, there are two methods of UFP exposure that have been and are being used in clinical studies: carbon ultrafine particles freshly generated in the laboratory, and concentrated ambient ultrafine particles. Additionally, a series of studies have been conducted with exposure to diesel exhaust, which is a complex mixture containing relatively high numbers of ultrafine particles, and exhaust gases.

One of the hypotheses tested in our clinical studies is that inhalation of UFP has both pulmonary and systemic vascular effects. We postulated that subtle vascular responses would be reflected as changes in the expression of adhesion molecules on the surface of leukocytes sampled from the peripheral blood, in addition to other measures of pulmonary and systemic vascular function.

We designed an exposure system for clinical studies of UFP, with particles generated in an argon atmosphere using an electric spark discharge between two electrodes of elemental carbon (Chalupa et al. 2002). Exposures were for two hours by mouthpiece. Initial exposures to 10 $\mu g/m^3$ ultrafine particles at rest showed no effects on symptoms, respiratory function, heart rate variability, changes in the ST segment of the ECG, or cardiac repolarization (Pietropaoli et

al. 2004b). There were also no effects on soluble markers of coagulation and inflammation (Pietropaoli et al. 2004a).

Having established the feasibility and safety of the exposure system during resting exposures, a subsequent series of clinical studies were undertaken involving intermittent exercise. Healthy subjects inhaled filtered air or freshly generated elemental carbon particles (count median diameter ~25 nm, geometric standard deviation ~1.6) for two hours, in three separate protocols: 10 $\mu g/m^3$ at rest, 10 and 25 $\mu g/m^3$ with exercise, and 50 $\mu g/m^3$ with exercise. In an additional protocol, subjects with mild asthma inhaled air and 10 $\mu g/m^3$ UFP.

In healthy subjects, exercise in air induced changes in leukocyte surface marker expression, including increased monocyte expression of CD54 (intracellular adhesion molecule-1) (Frampton et al. 2006). UFP exposure blunted this increase or reduced expression of CD54 in a concentration-response fashion. UFP also reduced expression of CD18 on monocytes and CD18 and CD49d on granulocytes. There were also concentration-related reductions in the numbers of blood monocytes, basophils, and eosinophils, and increased lymphocyte expression of the activation marker CD25. In subjects with asthma, UFP exposure reduced expression of CD11b on monocytes and eosinophils, and CD54 on granulocytes.

The increase in surface expression of adhesion molecules such as CD54 after air exposure likely reflects a "flushing out" of more highly expressing cells finding their way through the pulmonary circulation, as a result of increased pulmonary blood flow with the exercise during exposure. UFP exposure blunted or reversed this increase, suggesting the possibility that UFP exposure may have induced relative pulmonary vasoconstriction, or impaired exercise-related vasodilatation in the lungs.

In order to test this hypothesis, we measured UFP effects on the pulmonary diffusing capacity for carbon monoxide (DLCO), which is sensitive to changes in pulmonary capillary blood volume (Pietropaoli et al. 2004a). We observed a significant reduction in DLCO 21 hours after exposure to 50 $\mu g/m^3$ UFP when compared with filtered air. There were no effects of UFP on spirometry or exhaled nitric oxide, making it unlikely that the effects on DLCO were caused by airway epithelial injury or impairment in carbon monoxide diffusibility across the pulmonary membrane. This study therefore supported the hypothesis that inhalation of carbon UFP has subtle effects on pulmonary vascular function, possibly by transiently reducing or redistributing the pulmonary capillary blood volume.

Inhalation of UFP may also have transient effects on systemic vascular function. Sixteen healthy subjects inhaled air or 50 $\mu g/m^3$ carbon UFP for two hours with intermittent exercise

(Shah et al. 2008). To assess effects on systemic vascular function, venous occlusion plethysmography and reactive hyperemia of the forearm were measured before and at intervals after exposure along with blood venous levels of nitrate and nitrite. The peak forearm blood flow after ischemia increased 3.5 hours after exposure to air, but not UFP ($p=0.03$). There was no change in systemic blood pressure, and there was no UFP effect on pre-ischemia forearm blood flow. Venous nitrate levels were significantly lower after exposure to carbon UFP compared to air ($p=0.038$), suggesting that UFP exposure may deplete the availability of nitric oxide. We concluded that UFP inhalation transiently impaired peak forearm blood flow during reactive hyperemia.

