

University of Rochester – EPA  PM Center

**ULTRAFINE PARTICLES:
CHARACTERIZATION, HEALTH EFFECTS
AND
PATHOPHYSIOLOGICAL MECHANISMS**

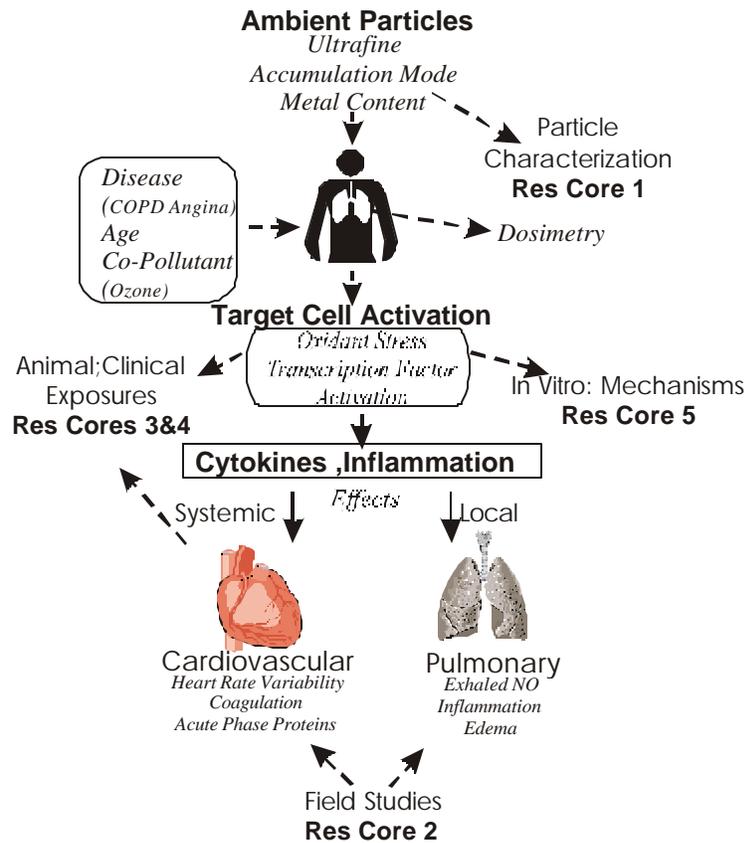
**PROGRESS REPORT
January, 2002**

TABLE OF CONTENTS

	<u>Page</u>
INTRODUCTION	3
Research Core 1: <i>Characterization of the Chemical Composition of Atmospheric Ultrafine Particles</i>	4
Research Core 2: <i>Inflammatory responses and cardiovascular risk factors in elderly subjects with angina pectoris or COPD in association with fine and ultrafine particles</i>	6
Research Core 3: <i>Clinical studies of ultrafine particle exposure in susceptible human subjects</i>	13
Research Core 4: <i>Animal models: Dosimetry, and pulmonary and cardiovascular events</i>	20
Research Core 5: <i>Ultrafine particle cell interactions: Molecular mechanisms leading to altered gene expression</i>	31
Pilot Project: <i>Development of an Electrodynamic Quadrupole Aerosol Concentrator</i>	35
Pilot Project: <i>Kinetics of clearance and relocation of insoluble ultrafine iridium particles from the rat lung epithelium to extrapulmonary organs and tissues</i>	37
Visiting Scientist Project: <i>Ultrafine oil aerosol generation for inhalation studies</i>	38

INTRODUCTION

The overall hypothesis of the Rochester PM Center is that ultrafine particles (UFP) occurring in the urban environment cause adverse cardiopulmonary health effects noted in the epidemiological studies independent of other particulate or gaseous co-pollutants. These studies have shown increased morbidity and mortality during episodes of increased ambient particle concentrations. The attached diagram shows the relationship between the information flow in our Center, including the 5 highly integrated research



cores, and the hypotheses under evaluation. The research is coordinated, *e.g.*, the field, clinical and animal studies measure the same cardiovascular and pulmonary endpoints. Results from one core are used to assist the design of studies from other cores.

To effectively test the UFP hypothesis more data are needed regarding particle size distribution, UP concentration, chemical composition of these size fractions, and relationship to source emissions. Particle characterization in Core 1 has expanded our understanding of these questions and provides instrumentation to carry out these analyses with high precision. The untimely death of the Core Director, Dr. Glen Cass, has resulted in our transferring responsibilities to his former associate, Dr. Ann Dillner, who recently accepted a faculty position at the Univ. of Arizona in Tempe.

The field studies in Epidemiology, Core 2 directly address the association between ambient particle exposure and biomarkers of inflammation and cardiac function in subpopulation suffering from chronic disease. As with the studies in Cores 3 and 4, this project tests the hypothesis that changes in ambient particles, particularly the UP phase is associated with changes in parameters of inflammation and systemic disease. Extending these studies to the cellular and molecular mechanism of injury is the goal of Cores 3, 4 and 5. Each examines an aspect of the hypothesis that particle induced oxidant stress leads to local and systemic inflammatory changes.

Core 3 addresses the hypothesis that epithelial activation leads to endothelial responses that results in activation of blood mononuclear cells in controlled clinical exposures. Core 4 addresses similar questions in animal studies, but more directly tests the hypothesis that age, chronic disease and oxidant co-pollutants are the key factors in determining susceptibility of populations. These studies provide a framework for Core 5 which extends the animal and controlled clinical exposures to the cellular and molecular level by examining the hypothesis that UP leads to activation of oxidant responsive transcription factors in specific pulmonary and extrapulmonary cell populations and that the degree of these activation processes are different between the young and aged organism.

RESEARCH CORE 1: Characterization of the Chemical Composition of Atmospheric Ultrafine Particles

Co-Investigators: the late Glen Cass, School of Earth and Atmospheric Sciences, Georgia Institute of Technology; Ann Dillner, Civil and Environmental Engineering Department, Arizona State University; Kimberly Prather, Department of Chemistry, University of California, San Diego

Summary of Progress to Date

The Cass/Dillner group has collected ultrafine particle samples in field experiments in a south central U.S. city (Houston, TX) and in a west coast city (Riverside, CA) and automated equipment that measures ultrafine aerosol size distributions. Professor Cass participated in The Royal Society's Discussion Meeting entitled "Ultrafine Particles in the Atmosphere" on March 15-16, 2000, which resulted in the published journal article listed below. The Prather group is in the final stages of developing an aerosol time of flight mass spectrometry instrument to measure the chemical composition of single atmospheric particles smaller than 100 nm in particle diameter.

During August and September, 2000, seven sets of 24-hour fine and ultrafine ambient samples were collected using cascade impactors at two sites in Houston, TX. The ultrafine concentration at the two sites ranged from 0.09 – 0.57 $\mu\text{g m}^{-3}$. The average composition of the ultrafine aerosol did not significantly differ between the two sites and, as shown in Figure 1a, consisted of 40% organic compounds, 17% sulfate, 5% metal oxides and trace amounts of elemental carbon, nitrate, ammonium, sodium and chloride. The dominant transition metal ions, obtained by ICP-MS analysis, were nickel, iron and vanadium with average concentrations less than 2 ng m^{-3} .

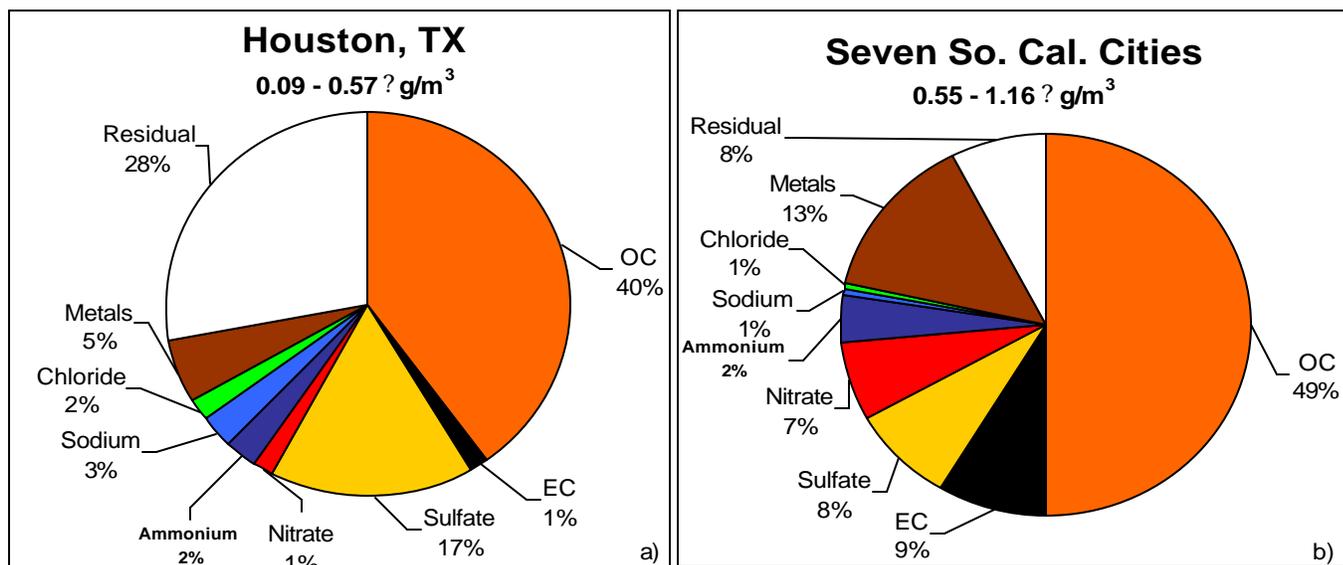


Figure 1. Speciation of ultrafine aerosol in a) Houston and b) Seven Southern California Cities.

Analyses of data obtained at seven sites in Southern California show average ultrafine particle concentrations ranged from 0.55 – 1.16 $\mu\text{g m}^{-3}$ and consisted of 50% organic compounds, 14% metal oxides, 8.7% elemental carbon, 8.2% sulfate, 6.8% nitrate and traces of ammonium,

sodium and chloride (Figure 1b). The Houston ultrafine concentrations based on mass metrics were roughly half of those obtained in the LA studies. Additionally, the ultrafine aerosol in Houston contained smaller percentages of organic compounds and metal oxides than LA but a larger percentage of sulfate. These results should assist researchers of the controlled clinical, animal and *in vitro* studies of the PM Center (Cores 3, 4, and 5) in the design of realistic exposure studies involving ultrafine particles.