Recently published clinical studies of exposure to diesel exhaust provide additional support for systemic vascular effects of air pollution exposure, although it remains unknown to what degree the ultrafine particle component of diesel exhaust is responsible for the observed effects. In one study (Mills et al. 2005), 30 healthy men were exposed to diluted diesel exhaust (300 $\mu g/m^3$ PM) or air for one hour with intermittent exercise. Forearm blood flow and inflammatory factors were measured before and during intra-arterial infusion of vascular mediators. In comparison with air exposure, diesel exhaust exposure attenuated the vasodilator response to bradykinin, acetylcholine, and sodium nitroprusside. Diesel exhaust also suppressed the increase in plasma tissue plasminogen activator stimulated by bradykinin. In another study (Mills et al. 2007), 20 men with prior myocardial infarction inhaled diesel exhaust and air for one hour under similar conditions, with intermittent exercise. Measurement of ST segment depression by ECG suggested an increase in the burden of ischemia to the myocardium following diesel exhaust compared with air exposure. These studies provide support for the hypothesis that exposures containing high numbers of ultrafine particles have transient systemic vascular effects that may be important for patients with coronary artery disease.

In summary, despite the practical and ethical limitations of clinical studies, human data have an important role in understanding air pollution health effects. Clinical studies of UFP present specific technical challenges. Our studies of the effects of laboratory generated UFP suggest that even relatively inert particles of elemental carbon induce subtle changes in both pulmonary and systemic vascular function. We have observed effects with exposures at mass concentrations an order of magnitude lower than in the studies of vascular effects of diesel exhaust. The findings suggest that these vascular effects are not a consequence of airway inflammation. Whether UFP act directly on vascular endothelium or blood cells via

translocation from the airways, or act indirectly through generation of soluble mediators, requires further studies.

Table 1. Clinical measures of cardiovascular effects.

	Cardiac	Vascular	Coagulation
ECG:	Heart rate Heart rate variability Repolarization Arrhythmias S-T segment depression (ischemia)	Forearm flow-mediated dilatation Digital plethysmography Soluble markers of endothelial function Diffusing capacity for carbon monoxide (DLCO) Soluble markers of vascular inflammation	Clotting factors Products of thrombosis & thrombolysis Platelet number, function, activation Thrombus formation Tissue Factor Circulating Microparticles
Echo-cardiography:	Cardiac output Ventricular function Pulmonary artery pressure		

5. Biokinetics and Pulmonary and Cardiovascular Effects Studies in Animals

Introduction

The animal studies were designed to be complementary to the field and controlled clinical studies and to provide a link to the mechanistic *in vitro* studies. Furthermore, they were designed to (*i*) determine pulmonary and systemic responses to inhaled laboratory-generated and real world ultrafine particles (UFP) and (*ii*) to develop rodent models of human disease to test our central hypothesis that UFP contribute to the increased morbidity and mortality of susceptible individuals seen in association with small increases in urban particles. Thus, the overall objective of the animal studies was to identify factors that are causally associated with adverse pulmonary and extrapulmonary health effects after low-level exposures to UFP. We hypothesized that these factors would include particle size, lung deposition and disposition, host susceptibility, and pollutant co-exposure (*e.g.* ozone).

Biokinetic Studies

Translocation of ^{13}C UFP to the Liver

Kinetics studies can provide insight into whether or not observed responses in animals may result from UFP retention in a particular organ system or if they are due to soluble mediators that are generated in distal organs. Rats were exposed to ultrafine ^{13}C particle-containing aerosols (CMD, 20-29 nm; GSD, 1.7) at concentrations of 180 and 80 $\mu g/m^3$. ^{13}C was analyzed by isotope ratio mass spectrometry. The ^{13}C retained in the lung at 0.5 hrs post-exposure was about 70% less than predicted by rat deposition models for UFP and did not change significantly over 24 hrs. Normalized to exposure concentration, the added ^{13}C per gram of lung during the post-exposure period was on average ~9 ng/g organ/$\mu g/m^3$ (Oberdörster *et al*., 2002). Significant amounts of ^{13}C accumulated in the liver within 30 mins after exposure to 180 $\mu g/m^3$ and after 18 and 24 hrs, the added liver ^{13}C concentration was more than one-third the concentration found in the lung. There was also a slight increase in added ^{13}C in the olfactory bulb, but no increase was detected in the other organs that were examined. These results demonstrated the effective translocation of elemental carbon UFP to the liver within one day after inhalation exposure. Potential translocation pathways include direct input into the blood compartment from UFP deposited throughout the respiratory tract as well as uptake into the blood via the GI tract after swallowing. In contrast to these results with ^{13}C UFP, we found in another study that only minimal amounts of ^{192}Ir UFP were translocated to extrapulmonary organs, including liver, after intratracheal inhalation exposures (Kreyling *et al*., 2002).

Differences in exposure methodology (whole body inhalation vs. intratracheal inhalation) and particle types (carbon vs. iridium) could be reasons for differences in biokinetics. Interestingly, the liver accumulation of 15 nm iridium particles was 5-10 times higher than for 80 nm iridium particles.