During June and July, 2001, ultrafine and fine aerosol mass samples and ultrafine and fine number size distributions were collected over five consecutive 24-hour periods in Riverside, CA. Ultrafine aerosol mass was collected by a nano-MOI (micro-orifice impactor) that allows for size segregation of the aerosol into four bins below 0.1 μm (0.01 – 0.018 μm , 0.018 – 0.032 μm , 0.032 – 0.056 μm , 0.056 – 0.1 μm). Analysis of impactor substrates for both the ultrafine and the fine (0.1 – 1.8 μm) aerosol is underway to determine the chemical composition of the aerosol. A nano-differential mobility analyzer (nano-DMA) and an ultrafine condensation particle counter (UCPC) as well as a standard DMA and CPC were automated and used to obtain particle concentration as a function of size for particle diameters between 0.003 and \sim .600 μm during the Riverside, CA study.

An ultrafine particle aerosol time of flight mass spectrometry instrument has been constructed. The aerodynamic lens system, which allows transmission of ultrafine particles into the instrument, has been successfully designed and installed. An effective method for detecting ultrafine particles in the systems is being developed. Further studies will be undertaken to assess the chemical characterization capabilities of the instrument.

Meanwhile, ambient UFP measurements have also begun in Rochester and in Erfurt (average conc. 12500 [cm^{-3}]). In Erfurt, the elemental composition has been measured, measurement or organic composition has been started and a device similar to particle aerosol time of flight mass spectrometer is under development at the GSF.

Future Plans :

In spring 2002, aerosol characterization experiments will be undertaken in Rochester, NY, using two nano-MOUDIs and ultrafine size distribution instrumentation along side the ultrafine single particle aerosol time of flight mass spectrometry instrument. Comparison of bulk and single particle chemical composition data for this eastern U.S. city will further enhance our understanding of the ambient ultrafine particles. Additional studies will be undertaken to characterize the ultrafine aerosol concentrator utilized in the animal studies performed by Rochester PM Center researchers. This characterization will provide a better understanding of the size and chemical composition of the ultrafine particles used in the exposure studies. Another planned study will be to characterize ambient ultrafine aerosol at a traffic-dominated site in LA.

Publications :

Cass, G. R., Hughes, L. S., Bhave, P., Kleeman, M. J., Allen, J. O., and Salmon, L. G. (2000) The Chemical Composition of Atmospheric Ultrafine Particles. The Philosophical Transactions of the Royal Society (Series A), 358, 2567-2580.

RESEARCH CORE 2: Inflammatory responses and cardiovascular risk factors in elderly subjects with angina pectoris or COPD in association with fine and ultrafine particles

Principal Investigators: H.-Erich Wichmann and Annette Peters

Co-Investigators: Angela Ibald-Mulli, Wolfgang Kreyling and Joachim Heyder in collaboration with Wolfgang Koenig, University of Ulm, Germany

Background:

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 the autonomic control.

In order to identify the mechanisms that lead from the deposition of particles in the lung to cardiovascular disease exacerbation, it is crucial to also show that the proposed mechanism is at play in diseased patients and not only a physiological response in healthy individuals. So far, all published evidence on effects of particulate air pollution on the autonomic nervous system function is based on panels of healthy elderly subjects (Pope et al. 1999a, Pope et al. 1999b, Liao et al. 1999, Gold et al. 2000, Creason et al. 2001), population based samples (Peters et al. 1999) or occupational cohorts (Magari et al. 2001). A European multi-center study on patients with CAD, the ULTRA study, did not observe an association between ambient air pollution and autonomic nervous system function (Timonen et al. 2001, Ibald-Mulli et al. 2001b). Nevertheless, particulate air pollution was associated with ST-segment depression as a sign for ischemia (Pekkanen et al. 2001). It might therefore be that ischemic events rather than altered autonomic control might be the main mechanism in patients with CAD.

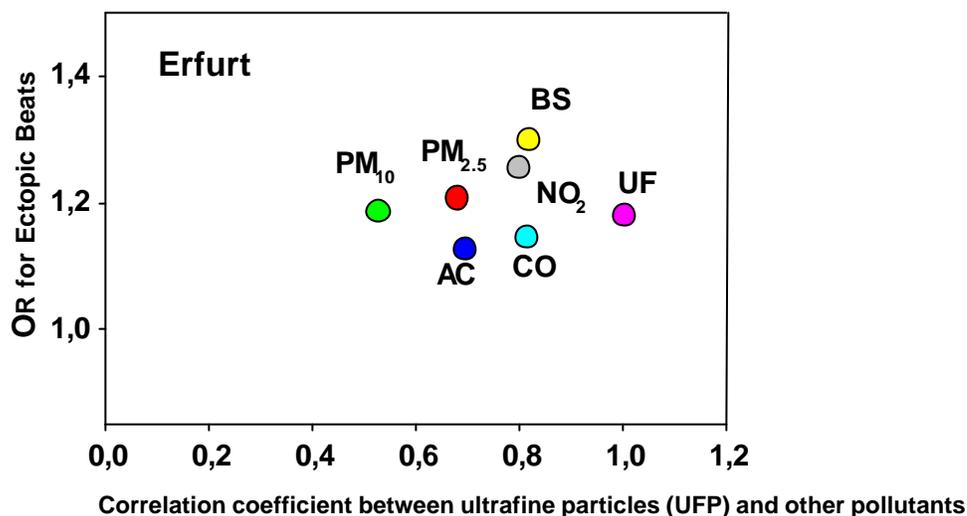


Figure 1: Effects of ventricular ectopic beats and the correlation between accumulation mode particles and other traffic related pollutants lagged one day (ULTRA Study, Erfurt data only, winter 98/99).

In Erfurt, results from the ULTRA study have also shown an increase in ventricular ectopic beats, a marker for arrhythmia, in association with all measures of particulate air pollution in

during the winter 98/99 (figure 1). As can be seen in figure 1 the correlations between the pollutants is a crucial factor which will determine the ability to distinguish between the effects of different particle fractions. The effect estimates ranged between odds ratios of 1.30 and 1.13 per interquartile range and were statistically significant for all particles. The correlation structure between the pollutants indicated that maybe traffic related particulate air pollution as indicated by ultrafine particles, PM_{2.5} and black smoke (BS) measured as reflectance of PM_{2.5} were responsible for the observed associations.

Recent work on the role of particles as a trigger of myocardial infarctions (Peters et al. 2001b) support the effects seen in the ULTRA study. In addition, interim analyses of an ongoing case-crossover study on myocardial infarctions suggested that in addition to fine particles also ultrafine particles might be able to induce myocardial infarctions. Therefore, the first set of analyses will address the following questions:

- (1) Do fine and ultrafine particles not affect blood pressure, heart rate and heart rate variability as seen in the ULTRA study?
- (2) Are signs of QTc reduction observed as in the human exposure studies of the Rochester Particle Center?
- (3) Are ventricular ectopic beats and arrhythmia more prevalent on days with high concentrations of fine and ultrafine particles as in the ULTRA study?
- (4) Are signs of ischemia present in 24-EKG readings during exercise periods in association with fine and ultrafine particles ?

So far, evidence on an association between ambient concentrations of particulate matter and markers of blood coagulability were only obtained in cross-sectional settings (Peters *et al.* 1997, Pekkanen *et al.* 2000, Schwartz 2001, Peters *et al.* 2001a). Only one study assessed this association in healthy elderly subjects and did not observe an effect (Seaton *et al.* 2000). Therefore, the second set of analyses will investigate systemic effects of fine and ultrafine particles in subjects with pre-existing disease based on changes in blood parameters of inflammation and coagulability. The following questions will be addressed:

- (5) Do white blood cell counts increase in association with fine and ultrafine particles reflecting the recruitment of leukocytes to the lung?
- (6) Do red blood cells decrease based on the process of sequestration?
- (7) Do acute phase reactants increase in association with fine and ultrafine particles?
- (8) Will plasma viscosity increase in association with fine and ultrafine particles?
- (9) Do clotting factors also increase in association with fine and ultrafine particles?

The first time-series study on mortality considering ultrafine particles in addition to particles showed relative risk estimates of comparable sizes in association with cumulated particle concentrations over the last four days (Wichmann *et al.* 2000). In addition, the data suggests that respiratory mortality increased immediately after elevated concentrations of particles while cardiovascular disease mortality showed a delay up to four days. Therefore, we will investigate the lag-structure carefully in all analyses.

Summary of Progress to Date:

Particles were characterised by an aerosol spectrometer (MAS, combining DMA/CPC and LAS-X) covering the size range from 10 nm to 2.5 μm . The size distribution of the ambient aerosol has changed in Erfurt substantially during the nineties. Figure 2 shows the changes in the average particle number distribution between winter 1991/92 and the winter 2000/01. While accumulation mode particle number concentrations with diameters between 100 and 1000 nm decreased during the nineties, increased the number concentrations of the ultrafine particles increased especially in the fraction between 10 and 30nm. This is taken as an indication that the potential health problems related to ultrafine particles might be increasing while the ambient concentrations of the particle mass is decreasing (Wichmann and Peters, 2000). These results were obtained in collaboration with the newly formed research program on aerosol science within the GSF which is headed by Wolfgang Kreyling, GSF-Institute of Inhalation Biology. Also in Erfurt, the elemental composition of ultrafine and fine particles in the 5 size fractions in the range 0.05 – 1.62 μm has been measured by PIXE (Proton-Induced X-ray Emission) and analysed by principal component (PC) analysis (Wichmann et al., 2002, Stoelzel et al. 2001 abstract). For UFP, 4 PCs explained 57% of the variance: traffic exhaust (Pb, Zn, K), resuspended crustal material (Al, Si, Ti), coal combustion (S) and oil combustion (V, Ni). PIXE measurements and PC analyses are continuing for the winters 2000/01 and 2001/02.

The field study with the CAD patients was conducted during the winter 2000/01. Concentrations during the field phase are given in table 1. Unfortunately, no aerosol spectrometer data are available between January 20th and February 13th due to a technical defect of the measuring device. The ultrafine particle number concentrations are dominated by the very fine particles with diameters between 10 and 30 nm. The correlation coefficient between UFP (= $\text{NC}_{0.01-0.1}$) and $\text{NC}_{0.01-0.03}$ was 0.96. Ultrafine particles are correlated with accumulation mode particles ($\text{NC}_{0.1-1.0}$) with a correlation coefficient of 0.67. However, the two different fractions of the ultrafine particles do show different correlation coefficients with the accumulation mode particles (0.90 for $\text{NC}_{0.03-0.1}$ and 0.47 with $\text{NC}_{0.01-0.03}$). The variation in exposure to particle number concentrations seems to be sufficient for epidemiological data analyses.

Table 1: Distribution of the measured 24 hour average particle number concentrations and $\text{PM}_{2.5}$ during winter 2000/2001 when the CAD panel study was conducted. The indices give the size range of the particles counted in μm .

	N	%	Mean	Median	95%	Max
$\text{NC}_{0.01-0.1} [\text{cm}^{-3}]^{1)}$	163	83.2	12540	10940	25190	34290
$\text{NC}_{0.01-0.03} [\text{cm}^{-3}]^{1)}$	163	83.2	8740	8240	17320	22530
$\text{NC}_{0.03-0.1} [\text{cm}^{-3}]^{1)}$	163	83.2	3800	2810	9290	12320
$\text{NC}_{0.1-1.0} [\text{cm}^{-3}]^{1)}$	163	83.2	1570	1220	3920	4910
Total number concentrations $[\text{cm}^{-3}]^{2)}$	196	100	20290	19310	36800	47000
$\text{PM}_{2.5} [\mu\text{g}/\text{m}^3]^{3)}$	189	96.5	15.2	11.3	38.0	66.3

¹⁾ MAS data, UFP = $\text{NC}_{0.01-0.1} [\text{cm}^{-3}]$

²⁾ CPC data

³⁾ Harvard impactor data

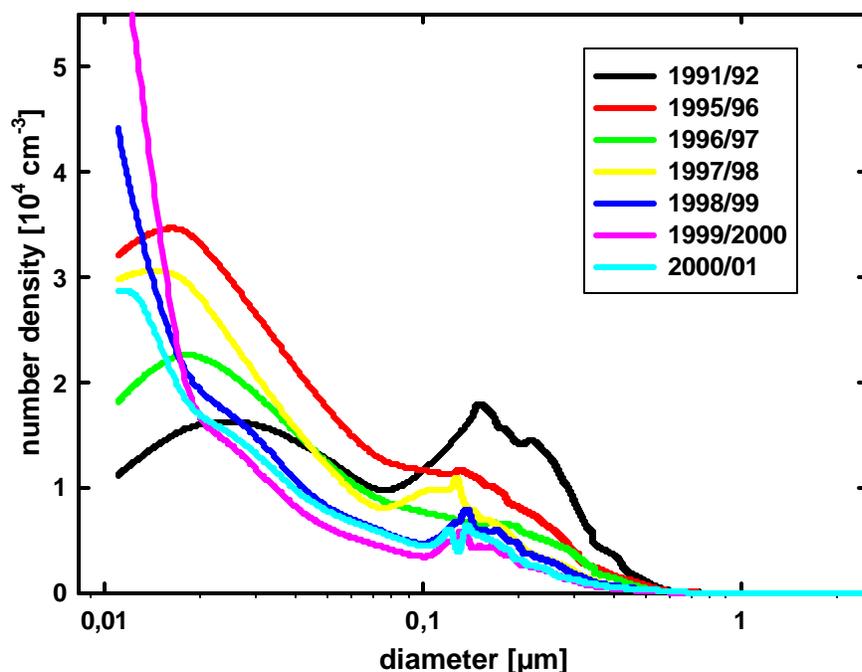


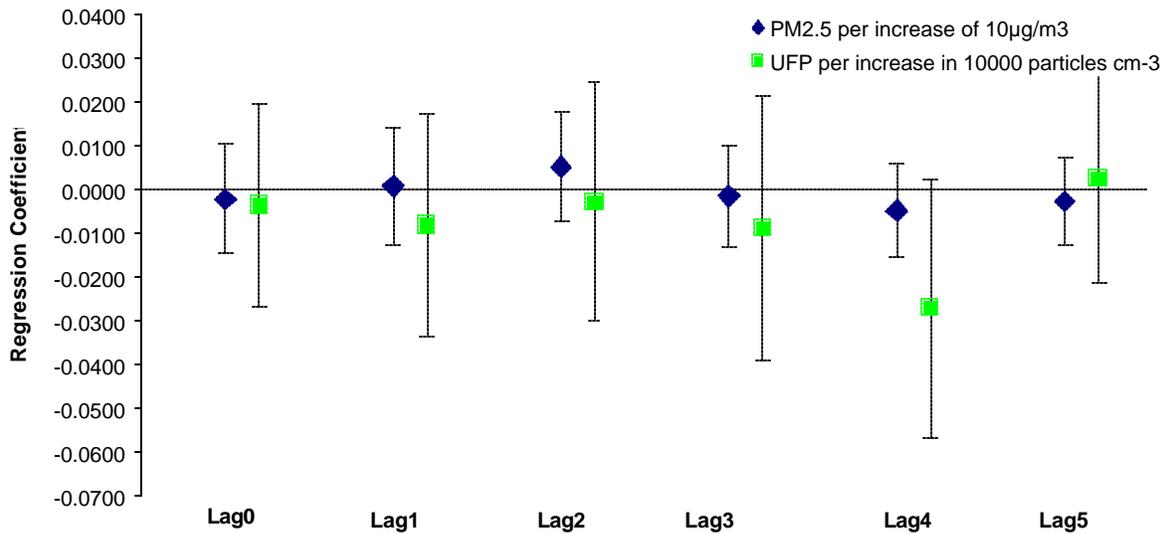
Figure 2: Size distribution of particle number concentrations in Erfurt during winter months including the Rochester Particle Center study period.

The field phase on 58 CAD patients aged between 51 and 76 years started in October 2000 and was completed in May 2001. The study protocol and outcome measures were designed to be as similar as possible to that of the clinical studies (Core 3). It comprised a maximum of 12 bimonthly clinical examinations with an interview, resting ECG, blood pressure measurement, urine sample and blood sample. Further, 6 monthly 24-hour holter recordings and daily blood pressure measurements for a period of 1 month were taken. Throughout the whole study period subjects were recording symptoms and medication use in a diary. 97% of all scheduled clinic visits were realized; a total of 683 clinical examinations, 279 24-hour holter recordings and 659 blood samples were completed. All questionnaire based data was entered and has been checked for plausibility.

Preliminary analyses of the blood cell counts and 24 hour averages of particle mass and number counts were conducted. Results indicate a decrease in red blood cells in association with fine particle mass (PM_{2.5}) and ultrafine particle counts (figure 3). Similar effects of particulate matter on red blood cell counts have been observed by Seaton and colleagues (Seaton *et al.* 2000). All estimates are given for 10 $\mu\text{g}/\text{m}^3$ PM_{2.5} or 10,000 ultrafine particles per cubic centimetre of ambient air. The decrease in red blood cells were strongest for the ultrafine particles at lag 4. The hematocrit showed results consistent with those observed for the red blood cells. However, the results were not statistically significant. Total white blood cell counts also decreased in association with ambient particle concentrations. For ultrafine particles a significant decrease is observed for lag 1 and lag 4 and fine particle mass shows a significant effect for lag 4 and 5. The lag structure observed in these analyses are partly consistent with those found by Wichmann and colleagues analysing the impact of ambient particle number concentrations on mortality (Wichmann *et al.* 2000). Cardiovascular disease mortality showed the strongest evidence of an association with ultrafine particles with a lag of 4 days (RR: 1.051 (95% CI:

0.990 to 1.115) while respiratory disease mortality showed also an increase in association with ultrafine particles. However, the relative risk was strongest for lag 1 (RR: 1.155 (95% CI: 1.055 to 1.264)). Platelet counts also decreased in association with ambient particle concentrations. The strongest association was observed with the pollutant concentrations delayed by two days. For an increase of 10,000 particles a reduction of $4.6 \text{ platelets} \cdot 10^9/l$ was observed (95% CI: -1.33 to -7.87). The consistency and plausibility of these effects need to be further evaluated. In particular it will be assessed whether the results are in accordance with findings from the analysis of more specific biomarkers such as clotting factors or soluble ICAM-1.

Red blood cells



White blood cells

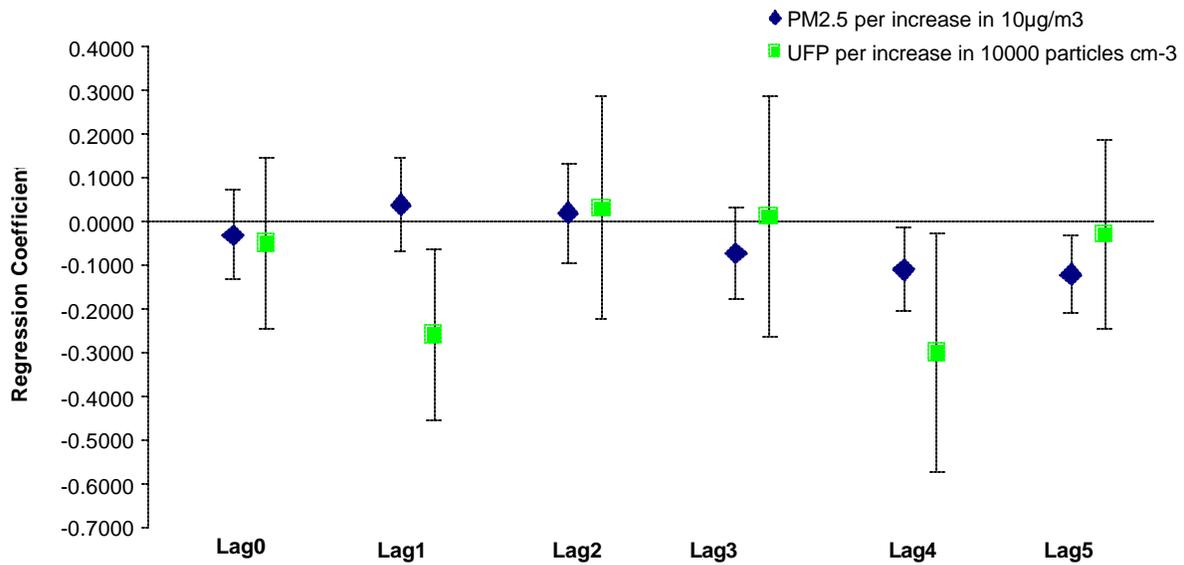


Figure 3: Red blood cells and white blood cells in association with 24 hour average concentrations of PM 2.5 and Ultrafine particles (UFP) of the CAD panel during the winter 2000/01 in Erfurt (Rochester Particle Center).