Translocation of UFP to the Brain

Subsequent studies in rats with inhaled ^{13}C UFP (CMD, 36 nm; GSD, 1.66; 160 µg/m^3, 6 hrs) resulted in pulmonary ^{13}C UFP deposition of 1.39 µg at day 1. Lung ^{13}C concentration decreased from this level to 0.59 µg/g by day 7d (Oberdörster *et al*., 2004). There was a significant and persistent increase in ^{13}C in the olfactory bulb of 0.35 µg/g (day 1) to 0.43 µg/g (day 7) throughout the 7-day post-exposure period with respective ^{13}C levels of 30-40 ng per organ. Day 1 ^{13}C concentrations in cerebrum and cerebellum were also significantly increased, but the change was not always significant over the following days. We concluded from this study that the CNS can be targeted by inhaled UFP and that a neuronal route of translocation of nasally deposited UFP via the olfactory nerve exists.

In order to confirm the olfactory translocation route for inhaled poorly soluble UFP we used another material in a subsequent study. We selected ultrafine Mn oxide because Mn can be sensitively detected by atomic emission spectroscopy (AES), is a known neurotoxicant, and is of importance for certain occupational exposures where these particles are generated as UFP (metal smelting, welding). Rats were exposed for 2 days, 6 hr/day, to Mn oxide UFP (CMD, 31 nm; GSD, 1.77) with the right naris occluded. Mn accumulated only in the left olfactory bulb, providing further support for the olfactory neuronal route of UFP translocation. Progressive and almost 4-fold increases in the Mn content of the olfactory bulb were found after 12 days of exposure in a subsequent study. Smaller increases were also seen in striatum and frontal cortex, structures that are anatomically close to the olfactory bulb (Elder *et al.,* 2006). Lung Mn content was slightly more than doubled, but there was no evidence of lung inflammation, as assessed by cellular and biochemical lung lavage parameters. An evaluation of the solubility of the mixed Mn oxides (61% Mn (II), 39% Mn (III)) revealed that only ~1.5% of the Mn was solubilized per day in physiological saline at neutral pH. We conclude from these data that the Mn oxide UFPs are transported in solid particle form by the olfactory neuronal axons rather than in solubilized form.

Effect Studies

Toxicology Studies with Laboratory-generated UFP

Studies in Young and Old Mice Exposed to Combinations of UFP and Ozone with Respiratory Tract Priming

Young (8-10 weeks) and old (20-22 months) mice were exposed to UFP (carbon or mixed carbon/Fe) with or without ozone for 6 hrs in compartmentalized whole-body chambers. Respiratory tract cells were primed with inhaled endotoxin (LPS). In agreement with our earlier studies in rats (Elder *et al*., 2000), all four factors (UFP, ozone, LPS, and age) had significant main effects on most of the respiratory and cardiovascular endpoints examined. The striking age effect was such that inflammatory and cell activation responses in old mice were greater than in young mice. For some endpoints (*e.g.* lavage PMNs, lavage AM surface ICAM-1 expression), the UFP effect was dependent upon the presence of LPS. This draws attention to the fact that there were several consistent interactions involving inhaled UFP, among them those involving LPS and age (response enhancement) and ozone (response suppression). Our findings that inhaled UFP can alter blood PMN surface ICAM-1 expression are in agreement with results from PM Center-related clinical studies. Some of the most striking effects were observed in lung and heart tissue gene expression changes. Not only were significant alterations in heart tissue gene expression observed, indicating extrapulmonary effects of inhaled UFP and ozone, but the data also suggests an imbalance in old animals between pro- and anti-inflammatory species production (Elder *et al*., 2004a). Similar results were obtained from studies in which influenza virus was used as the priming agent. When the results from these studies are considered as a whole, a consistent pattern of main effects and factor interactions emerges. Ozone, the priming agent, and age almost always had significant main effects on the pulmonary and vascular endpoints examined. In addition, independent and significant main effects of UFP were frequently found. UFP also consistently interacted with the respiratory tract priming agent and ozone to alter physiological response. Additionally, young and old animals differently modulated their responses to UFP in combination with the other factors. Given the fact that there were so many endpoints analyzed in these two sets of studies, the consistency of these interactions is remarkable and strengthens the causality of associations that were found in the statistical analyses. Moreover, the results are also consistent with those of our earlier multigroup studies in young and old rats, showing that effects are not species specific.

Effects of Ultrafine Particle Exposure on the Formation of Thrombi in Rat Ear Veins

Intravenous administration of ultrafine aminated-polystyrene (positively charged) particles (0.02, 0.5 and 50 mg/kg) 30 min after the start of the rose Bengal (RB) infusion significantly shortened the time of thrombus formation in rat ear vein. However, carboxylated PS (negatively charged) particles of the same size failed to affect thrombus formation at any dose. These data suggest that UFP can affect coagulation directly, but that the effect will depend on particle charge. Intravenous administration of positively-charged UFP without RB also resulted in a significant reduction in thrombus formation time. Likewise, intratracheal instillation of the 60 nm PS particles shortened thrombus time to about 42% of the baseline level. These data indicate that particles can induce changes in thrombus formation without RB and that they might translocate from the lung into the blood stream to produce thrombi in the ear vein model (Silva *et al*., 2005).