Analyses of the blood parameters by the vascular core are still ongoing because shipment of the blood samples from Germany was postponed after the September 11th terrorist attack. The potential role of ECG parameters in air pollution epidemiology has been established (Zareba et al. 2001, Zareba, Couderc, and Nomura, 2001) and based on these concepts ECGs obtained in Erfurt during the first field phase are currently analysed by the cardiac core. The same blood parameter and EKG parameter will be determined by the same core facilities for the epidemiological studies and the human exposure studies. Therefore, the results will be directly comparable to those of the human exposure studies.

Following the angina pectoris panel the pilot study for the COPD panel took place in June 2001 in order to test the protocol for the main COPD panel study in the winter 2001/2002. For the pilot study 15 non-smoking males, aged between 50 and 80 years with physician diagnosed COPD were recruited from local practitioners. The main purpose of the pilot study was to test acceptance, feasibility and significance of additional measurements to assess the responses of the lung to ambient particles. The final protocol includes impulse oscillometry, body plethysmography, and spirometry. Additional components such as a 6-minute-submaximal exercise on a bicycle ergometer and oxygen saturation measurements were added to the clinical protocol. The main panel study started with a total of 38 COPD patients in October 2001 and will be completed in April 2002. Local expertise from the GSF-Aerosol Research Program was provided to implement the additional measurements for the COPD panel.

Future Plans:

The COPD field study will be completed within the next months. The statistical analyses of the CAD and COPD panel will be done based on the concepts outlined in the background section. This is a major task given the large database. Effort will be spent on the relationship between blood biomarkers and EKG parameters, as these relations have not been assessed well in the current medical literature. However, this knowledge will be crucial in order to understand the results with respect to air pollution. The CAD and the COPD panel will first be summarized in terms of internal consistency and plausibility and then compared between each other. Comparable or additional information obtained by the human studies of the Rochester Particle Center and epidemiological as well as human studies by the other centers will be used to substantiate the results observed.

Toxicological experiments using particles collected on filters from Erfurt during the winters of 2000/01 and 2001/02 are planned by Core 5.

Additional blood analyses applying recently developed techniques in proteomics will be conducted through the GSF-EPA collaboration with Robert Devlin and Lucas Neas at NHEERL.

Publications of Core Investigators:

Heinrich J, Hölscher B, Wichmann HE (2000) Decline of ambient air pollution and respiratory symptoms in children. *Am. J. Resp. Crit. Care Med.* 161: 1930-1936

- Ibald-Mulli A, Stieber J, Wichmann HE, Koenig W, Peters A. (2001) Effects of air pollution on blood pressure: A population based approach. *Am J Public Health* 91:571-577.
- Kotesovec F, Skorkovsky J, Brynda J, Peters A, Heinrich J (2000) Daily mortality and air pollution in northern Bohemia: different effects for men and women. *Cent Eur J Public Health* 8: 120-127
- Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh JA, Allen G, Monahan K, Dockery DW (2000) Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11: 11-17
- Peters A, Perz S, Döring A, Stieber J, Koenig W, Wichmann HE (2000) Activation of the autonomic nervous system and blood coagulation in association with an air pollution episode. *Inhal Toxicol* 12: 51-61
- Peters A, Skorkovsky J, Kotesovec F, Brynda J, Spix C, Wichmann HE, Heinrich J (2000) Associations between mortality and air pollution in Central Europe. *Environ Health Perspect* 108: 283-287
- Peters A, Fröhlich M, Döring A, Immervoll T, Wichmann HE, Hutchinson WL, Pepys MB, Koenig W. (2001) Particulate air pollution is associated with an acute phase response in men. *Eur Heart J* 22:1198-1204.
- Pitz M, Kreyling WG, Hölscher B, Cyrus J, Wichmann HE, Heinrich J (2001) Change of the ambient particle size distribution in East Germany between 1993 and 1999. *Atmospheric Environment* 35:4357-4366.
- Ruuskanen J, Tuch T, ten Brink H, Peters A, Khlystov A, Mirme A, Kos GPA, Brunekreef B, Wichmann HE, Buzorius G, Vallius M, Kreyling WG, Pekkanen J. (2000) Concentration of ultrafine, fine and PM_{2.5} particles in three European cities. *Atmospheric Environment* 35: 3729-3738
- Stoelzel M, Cyrus J, Wichmann HE (2001) Source apportionment of fine and ultrafine particles in Erfurt, Germany. ISEE 2001, Book of Abstracts A47
- Tuch T, Mirme A, Tamm E, Heinrich J, Heyder J, Brand P, Roth C, Wichmann HE, Pekkanen J, Kreyling WG (2000) Comparison of two particle-size spectrometers for ambient aerosol measurements. *Atmos. Environ.* 34: 139-149
- Wichmann HE, Spix C, Tuch T, Woelke G, Peters A, Heinrich J, Kreyling WG, Heyder J (2000) Daily mortality and fine and ultrafine particles in Erfurt, Germany. Part I: Role of particle number and particle mass. *Health Effects Institute Research Report* 98.
- Wichmann HE, Peters A (2000) Epidemiological evidence on the effects of ultrafine particle exposure. *Phil. T. Roy. Soc. A* 358: 2751-2769
- Wichmann HE, Cyrus J, Stölzel M, Spix C, Wittmaack K, Tuch T, Peters A, Wölke G, Menzel N, Hietel B, Schulz F, Heinrich J, Kreyling W, Heyder J (2002, in press) Sources and elemental composition of ambient particles in Erfurt, Germany. Ecomed Verlag Landsberg

Research Core #3: Clinical Studies of Ultrafine Particle Exposure in Susceptible Human Subjects

Principal Investigator: Mark W. Frampton

Co-Investigators: Mark J. Utell (co- P.I.), William Beckett, Günter Oberdörster, Paul Morrow, Wojciech Zareba, Christopher Cox

Background

These studies utilize controlled human exposures to examine, in healthy and potentially susceptible subjects, the deposition and fate of inhaled ultrafine carbon particles (UFP), and the role of UFP in inducing health effects.

Progress Report to Date:

Development of an UFP Exposure Facility. We have developed a facility for experimental exposure of humans to ultrafine particles, which permits the quantitative determination of the exposure levels, respiratory intakes, and depositions of the aerosol. Because our initial exposure mass concentrations were within the range of PM measurements outdoors, it was important to know numbers and mass concentrations of particles within the Clinical Research Center and the Environmental Chamber where the facility is located, as well as in the intake air for the exposure facility.

We measured particle number, UFP size distribution, and total suspended particulate mass in three locations: within the General Clinical Research Center, within our environmental exposure chamber, in which the intake air is purified, and from outdoor air above a construction site outside the hospital. Mean \pm SD particle numbers were $3.63 \pm 1.15 \times 10^3$ particle/cm³ in the GCRC, with a peak number of 2.78×10^4 particle/cm³ in the morning when activity on the nursing floor was most intense. Outdoor fine particle numbers reached peaks of nearly 2×10^6 particles/cm³. These were higher than in our partner location in Erfurt, where health effects of UFP have been observed, see Core 2. Both indoors and outdoors, peaks in particle number occurred without corresponding peaks in mass, indicating predominance of UFP, which contribute little to mass. These were the first published measurements of particle number within an acute care hospital (Riesefeld et al., 2000), and indicated that monitoring of particle mass does not provide an accurate estimate of exposure to ultrafine particles indoors.

Our objectives in designing an exposure system for clinical studies of UFP were as

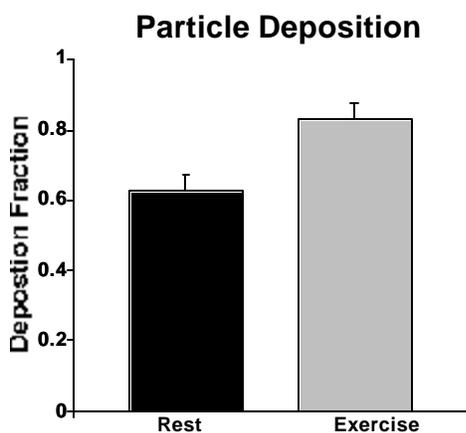


Figure 1

follows: 1) Allow short-term controlled exposures to particles less than 100 nm; 2) measure respiratory tract deposition of UFP both at rest and with exercise; 3) monitor changes in respiratory pattern and minute ventilation during exposure. A mouthpiece system best met these needs. Although whole chamber or face-mask exposures would better simulate natural oral-nasal breathing, quantitative deposition measurements are more difficult with these exposure modes. Characteristics of the exposure system have been described (Chalupa et al., 2002b).

Respiratory Deposition of UFP. Modeling predictions (International Commission on Radiological

Protection (ICRP)) indicate that particles in the ultrafine size range, especially those less than 50 nm geometric diameter, have a predicted high deposition efficiency. Thus, for a given exposure level, a greater percentage of UFP are retained in the lung than for larger (accumulation mode) particles. Our preliminary findings (Frampton et al., 2000a) confirm predictions for healthy subjects, and show that UFP deposition increases further with exercise. In 12 healthy subjects (6 female) exposed at rest to $10 \mu\text{g}/\text{m}^3$ UFP for 2 hours, the overall number deposition fraction exceeded 0.6, and did not differ by gender. Deposition decreased with increasing particle size within the particle size distribution, findings which conform reasonably with ICRP predictions. In a subsequent study of healthy subjects exposed to 10 and $25 \mu\text{g}/\text{m}^3$ UFP, deposition increased further with exercise (Figure 1). Thus, not only does exercise increase particle dose because of increased particle intake, but the fraction of inhaled particles that deposits is increased as well. Our preliminary results of a study of subjects with mild asthma exposed in a similar fashion indicates that even mild obstructive lung disease is associated with further enhanced UFP deposition, both at rest and with exercise. Remarkably, the number deposition fraction in asthmatics at rest was 0.77 ± 0.05 , increasing during exercise to 0.86 ± 0.04 (Chalupa et al., 2002a).

Effects on Vascular and Leukocyte Activation. For our initial studies, exposures were conducted at rest with a relatively low concentration of carbonaceous UFP ($\sim 10 \mu\text{g}/\text{m}^3$, $\sim 2 \times 10^6$ particles/ cm^3 , count median diameter 26.4 nm, GSD 2.3). Twelve healthy non-smoking subjects (6 female) inhaled either filtered air or UFP by mouthpiece for 2 hours at rest, with a 10 min break after the first hour. Exposures were double blinded, randomized, and separated by at least 2 weeks. The total respiratory tract deposition fraction (DF) was determined for 6 different particle size fractions after correction for system losses, and the overall DF was calculated for both number and mass for each subject. Respiratory symptoms, spirometry, blood pressure, pulse-oximetry, and exhaled NO were assessed before and at intervals after the exposure. Sputum induction was performed 18 hours after exposure. Continuous 24-hour, 12-lead Holter monitoring was performed on the day of the exposure and analyzed for changes in heart rate variability and repolarization phenomena.

The results of this initial exposure study have been presented in abstract form (Boscia et al., 2000; Frampton et al., 2000a; Frampton et al., 2000b). Analysis of variance indicated no significant differences in respiratory symptoms, blood pressure, pulse-oximetry, spirometry, exhaled NO (using methods developed in our laboratory (Geigel et al., 1999; Pietropaoli et al., 1999; Perillo et al., 2001)), blood markers of coagulation and endothelial activation, leukocyte activation, or sputum cell differential counts. Overall, we concluded that exposure to $10 \mu\text{g}/\text{m}^3$ carbonaceous UFP at rest did not cause significant respiratory or cardiac effects in healthy nonsmokers.

Having established the feasibility and safety of the exposure system, the next study incorporated intermittent exercise and a dose-response evaluation. 12 subjects (6 female) were exposed to air, $10 \mu\text{g}/\text{m}^3$ and $25 \mu\text{g}/\text{m}^3$ UFP (Frampton et al., 2001). During

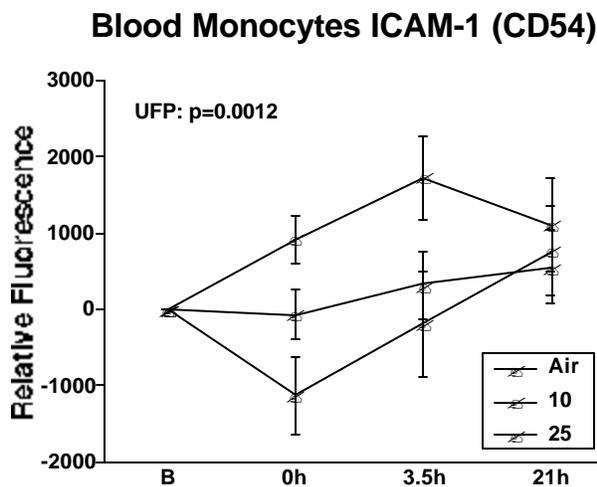


Figure 2

exposure, subjects alternated rest with 15 minutes of moderate exercise (minute ventilation ~25 L/min/m² BSA). Each exposure was randomized, double-blinded, and separated by at least two weeks. The randomization scheme was balanced, with equal numbers of males and females assigned to each order sequence. As in the previous study, data were analyzed by ANOVA, including a term for gender.

As in the resting study, there were no symptoms, changes in lung function, or evidence for airway inflammation (induced sputum or exhaled NO) associated with the exposures. Interestingly, pulse oximetry showed a small but statistically significant decrease in oxygen saturation in females after exposure to 25 µg/m³ UFP (p=0.02). Studies of blood leukocytes

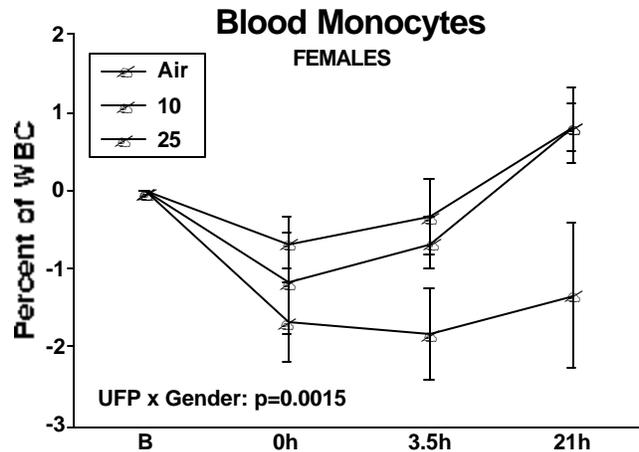


Figure 3

showed early reduction in blood monocyte expression of intercellular adhesion molecule-1 (ICAM-1, CD54) in both males and females (Figure 2), and later reductions in the percentage of blood monocytes in females (Figure 3) that was greatest 21 hours after exposure. There was no significant change in the total white count. There was increased lymphocyte expression of CD25 (an epitope of the IL-2 receptor, a marker of activation), again only in females (p=0.0024).

Taken together, these findings are most consistent with particle effects on vascular endothelium, leading to subtle changes in pulmonary capillary perfusion, sequestration of monocytes that are expressing higher levels of ICAM-1 (leaving the low-expressing cells in the circulation), and shifts in circulating lymphocyte populations. The increased lymphocyte CD25 expression may represent mobilization of activated cells to the blood, or alternatively, sequestration of less activated cells in tissues.

We conclude that exercise increases the already high respiratory deposition of UFP, and our data suggest that exposure to 10-25 µg/m³ UFP is associated with effects on circulating leukocytes. We have now initiated studies of UFP exposure in subjects with asthma. Because of the possibility of “carry-over” effects suggested by the analysis of variance, and by the effects seen in Erfurt (Core 2), where a decrease of leukocytes was observed after 1 and 4 days, we have altered the protocol to detect delayed effects. The interval between exposures was increased to 3 weeks, and, subjects now return 48 hours after exposure additional studies, including cardiac monitoring, to determine if there are delayed effects. Future studies are planned in elderly healthy subjects, and in subjects with COPD and coronary artery disease.

Cardiovascular Effects of UFP

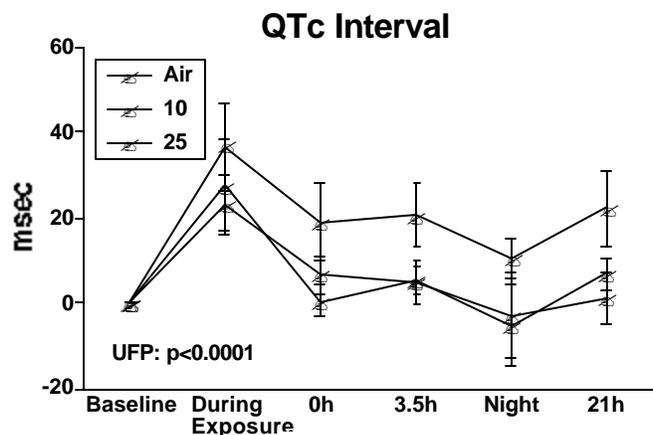


Figure 4

Exposure. The focus in these studies is to examine effects of inhalation of UFP on HRV and repolarization. In the studies described above, subjects wore a continuous 12-lead ECG monitor, which was started prior to exposure and ended the following morning.

Analyses of cardiac monitoring included a detailed analysis of HRV and repolarization intervals before exposure, during one of the exposure exercise periods, at time points after exposure, and during sleep. The resting exposures revealed no evidence for significant cardiac effects (Boscia et al., 2000; Frampton et al., 2000b; Frampton, 2001). In the study with intermittent exercise, frequency-domain HRV analysis indicated that the response of the parasympathetic nervous system (measured by normalized units of high-frequency [HF] components) was blunted during recovery from exercise immediately after exposure to UFP in comparison to pure air exposure. This diminished vagal response was not observed 3.5 hour later. Our preliminary finding suggesting an effect of UFP on parasympathetic modulation of the heart is in agreement with observation by Gold et al. (Circulation 101:1267, 2000), who found reduction in parasympathetic (vagal) tone in elderly subjects exposed to ambient pollution levels. In our analysis, none of the time-domain HRV parameters or low-frequency components showed significant changes induced by UFP.

The analysis of the corrected QT interval (QTc) and T wave amplitude, conducted in the same healthy subjects, also showed a blunted response after UFP exposure in comparison to pure air exposure. Figure 4 shows that the QTc shortened during exercise more substantially during UFP particle exposure than during pure air exposure and remained shortened for several hours after exposure. Simultaneously, T wave amplitude was higher after exercise with UFP than after exercise with air. Interestingly, we did not observe these repolarization changes in subjects undergoing exposures at rest.

The reduction in QTc duration with concomitant increase in T wave amplitude after UFP exposure represents the first evidence that repolarization is affected by inhalation of particles. Both lengthening and shortening of the QT interval have been implicated in increasing risk for cardiac arrhythmias, and these preliminary findings are of particular interest in light of the ectopic beats observed in the Erfurt field studies (Core 2).