The thrombogenic potential of more environmentally relevant particles was also assessed using the ear vein model in preliminary studies. Laboratory-generated carbon UFP (30 nm; 4, 20, 100 and 500 μg/kg) were administered intravenously to rats and their effects on coagulation studied. It was shown that doses as low as 4 μg/kg (1 μg per rat) significantly shortened the time of thrombus formation. Although different doses are not significantly different from each other, there seems to be a trend indicating that the lower the dose of UFP in the system, the greater the effect. Similar results were obtained when particles were intratracheally instilled. In this case, even a lower dose (0.8 μg/kg or 0.2 μg per rat) was able to produce a significant effect. As with these studies, we also observed a decrease in thrombus formation time in rat ear vein following inhalation exposure to either 70 or 200 $\mu g/m^3$ carbon UFP.

Toxicology Studies with Ambient UFP

Studies with Freshly-Generated On-Road Ultrafine /Fine Particles

For toxicological studies with real-world UFP, diluted exhaust from stationary engines or concentrated ambient UFP have been used, yet questions remain about how well these particles model those found in ambient air. Freshly-generated UFP are present at high concentrations on highways and passengers are directly exposed to them when traveling behind other vehicles. Rats were exposed in a mobile emissions laboratory (MEL) designed at the University of Minnesota to test the potential of freshly-emitted highway aerosols diluted at realistic levels (1:400 to 1:800) to evaluate pulmonary and cardiovascular effects. Three exposure atmospheres were separated for these studies: highway aerosol + gas phase, gas phase only, or particle-filtered, gas-denuded (filtered) air. The exposures in compartmentalized whole-body chambers consisted of 6 hr driving periods on I-90 between Rochester and Buffalo once or 3 days in a row. The

daily average number concentration in the control (filtered air) chamber was 0.01-0.12 x 10^5 particles/cm^3. The exposure chamber number concentrations were 0.95-3.13 x 10^5 particles/cm^3. Daily averages for co-pollutant gases ranged from 6.9-9.6 ppm for CO, 401-423 ppm for CO_2, and 0.10-0.24 ppm for NO. Further details about the system itself and the aerosol characterization are reported by Kittelson and colleagues (2001, 2004).

Old rats were pretreated with a low dose of inhaled endotoxin or with instilled influenza virus to induce lung inflammation as a model of susceptibility, as in our studies with laboratory-generated UFP. Endpoints related to lung inflammation, inflammatory cell activation, and acute phase responses were measured after exposure. Since such exposures had not been done, our objectives were to: (i) demonstrate the feasibility of on-road exposure studies; (ii) determine if there were significant effects of exposure in aged rats; and (iii) determine if priming (endotoxin; influenza) of respiratory tract modulated the responses to inhaled aerosols.

The exposures were well-tolerated by rats, baseline values from sham-exposed animals did not differ from what has been previously published for old F-344 rats; in addition, there were no effects of exposure on body weight. We also observed the expected increases in response (inflammation, inflammatory cell activation) to the priming agents. Interestingly, in general the results suggested no differences in rats exposed to gas-phase components alone *vs.* the gas-phase + particle mixture. However, we found a significant particle-associated increase in plasma endothelin-2 in the group exposed to the mixture, suggesting alterations in vascular endothelial function. In addition, we observed main effects of particles related to the acute-phase response and inflammatory cell activation (increased ICAM-1 on macrophages). Interactions between on-road particles and the priming agents were also found (Elder *et al.*, 2004b).

Analysis of HRV in Unrestrained, Telemetered SH Rats Exposed to On-Road Aerosols

In parallel to the evaluation of ECG recordings in the studies of the epidemiological and clinical cores of the Center, our collaborators in the cardiac core developed and tested an algorithm for analysis of ECG and blood pressure signals recorded from rats. An important finding from the development of this algorithm was that at least 1500 beats (~5 mins) are needed to obtain reliable and reproducible estimations of HRV parameters in rats (Couderc et al., 2002). Because of this finding, we established a protocol in which data could be recorded continuously from telemetered rats following on-road exposures. The results showed that heart rate, SDNN, and vagosympathetic balance decreased and the high frequency (HF) component of the power spectrum increased in response to on-road aerosols, suggesting a dysregulation of autonomic input (Elder et al., 2007). Although we did not evaluate arrhythmias, it is possible that a

prolonged imbalance favoring parasympathetic input to the heart rate might lead to bradyarrhythmias. Given evidence of parasympathetic activation and the fact that pulmonary irritant receptors are under vagal control, it is conceivable that the observed effects were mediated either directly or indirectly by inhaled ultrafine on-road particles and their gaseous co-pollutants at the level of the lung as opposed to a systemically-derived response. However, since companion studies in aged F-344 rats revealed significant increases in plasma fibrinogen and endothelin-2 in response to the UFP/gas mixture, a systemic origin for the HR changes cannot be completely ruled out. Although HRV analyses in humans and animals exposed to PM tend to produce variable outcomes, our findings of decreased HR and vagosympathetic balance agree with other recent reports (Gold et al., 2000; Ibald-Mulli et al., 2004; Timonen et al., 2005).