UFP Effects in Asthma. In the past year we have extended our studies to subjects with asthma. People with underlying airway inflammation, as in asthma, may be more susceptible to the systemic as well as pulmonary effects of particle inhalation. We are currently studying subjects with mild asthma, exposed to $10 \mu\text{g}/\text{m}^3$ carbonaceous UFP for two hours with intermittent exercise. In order to understand the duration of effects, subjects are assessed immediately after exposure and at 3.5, 21, and 45 hours after exposure. Eight subjects out of a planned 16 have been completed. We are seeing a significant reduction in monocyte expression

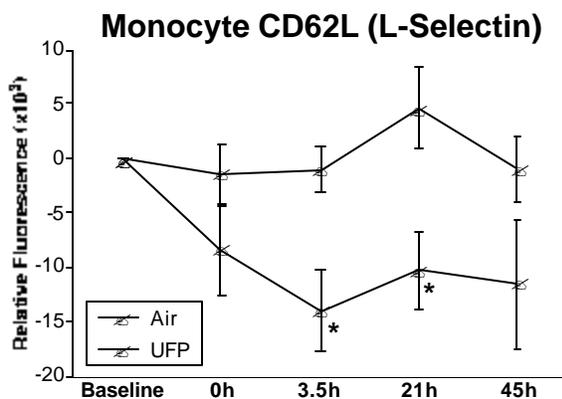


Figure 5

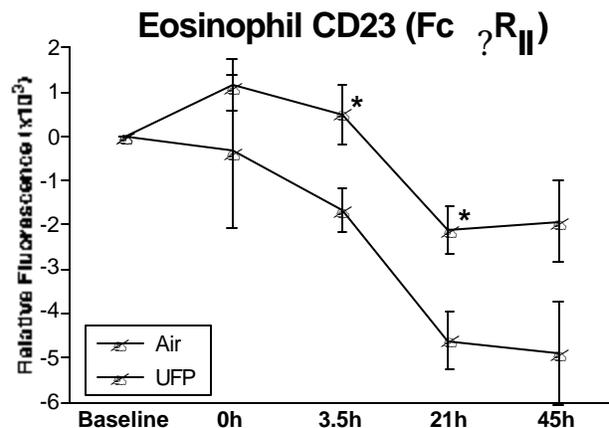


Figure 6

of L-selectin in association with UFP exposure (Figure 5). and in PMN expression of the low affinity Fc γ receptor. Because these asthmatic subjects had mild peripheral eosinophilia, we were able to separately analyze eosinophil expression of surface markers. The low affinity receptor for IgE increased on eosinophils relative to air exposure (Figure 6), suggesting that eosinophils may be differentially activated by UFP inhalation in asthmatic subjects. Finally, expression of CD40 ligand (CD154) increased on monocytes in association with UFP exposure (Daigle et al., 2002). These findings suggest a potential mechanism (enhanced eosinophilic inflammation) for the decreases in lung function observed in Erfurt in association with UFP (Peters et al. 1997).

The Leukocyte Oxidative Burst. Pollutant effects may be mediated in part by ROS generation by leukocytes, both in the lung and in the blood. We hypothesize that inhalation of UFP may prime circulating leukocytes, enhancing ROS production via the oxidative burst. In preparation for testing this hypothesis, we are currently developing methodology for assessing ROS generation by blood PMN.

Flow cytometric techniques have been developed for quantitation of the oxidative burst activity in leukocytes at the single cell level using 2',7'-dichlorofluorescein (DCFH). We have adapted this technique for measure of the oxidative burst in blood leukocytes. However, it became important for us to determine which specific oxidant species were being measured with this technique. We found that inhibition of NADPH oxidase activity using a calmodulin antagonist (W-13) decreased PMA-induced DCFH oxidation by 70%. In contrast, inhibition of NO synthase using N^G-monomethyl-L-arginine (NMMA) did not significantly reduce DCFH oxidation. Addition of superoxide dismutase had no effect, but catalase markedly suppressed DCFH oxidation. These data indicated that hydrogen peroxide, and not NO, is primarily responsible for PMA-induced oxidation of DCFH in human blood PMN (Azadniv et al., 2001a).

We developed a technique for measurement of DCFH oxidation in PMN in whole blood, in order to avoid priming or activation of PMN in the isolation process. We were surprised to

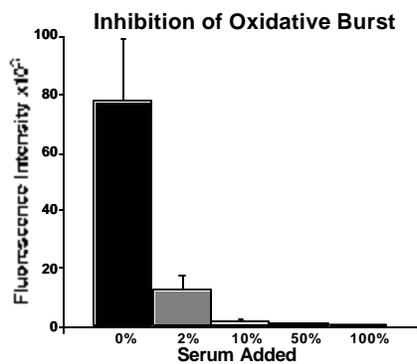


Figure 7

find that whole blood markedly inhibited the PMA- and FMLP-induced oxidative burst. As shown in Figure 14, human serum reproduced this effect, at concentrations as low as 2% (Azadniv et al., 2001b). This did not occur in a cell free oxidant generating system (xanthine + xanthine oxidase), indicating that serum was not quenching ROS directly. Inhibitory activity was confined to serum proteins with molecular weight greater than 10 kD. Serum albumin had a significant inhibitory effect but did not completely reproduce the serum effect. The importance of these observations is that serum proteins, and possibly other factors, regulate the oxidative burst in circulating leukocytes, keeping ROS production suppressed, effectively

protecting endothelium from oxidant injury. Inhaled particles may alter the dynamics of this inhibition, enhancing ROS production and endothelial injury by migrating leukocytes. Future studies will quantitate baseline and stimulated ROS production in PMN and monocytes isolated from buffy coat, and also examine suppression by autologous serum.

Future Plans

Our findings to date indicate that inhalation of low concentrations of UFP have subtle effects on circulating leukocytes, and on myocardial repolarization. Confirmation of these findings and investigation of concentration response relationships is needed, and was recommended by our Scientific Advisory Committee. Our future plans therefore include the following:

- ?? Complete the current study of subjects with asthma
- ?? Initiate a new study in healthy subjects, with the following:
 - ?? Increase the exposure concentration to 50 $\mu\text{g}/\text{m}^3$
 - ?? Monitor subjects for 48 hours after exposure
 - ?? Perform direct measurements of endothelial function (forearm ischemia-reperfusion test)
 - ?? Common endpoints with core II
- ?? Study susceptible subject groups, including patients with COPD
- ?? Extend studies using concentrated ambient UFP, for comparison with laboratory-generated carbonaceous UFP

Publications/Presentations of Core Investigators:

1. Azadniv, M., Torres, A., Boscia, J., Speers, D. M., Frasier, L. M., Utell, M. J., and Frampton, M. W. (2001a). Neutrophils in lung inflammation: which reactive oxygen species are being measured? *Inhalation Toxicol.* 13:485-495.
2. Azadniv, M., Torres, A., Frasier, L. M., Daigle, C., Speers, D. M., Utell, M. J., and Frampton, M. W. (2001b). Serum inhibition of the oxidative burst in human polymorphonuclear leukocytes. *Am J Respir Crit Care Med.* 163:A991 (Abstract).
3. Boscia, J. A., Chalupa, D., Utell, M. J., Zareba, W., Konecki, J. A., Morrow, P. E., Gibb, F. R., Oberdörster, G., Azadniv, M., Frasier, L. M., Speers, D. M., and Frampton, M. W. (2000). Airway and cardiovascular effects of inhaled ultrafine carbon particles in resting, healthy, nonsmoking adults. *Am J Respir Crit Care Med.* 161:A239 (Abstract).
4. Chalupa, D. C., Morrow, P. E., Oberdörster, G., Speers, D., Daigle, D., Utell, M. J., and Frampton, M. W. (2002a). Deposition of ultrafine carbon particles in subjects with asthma. *Am J Respir Crit Care Med* (Abstract, in press).
5. Chalupa, D. F., Gibb, F. R., Morrow, P. E., Oberdörster, G., Riesenfeld, E., Gelein, R., Utell, M. J., and Frampton, M. W. (2002b). A facility for controlled human exposures to ultrafine particles (in press).
6. Daigle, C. C., Speers, D. M., Chalupa, D., Stewart, J. C., Frasier, L. M., Azadniv, M., Phipps, R. P., Utell, M. J., and Frampton, M. W. (2002). Ultrafine particle exposure alters expression of CD40 ligand (CD154) in healthy subjects and subjects with asthma. *Am J Respir Crit Care Med* (Abstract, in press).
7. Frampton, M. W. (2001). Systemic and cardiovascular effects of airway injury and

inflammation: ultrafine particle exposure in humans. *Environ Health Perspect.* 109(Suppl. 4):529-532.

8. Frampton, M. W., Azadiv, M., Chalupa, D., Morrow, P. E., Gibb, F. R., Oberdörster, G., Boscia, J., and Speers, D. M. (2001). Blood leukocyte expression of LFA-1 and ICAM-1 after inhalation of ultrafine carbon particles. *Am J Respir Crit Care Med.* 163:A264 (Abstract).
9. Frampton, M. W., Chalupa, D., Morrow, P. E., Gibb, F. R., Oberdörster, G., Boscia, J., Speers, D. M., and Utell, M. J. (2000a). Deposition of inhaled ultrafine carbon particles in resting healthy nonsmoking adults. *Am J Respir Crit Care Med.* 161:A257 (Abstract).
10. Frampton, M. W., Chalupa, D., Morrow, P. E., Gibb, F. R., Oberdörster, G., Speers, D. M., and Utell, M. J. (2000b). Deposition and effects of inhaled ultrafine carbon particles in healthy subjects at rest. *Particulate matter and health, Charleston, South Carolina, January 24-28* (Abstract).
11. Geigel, E. J., Hyde, R. W., Perillo, I. B., Torres, A., Perkins, P. T., Pietropaoli, A. P., Frasier, L. M., Frampton, M. W., and Utell, M. J. (1999). Rate of nitric oxide production by the lower airways of human lungs. *J Appl Physiol.* 86:211-221.
12. Perillo, I. B., Hyde, R. W., Olszowka, A. J., Pietropaoli, A. P., Frasier, L. M., Torres, A., Perkins, P. T., Forster II, R. E., Utell, M. J., and Frampton, M. W. (2001). Chemiluminescent measurements of nitric oxide pulmonary diffusing capacity and alveolar production in humans. *J Appl Physiol.* 91:1931-1940.
13. Pietropaoli, A. P., Perillo, I. B., Torres, A., Perkins, P. T., Frasier, L. M., Utell, M. J., Frampton, M. W., and Hyde, R. W. (1999). Simultaneous measurement of nitric oxide production by conducting and alveolar airways of humans. *J Appl Physiol.* 87:1532-1542.
14. Riesenfeld, E., Chalupa, D., Gibb, F. R., Oberdörster, G., Gelein, R., Morrow, P. E., Utell, M. J., and Frampton, M. W. (2000). Ultrafine particle concentrations in a hospital. *Inhalation Toxicol.* 12 (Supplement 2):83-94.

RESEARCH CORE 4: Animal Models: Dosimetry, and Pulmonary and Cardiovascular Events

Principle Investigator: Günter Oberdörster

Co-Investigators: Alison Elder, Jacob Finkelstein, Robert Gelein, Jean Phillippe Couderc, Wojciech Zareba, Christopher Cox, Mark Frampton, Mark Utell, Wolfgang Kreyling, Paul Morrow, Zachary Sharp

Summary of Progress to Date:

Based on extensive critical discussions among the scientists of all five Research Cores and input from our SAC members, a major effort in this Core was devoted to improving the generation system for laboratory-generated ultrafine carbon particles with respect to eliminating organic contaminants and setting up a separate system for generation of ultrafine organic particles and of mixed ultrafine carbon/iron particles. We continued work on our general hypothesis that pre-existing inflammation and old age are important priming factors to sensitize the host for subsequent pulmonary and cardiovascular effects of inhaled ultrafine particles. The same or similar endpoints of pulmonary and cardiovascular effects are selected as those that are used in the epidemiological (Core 2) and clinical (Core 3) studies and are also the focus of the mechanistic *in vitro* studies of Core 5. These *in vivo* endpoints include pulmonary inflammatory markers, analysis of blood white cell adhesion molecules, blood coagulation factors, heart rate variability and blood pressure variability. A general study design for rats and mice includes an inflammatory priming event (inhaled low dose endotoxin [LPS] or intratracheal human influenza virus) followed by ultrafine particle inhalation exposure (ultrafine carbon with or without mixed in iron) and with or without ozone (as a frequently occurring co-pollutant). The priming of the respiratory tract is done to mimic the group of elderly with respiratory tract inflammation who have been identified in PM epidemiological studies as showing increased morbidity/mortality associated with PM exposure (e.g., Pope, *Environmental Health Perspectives* 108 (Suppl. 4): 713-723, 2000). We selected endotoxin as a priming agent because gram-negative bacteria, the source of endotoxin, have been found in several studies as the cause for pneumonia and bronchitis in people with COPD (Barreiro *et al.*, *European Resp. J.* 5(6): 675-679, 1992; Hamacher *et al.*, *J. Antimicrobial Chemotherapy* 36 (Suppl. A): 121-133, 1995; Chendrasekhar, *Amer. Surgeon* 62(5): 373-376, 1996, Klein and Cunha, *Seminars in Respiratory Infections* 12(1): 54-56, 1997).

The general design of these animal studies comprised a total of 16 different groups, including a sham-exposed control group. Young (8-10 weeks) and old (20-22 months) rats and mice were used and exposed for 6 hrs. after priming to the ultrafine particles, with or without ozone, and sacrificed 24 hrs. later. Endpoints included analysis of cellular (including chemiluminescence) and biochemical lung lavage parameters, blood cell and plasma cytokine and acute phase proteins. Results were analyzed by a 4-way ANOVA, with ultrafine particles, ozone, age and the priming agent being the four factors. This study design based on the results from 16 experimental groups represents a powerful tool to evaluate main effects and interactions. Only by examining the three treatments in combination with each other and with age can possible interactive or synergistic effects be understood. Such interactive effects will provide a basis for designing subsequent mechanistic studies.

We also extracted RNA from lungs, hearts, and livers for analysis of gene expression using micro-array technology. We were encouraged by our SAC to initiate such genomic

analysis to identify changes in oxidative stress-related genes and others as a basis for subsequent mechanistic studies.

A continued focus of this Core was the development, characterization and use of compromised animal models which included improving the analysis of rat ECG recordings from radio-transmitter implants. Furthermore, we continued our dosimetry studies using ^{13}C ultrafine particles to determine deposition in the lung and translocation to extrapulmonary tissues. We have also performed preliminary experiments using ultrafine fluorescent beads to evaluate ultrafine particle translocation from the conducting airways to ganglia in the neck along sensory nerves. Finally, we have initiated a collaborative study with the Harvard PM Center using their ultrafine ambient particle concentrator to expose hypertensive old rats (SHR rats) implanted with radio-transmitters for recording of ECG, blood pressure and body temperature. A summary of these studies is provided below.

A. STUDIES WITH MIXED INORGANIC AND WITH ORGANIC ULTRAFINE PARTICLES.

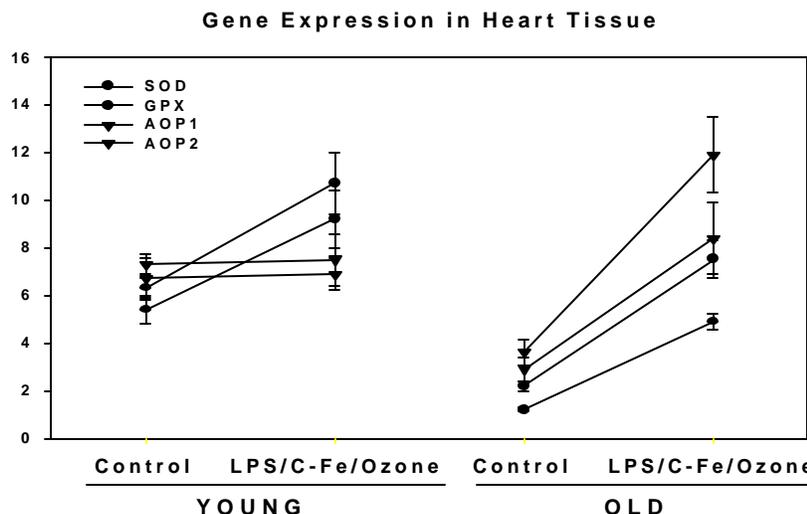
We pursued our initial plans to add a transition metal into our ultrafine carbon particles in order to investigate the impact on pulmonary inflammation, first in animal and *in vitro* (Core 5) studies as the basis for subsequent controlled clinical studies (Core 3). Generation of mixed carbon/iron ultrafine particles involved the mixing of carbon black powder and metallic iron powder and adding glucose and distilled water to form a paste. This mix was extruded through a 3 mm ID glass tube. The resulting cylinders were first dried and then graphitized by slowly raising temperatures up to 2300°C to be used as electrodes for mixed ultrafine C/Fe particles.

The graphitized electrodes of the C/Fe mix were inserted into the PALAS soot generator for generation of mixed ultrafine particles. Particle size distribution was about 25 nm (count median diameter) with a geometric standard deviation of 1.7. At a particle number concentration of 1×10^7 particles/cm³ the mass concentration was $\sim 100 \mu\text{g}/\text{m}^3$. Results from an *in vitro* citrate assay to determine bioavailability (performed by Dr. Aust, Utah State University) showed that these particles exhibited very high biological activities (370 nmol of bioavailable iron/mg of particle). ESR spin trapping analysis of the ultrafine particles was also performed (in collaboration with Dr. Castronova, NIOSH, Morgantown, WV) and revealed that the addition of iron to the ultrafine carbon particles resulted in the generation of OH radicals in the presence of H₂O₂.

In parallel with *in vitro* studies of Research Core 5, we have performed 6 hr. exposures of groups of aged (18 months) and young (8 weeks) mice to the mixed C/Fe ultrafine particles at $\sim 100 \mu\text{g}/\text{m}^3$, with and without prior priming by inhaled low dose LPS and with and without additional exposure to ozone (0.5 ppm). In agreement with our earlier studies in rats (Elder *et al.*, 2000b), age and the ultrafine particles showed a significant main effect in that lavaged inflammatory cells from old mice showed greater release of reactive oxygen species in an *ex vivo* assay performed 24 hrs. post-exposure. We also have first results of the toxicogenomic RNA analysis of lungs and heart which again show striking differences between responses of old and young mice: In the lung, young animals exposed to the combination of ultrafine particles and ozone after priming with inhaled LPS, showed a significant increase not only in inflammatory cytokines and chemokines but also in anti-inflammatory genes (IL-6, IL-10). This was not found in the old mice. This implies that the old organism may not be able to mount an efficient anti-inflammatory response as does the young organism. RNA from the heart also showed differences between young and old mice (Fig. 1): Expression of antioxidant proteins 1 and 2 (AOP1; AOP2) were significantly increased in old animals but not in young animals at 24 hrs.

post-exposure. In addition, two-fold and greater expression of many more oxidative stress-related genes was found in the hearts of old mice than in the hearts of young mice after exposure, indicating that the old organism is much more susceptible to cardiac effects than the young organism following inhalation of air pollutants.

Figure 1:
Micro-array results of cardiac gene expression in old and young LPS-primed mice following inhalation exposure to ultrafine mixed carbon/iron particles in combination with ozone



The results of the micro-array analysis were confirmed by Northern slot blot analysis. Once all the results of this study are available, we will subject them to a 4-way ANOVA to determine main effects of ultrafine particles, age, ozone and LPS priming as well as their interactions. We plan to perform additional studies with inclusion of sacrifice times prior to and beyond 24 hrs. Results of these studies will be an important feedback for both our ongoing controlled clinical and the epidemiological studies with regard to inclusion of potential additional endpoints in those studies.

A second study was performed with the same study design of 6-hr. exposures of old and young mice to ultrafine carbon/Fe particles with and without ozone and with and without priming using intratracheal influenza virus. The results of the 4-way ANOVA showed (i) that the ultrafine C/Fe particles had a main effect for the endpoints lavaged PMN's and reactive oxygen species (ROS) release of lavage cells; (ii) that again age had a main effect, (iii) that additional significant interactions with the ultrafine particles were present leading to greater responses in the aged organism; and (iv) that ozone and virus priming also had significant main effects and interactions with the ultrafine particles. Some of the endpoints are still being analyzed and results are forthcoming. These are ICAM-1 expression on PMN's, monocytes, and lymphocytes of the blood and of lavage cells; acute phase proteins, and coagulation factors in plasma which will allow us to compare responses seen in the animal studies with the clinical (Core 3) and epidemiological studies (Core 2).