CNS Effects of Inhaled UFP

Because our biokinetics studies have demonstrated that UFP deposited in the upper respiratory tract can translocate to certain regions of the brain, we assessed UFP toxicity in these regions. Brain tissues from our Mn oxide UFP-exposed rats were screened for gene and protein expression changes *via* microarray and proteomic analyses. Increases in glial fibrillary acidic protein and tumor necrosis factor-α, for example, were found in olfactory bulb (8-fold increase in message, 30-fold increase in protein) and, to a lesser extent, in other brain regions where Mn levels were also increased as a result of exposure (Elder et al., 2006). Furthermore, we found increases in mRNA expression for tumor necrosis factor-a and its receptor I in olfactory bulb tissue from old rats exposed on-road to freshly-generated exhaust emission aerosols in combination with endotoxin or influenza virus. These results are intriguing and imply that inhaled UFP – most likely depending on their chemistry – may affect CNS function, in particular if there is continuous accumulation under even low environmental exposure conditions. The relationship between the observed responses and UFP physicochemical characteristics remains to be determined.

6. Mechanistic *In Vitro* Studies

Numerous epidemiological studies have found a correlation between exposure to respirable airborne particulate matter (PM) and increased mortality and adverse respiratory health effects, including the development of emphysema, chronic bronchitis, and asthma (Dockery, 1993, Dockery et al., 1993, Morris et al., 1995, Pope et al., 1995) and acute and chronic cardiovascular effects. On the tissue and cellular level, PM-deposition insults can result in pulmonary inflammation, airway hyperreactivity, epithelial cell damage, and increased

epithelial permeability (Fabbri et al., 1984). A key biological effect of inhaled PM has been the recognition of cardiac and cardiovascular effects (Bagate et al., 2004, Devlin et al., 2003, Ulrich et al., 2002). The mechanism of effects of inhaled PM on the cardiovascular system were more difficult to discern until recent data indicating the transport of ultrafine PM across the pulmonary epithelium into the vascular bed (Kreyling et al., 2002, Oberdörster et al., 2002, Oberdörster and Utell, 2002). Thus the possibility of direct particle cell interaction with the vascular endothelium becomes a distinct mechanistic possibility.

Work by a number of authors (Breen *et al*. 1992; Crestani *et al.* 1994; Driscoll *et al.* 1996; Dunsmore *et al*. 1996; Finkelstein, 1990) has suggested that production of both inflammatory and fibrotic mediators following particle interaction is not limited to classic inflammatory cells, that pulmonary parenchymal elements including epithelial cells (type II, Clara cells), vascular endothelial cells and fibroblasts my also contribute to the response.

The formation of reactive oxygen species and subsequent lipid peroxidation is believed to play a major role in toxicity; however, the rate of formation of reactive oxygen species can depend on synergistic effects between components of PM and on the presence of relatively benign materials. The direct mechanisms by which the wide variety of airborne PM types impact target cells in the respiratory and cardiovascular system is diverse, thus severely complicating schemes to monitor the potential impact of the release of such particulates into the atmosphere. One approach to dealing with these complexities and potentially formulating specific mechanisms relies on reducing the interacting systems to relatively simplistic experiments. Experiments in which cultured cells were incubated with various forms and concentrations of PM, are of necessity simplistic and subject to criticism. In formulating such experiments one needs to carefully consider a vast array of potential variables. Among these are the choice of PM, cell type, the biological endpoint, (influenced in no small part by the choice of cell) and finally the exposed dose. Each of these has the potential to influence the result of such studies and limit their applicability for formulating generalizable mechanisms.

To test the hypothesis that increased susceptibility of aged animals is due to cell intrinsic differences in oxidant sensitivity, experiments evaluating the effect of age on the response of cells to particles have been performed. Comparing the effects of endotoxin and various characterized particles added to cells from 22-27 month old rats or from 10-12 week old rats revealed increased production of PGE2 by cells from “aged” animals, used as a maker of the ability to induce an inflammatory response. Also production of MIP-2 and TNF was significantly elevated in cells from “old” animals.

When particles and LPS were combined as a stimulus an enhanced effect is observed only in the "old" cells except at the highest dose of particles. Most significant, in the context of our investigation of age effects and the ability of particles to induce effects at low dose, was the fact that in the aged animals co-administration of particles and LPS leads to synergistic effects at the lowest dose of particles. This result is somewhat similar to results obtained in the *in vivo* studies in which enhanced response to combined insult was noted in aged rats.

Particle Effects on Vascular Endothelium

Monolayer cultures of human umbilical vein endothelial cells (HUVEC) have been used to mimic studies of cardiovascular effects of PM. Vascular endothelial cells (HUVEC) exposed to conentrated fine and ultrafine particle respond through increased production of IL-6. This cytokine was chosen as a possible acute phaase response following particle inhalation in vivo . Interestingly, using this marker and cell type we revealed a differential response from particles of varying origin. It is hypothesized that this relates to the abundance of vehicle emissons at these different sites. Studies of these same particles using human epithelial cells revealed an important principle of *in vitro* modeling. When epithelial cells (A549 cells) were similarly exposed to these materials no differential response based on site selection was noted. This emphasizes the point that it is critically important to choose the test cell system carefully and not to overinterpret the results from a single cell type

Role of oxidative stress in the response to particles

A major hypothesis of these studies on PM is that cellular activation of cells following interaction with particles is a result of cellular oxidative stress. This may be an important characteristic that differentiates the activity of various particle types but does not directly address the question of whether particles increase the oxidative stress response inside cells and whether particle uptake is a critical mechanism in cellular activation. These studies have been addressed by investigators in a number of ways. Fluorescent dyes that vary in their cellular permeability can be used to look at both intracellular and extracellular dye oxidation after adding particles. In addition one can assess oxidative stress under conditions in which particle uptake is blocked. The result of these studies suggests that with ultrafine particles, uptake is required to induce cellular oxidative stress, regardless of composition. How this oxidative stress manifests injury is the subject of intense investigation and a number of hypotheses

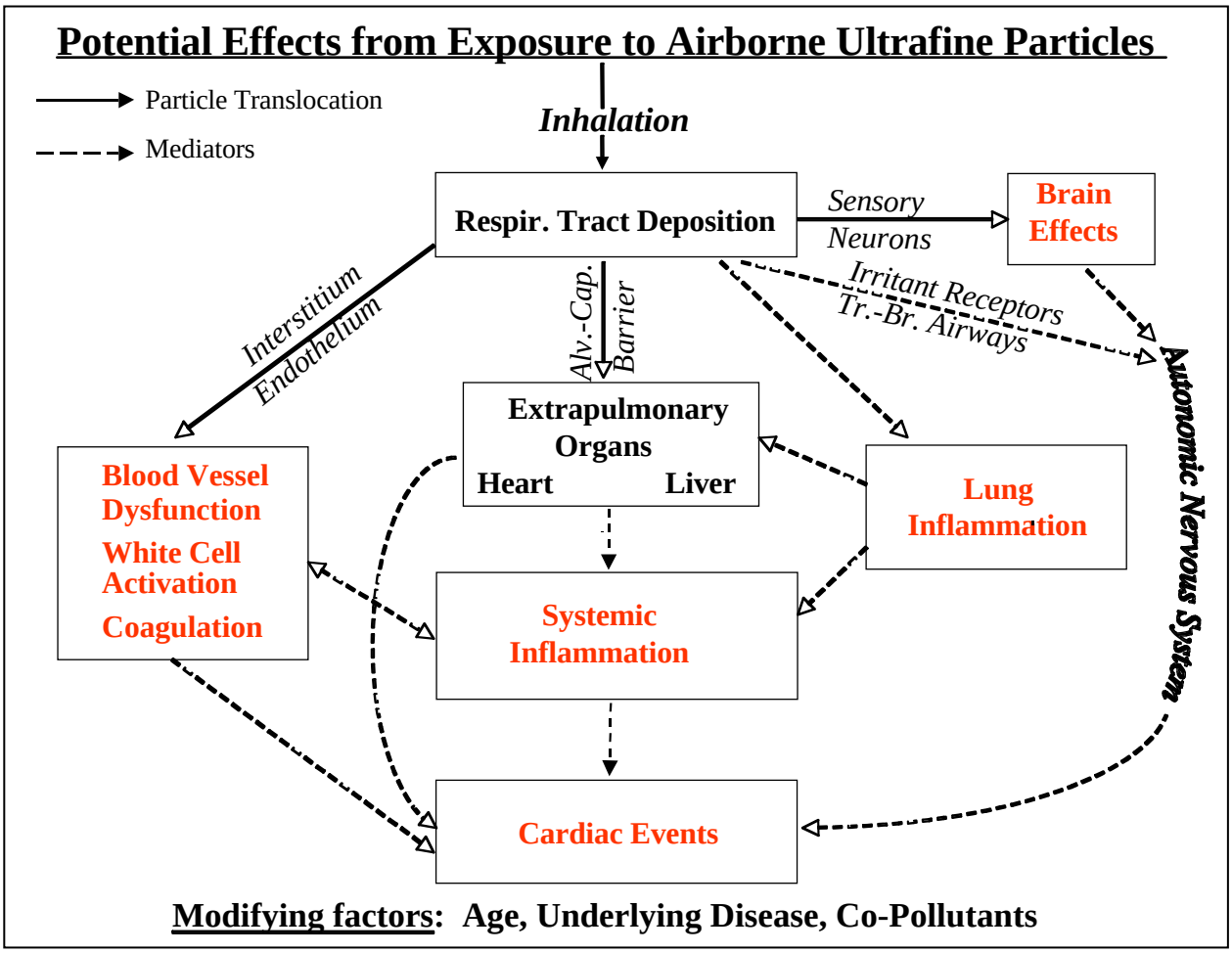
Cell Culture Models of Disease

Among the susceptible populations to PM cardiovascular effects are type 2 diabetics. The possibility to be able to model the effect of diabetes in a cell specific manner could be beneficial. Recent studies in the Rochester Center have begun to investigate various possible models of diabetic cells. It has recently been suggested that culture of cells in high glucose can mimic some of the altered responses seen in diabetes. This phenomenon appears to be reasonably well established in the diabetes literature. We have extended these studies to combine the effects of high glucose on the expression of IL-6 by vascular endothelial cells in response to standard inflammatory stimuli as well as effects of culture with PM. In these studies IL-6 protein production was suppressed when glucose is raised to 10 or 30 mM.

Concluding Remarks

Research conducted at the Rochester PM Center in the six years since its inception in 1999 has significantly advanced our knowledge of ultrafine PM induced effects and underlying mechanisms. Table 1 summarizes some of our findings. Based on these results as well as on additional data from the literature, we suggest that ambient ultrafine particles have the potential to induce or contribute to adverse health effects through a network of four separate mechanistic pathways (Figure 1): (*i*) pulmonary inflammatory effects initiating a subsequent systemic acute phase response; (*ii*) translocation of UFP into the blood circulation, leading to interaction with cells of the endothelium and with white blood cells and to distribution to extrapulmonary organs (e.g., liver, heart) thereby inducing vascular effects and cardiac events; (*iii*) translocation of UFP along sensory neuronal pathways to the CNS causing inflammation (neurodegenerative events) or affecting sympathetic and vagal signals, and (*iv*) activation of irritant receptors in the conducting airways affecting vagal input to the automonic nervous system.

There are still a number of obvious gaps that we have now identified that need to be filled in order to get a more complete understanding of UFP-induced effects. There are four broad categories requiring more research: Source-specific UFP characterization; mechanistic studies of UFP effects; UFP exposure in susceptible parts of the population; and UFP biokinetics. The first concerns the need for more detailed physico-chemical characterization of specific UFP sources and source-specific effects as defined by *in vitro* and *in vivo* studies. The second relates to the mechanistic understanding of UFP-induced effects, specifically the role of oxidative stress for inducing cardiovascular effects and effects in the CNS, but also examining the role of UFP-induced electrophysiological alterations that might lead to cardiac events. A third category



involves questions about lowered resistance against UFP-induced effects in susceptible populations, such as those with underlying disease, genetic susceptibility, previous coronary events, and those at an early developmental stage. The fourth has to do with aspects of UFP biokinetics/toxicokinetics which requires additional studies in order to identify extrapulmonary target organs and quantify translocation processes.

We are addressing these gaps in ongoing studies at the Rochester PM Center by continuing our integrated multidisciplinary team approach. Studies include the refinement of our ability to characterize in real-time single particles of less than 50 nm, comprising both physico-chemical characterization and measuring ROS-inducing capacity of PM. Collection of size selective PM from specific sources has begun including motor vehicle traffic and marine diesel emissions for further chemical analysis by advanced methodologies; by advanced methodologies and for evaluating source-specific effects and underlying ROS-based mechanisms in *in vitro* and *in vivo* experiments. Determination of coherence in time series of UFP distributions across Rochester as measured at multiple sites is of particular importance for our current studies

evaluating PM effects with an acute coronary event. Likewise, information from central PM monitoring sites and personal monitors will be collected to study the correlation of source-specific fine and ultrafine particles with observed effects in epidemiological studies.

Diesel exhaust has been one UFP source of particular interest, and we are continuing our on-road studies to assess the impact that switching to ultra-low sulfur fuel has on exhaust composition and on respiratory, cardiovascular and CNS effects. We are using diabetic rats as a model that is known in humans to be a susceptible group. In parallel, current controlled clinical studies with exposure to concentrated Rochester UFP assess markers and mechanisms of cardiovascular effects, measuring blood microparticles, pulmonary capillary blood volume and cardiac ventricular function. With respect to cardiac electrophysiology, one benefit from these corresponding human and rat studies is to gain a better understanding of how to interpret changes in rodent heart rate and heart rate variability in response to real-world UFP aerosols. At the same time, we will learn more about similarities and differences of oxidative stress based mechanisms of UFP induced vascular responses between rodents and humans, providing justification for extrapolating results observed in rodents to humans. Type 2 diabetics, subjects with impaired glucose tolerance, and healthy subjects with suspected genetic susceptibility are also the focus of present epidemiological studies dealing with the issue of specific mechanisms for PM susceptibility.

Expanding our knowledge about the biokinetics of inhaled UFP is of great importance. It appears that translocation rates from the portal-of-entry, the respiratory tract, to secondary organs are very low. However, only very few data are available. Thus, a major effort will be devoted to obtaining more quantitative information, which will greatly help to answer questions as to whether direct UFP-induced effects are likely to occur in remote organs. In this context, we are evaluating translocation pathways to, and effects in, the CNS, using both model surrogate UFP as well as real-world UFP. Hypotheses about suggested neurodegenerative effects of inhaled UFP will be tested, using specific rodent models and both acute and repeat exposures. Of related interest in this regard is the question about the impact of UFP exposure in the developing organism, which is addressed in ongoing studies with exposures of newborn rodents.

The integrated team effort of our PM Center in designing and executing these studies will provide significant new scientific information. This may be useful to EPA's mission. In1998, a Committee of the National Research Council (NRC) published the first of a 3 volume report entitled: "Research Priorities for Airborne Particulate Matter" identifying the 10 highest priority targets for PM research (NRC 1998). The Rochester PM Center has addressed virtually all of the

10 priority targets with a special emphasis on susceptibility of human populations, dosimetry and mechanisms. A major focus for the future of our PM Center will be to better understand susceptibility whether through animal models, performing panel studies of subjects with compromised health status, or using our large established cohorts to identify how PM-associated risk factors (size, composition, source) for adverse health effects may be modified by individual factors such as pre-existing conditions, medication use, diet and/or genotype.

Table l: Summary of Rochester PM Center Studies

Type of Study	Design of Study	Particle Source & Size	Findings	Reference
Field studies in Rubidoux (2003) and New York City (2004) Studies in Rochester (2002) and Philadelphia (2002)	Measurement of particle-bound ROS in size segregated samples Semicontinuous measurement of particle compositions	Particle sizes range from 10 nm to >18 µm PM2.5; traffic and wood smoke	Significant amounts of particle-bound ROS were present in both locations and were correlated with the general level of gaseous oxidants in the local atmosphere	Venkatachari et al., 2005; 2007
Laboratory study	Measurement of the particles entering and leaving ultrafine particle concentrators	For the HUCAP and VACES ultrafine concentrators, particle sizes are <180 nm	The ultrafine concentrators significantly increased the amounts of organic carbon associated with the concentrated ultrafine aerosol	Su et al., 2006
Cohort study	Coronary artery disease; myocardial infarction survivors COPD patients	Traffic exposure, PM UFP numbers, source apportionment based on number concentrations	MI one hr. later ↑ Inflammatory markers, endothelial dysfunction Altered cardiac function, repolarization	Peters *et al*. 2004 Berger *et al*. 2006; Henneberger *et al*. 2005; Rückerl *et al*. 2006, 2007; Yue *et al*. 2007
Clinical study	Randomized, double-blind, crossover, inhalation of air and UFP, healthy, asthmatic & diabetic subjects	PALAS carbon UFP, 25 nm CMD	▪ High UFP deposition ▪ Pulmonary vascular effects ▪ Systemic vascular effects ▪ Reduced NO	Chalupa et al. 2002 Daigle et al. 2003 Chalupa et al. 2004 Pietropaoli et al. 2004a Pietropaoli et al. 2004b Frampton et al. 2006 Shah et al. 2008

Table 1: continued

Type of Study	Design of Study	Particle Source & Size	Findings	Reference
Animal study - Rats	Inhalation, rats	Highway UFP Diesel exhaust; 19 nm CMD	Endothelin ↑, HRV changes; ICAM-1 ↑	Elder *et al.*, 2004a.b; 2007
Rats	1-12 day inhalation, rats; biokinetics of UFP	Lab-generated carbon and Mn-oxide UFP 20-30 nm	Translocation to liver, CNS	Oberdörster et al., 2002; 2004; Elder et al., 2006
Mice	Inhalation, young and old mice; influence of LPS and influenza priming on UFP effects, in combination with O_3	Lab-generated carbon UFP, ~25 nm	O_3, age and priming have significant effect and interact with UFP	Elder et al., 2004
Rats	Inhalation; exposure, UFP effect on CNS	Highway UFP and Mn UFP (~20-30 nm)	Increase of inflammatory markers in olfactory bulb and other brain regions	Elder et al., 2006
Rats	Ear vein thrombus formation, rats; i.t. i.v. inhalation dosing with carbon UFP and positive and neutral charged polystyrene UFP	Lab-generated carbon UFP, ~30 nm; polystyrene UFP, 60 nm	Positively charged polystyrene particles and carbon UFP were thrombogenic after all modes of administration	Silva et al., 2005
In vitro study	Lung epith. cell Endoth. cell	Ambient $PM_{0.1}$; $PM_{2.5}$; PM_{10}	IL6 ↑ ; TNF ↑	Finkelstein *et al.*, 2007

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