Other studies with laboratory-generated ultrafine carbon particles were performed in old rats with and without prior endotoxin priming *via* i.p. injection, to simulate the early phase of a systemic inflammatory stimulus. Twenty-month old normal Fischer rats and 15-month old spontaneously hypertensive rats (SHR) were used for a 6-hr. ultrafine particle exposure, with or without the i.p. LPS priming treatment. Inflammatory lung lavage and blood parameters were determined, including measurement of intracellular ROS generation by inflammatory pulmonary

and blood cells. Results confirmed a significant main effect for the ultrafine particles as well as the priming treatment. Results of several other endpoints are not yet available and will be updated with the next report.

Table 1 summarizes main effects of ultrafine particles for some endpoints of our rodent studies after respiratory tract or i.p. priming. Significant respiratory and systemic effects have been found, which together with results from the controlled clinical studies confirm that laboratory-generated ultrafine carbon particles and carbon/iron particles can, indeed, cause acute responses. Our focus in future studies is the use of our animal models for exposures to ambient ultrafine particles and organic ultrafine carbonaceous particles.

Table 1: Summary of main effects (+) of inhaled ultrafine carbon or carbon/iron particles (UFP); age; ozone; and priming in rodents for some pulmonary and vascular endpoints.

	UFP	Primer	Age	O ₃
<u>Respiratory tract priming</u> (LPS; virus)				
Lavage PMN's	+	+	+	+
Lavage Cell Extracellular ROS release	+	+	+	+
Blood PMN's		+	+	+
Lavage Protein		+	+	+
<u>i.p. priming (LPS)</u>				
Intracellular ROS generation:				
blood cells	+	+	not done	not done
lavage cells	+	+	not done	not done
Blood PMN's	+	+		
Fibrinogen (<i>plasma</i>)		+	not done	not done
IL-6 (<i>plasma</i>)		+	not done	not done

ROS = reactive oxy gen species: extracellular – measured by chemiluminescence (luminol) assay
intracellular – measured by DCFD (2'7'-dichlorofluoresceindiacetate)
oxidation by flow cytometry

A concerted effort of investigators in all five Research Cores of the Center was devoted to the characterization of our spark discharge generated ultrafine carbon particles. This was prompted by an observation of investigators at the GSF (Munich, Germany) collaborating with our Core 2 scientists that these particles contain significant amounts of organics. Measurements (Dr. G. Cass' laboratory of Research Core 1) of the composition of the ultrafine particles generated with the electric spark discharge soot generator (PALAS generator) confirmed that these particles before we began our studies consisted of more than 30% organic compounds.

Additional collaborative efforts with investigators from Lovelace Respiratory Research Institute (Albuquerque, NM) and from GSF in Germany led to further characterization of the organic compounds. One source for these appeared to be off-gassing from plastic materials. A major effort of our Center was subsequently devoted to "cleaning up" the PALAS generator's plastic components inside the generator by replacing them with either stainless steel, Teflon® or ceramic parts. Despite these efforts, there are still some organic carbonaceous materials on the emitted ultrafine particles. These could be coming from small contaminants in the diluting air which may adsorb rapidly onto the large surface area of the ultrafine carbon particles. The surface area of the electric spark discharge generated ultrafine carbonaceous particles was determined in collaboration with Dr. B. Fubini (Turino, Italy) to be 580 m²/g.

A new research initiative was started based on the suggestion by SAC members to investigate effects of organic ultrafine particles which are a major fraction of urban ultrafines, as determined by our Core 1 studies. We developed methodologies for generating an ultrafine condensation aerosol from C20 and C30 compounds and from fresh as well as used motor oil. Methods included use of an electrospray nozzle, heating in a tube furnace and subsequent cooling with and without seed nuclei. This resulted so far in ultrafine particles of C30-alkane (Triacotane) below 100 nm, and of used motor oil of ~100 nm. Pilot studies of exposures of rats to these particles did not show significant changes in lung lavage parameters. These early efforts to generate organic ultrafine particles have been continued by Dr. John Veranth, University of Utah, as a Visiting Scientist supported by our PM Center's Visiting Scientist Program. His summary of these studies as Visiting Scientist is included at the end of this report.

B. DEVELOPMENT AND CHARACTERIZATION OF COMPROMISED ANIMAL MODELS.

1. Analysis of HRV and repolarization segment in unrestrained rats using a telemetry system.

In parallel to the evaluation of the ECG recordings from the epidemiological and clinical PM Center studies, scientists in our cardiac core have developed an algorithm for analysis of recorded ECG and blood pressure signals of rats. In our preliminary analysis of heart rate variability (HRV) in rats, we developed a program to analyze ECG recordings obtained using a telemetry system with implantable transmitters. The analysis of variability of the HRV parameters led us to conclude that at least 1500 beats are needed to obtain reliable and reproducible estimation of HRV parameters [Couderc *et al.*]. Thus in our current experiment, we increased the length of the ECG recordings in order to insure better estimation of HRV parameters.

Our program has been modified to analyze both electrocardiographic (ECG) and blood pressure (BP) signals. The BP signal is often easier to analyze and often of better quality (with less electromyogram) than the ECG [Cerutti *et al.*, *Am J Physiol* 1991;H1292-H1299]. From BP, similar HRV estimators than the one based on ECG signals can be computed. Currently, our program analyzes long-term recordings of ECG and BP signals (24-hours) with the possibility of scanning the entire period in order to locate stable and noise-free signal sequences.

In addition, we are currently working on a new tool for the analysis of the repolarization interval from the ECG signals of rats. Recent experimental findings show that modifications of ionic channel functions by pharmacological agents, ischemia, or electrolyte abnormalities generate repolarization abnormalities with increased heterogeneity of repolarization. These repolarization abnormalities are associated with increased risk of ventricular arrhythmias leading to episodes of torsades de pointes with subsequent ventricular fibrillation [Burashnikov and Antzelevitch, *J. Cardiovasc. Electrophysiol.* 2000; 11(4): 458-465; el Sherif and Turitto, *Pacing Clin.*

Electrophysiol. 1999; 22 (1 Part 1): 91-110]. These experimental data confirm long-term clinical experience indicating the association between prolonged (and abnormal) repolarization and sudden cardiac death. Exposure to particulate air pollution has been shown to be associated with an increased risk of cardiac death in animals where predominantly elderly patients with existing cardio pulmonary disease appear to be at risk. Air pollution may affect the myocardium at the cellular level by modifying intrinsic electrical properties of the myocardial cell through direct (blood-born or reflex-based) or indirect (inflammatory) mechanisms [Arnoow, *Amer. Heart J.* 1981; 101(2): 154-157; Gold *et al.*, *Am. J. Respir. Crit. Care Med.* 1998; (A262): 157; Samet *et al.*, *N. Engl. J. Med.* 2000; 343(24): 1742-1749].

Our program will measure QT and QT peak interval duration by identifying the beginning of the QRS complex and the peak and end of the T-wave [Zareba *et al.*, 2001]. Normal QT and QT peak values will be determined based on a series of baseline recordings in normal rats and using heart rate adjustment formula. The T-wave area analysis will be used to quantify repolarization morphology by tracking the distribution of the amplitude along the time axis. The measure of T-wave morphology has the benefits of being less noise dependent and is also less dependent on accurate detection of the end of the T-wave.

To conclude, our work focuses on the design of tools for the analysis of HRV and repolarization intervals in unrestrained rats based on long-term ECG recordings. The program will be applied to the ECGs recorded during the experiment currently in process to identify potential cardiac abnormalities after exposure to be comparable with the ECG measurements performed in Cores 2 and 3.

The methods described above for heart rate variability (HRV) analysis were applied to data from a cross-over pilot study on the effects of inhaled ultrafine particles (carbon/20% Fe) with and without inhaled endotoxin priming. Six SHR rats (18-20 months) with radiotelemetry implants were exposed to ultrafine particles and endotoxin priming, alone and in combination, which was followed by ECG, body temperature, activity, and blood pressure signals measurements in intervals through the fifth post-exposure day. Analyses have not revealed any changes in HRV associated with exposure. However, we found that the length of recording time (5 mins.) was insufficient for drawing meaningful conclusions. We are presently in the last phase of a second cross-over study in aged SH rats with continuous recording times. Data are being reanalyzed now. As a positive control, the rats of our pilot study were exposed systemically (ip) to endotoxin prior to sacrifice. Preliminary analyses suggest dramatic HRV changes in these animals and the results will be used in future experiments to 1) target the appropriate post-exposure times for analyses and 2) gauge the magnitude of expected changes in HRV.

The endotoxin inhalation model was used to examine the effects of ultrafine carbon/Fe particles in combination with ozone in young and old mice (see section A above). Separate studies were performed to assess the effect that systemic priming with endotoxin has on the response to inhaled ultrafine particles (see section A above).

2. *Studies with endotoxin priming.*

Endotoxin (LPS) priming involves two different models, administration by inhalation and administration by i.p. injection, followed by inhalation exposure to the ultrafine particles within 30-mins. after LPS dosing. The former should serve as a model of the early stage of a respiratory tract infection with gram-negative bacteria, the latter as a model of extrapulmonary systemic infection.

The methods described above for heart rate variability (HRV) analysis were applied to data from a cross-over pilot study on the effects of inhaled ultrafine particles (carbon/20% Fe) with and without inhaled endotoxin priming. Six SHR rats (18-20 months) with radiotelemetry implants were exposed to ultrafine particles and endotoxin priming, alone and in combination, which was followed by ECG, body temperature, activity, and blood pressure signal measurements in intervals through the fifth post-exposure day. Analyses have not revealed any changes in HRV associated with exposure. However, we found that the length of recording time (5-10 mins.) was insufficient for drawing meaningful conclusions. We are presently in the last phase of a second cross-over study in aged SH rats with continuous recording times. Data will be reanalyzed in the future. As a positive control, the rats of our pilot study were exposed systemically (ip) to endotoxin prior to sacrifice in a pilot study. Preliminary analyses suggest dramatic HRV changes and the results will be used in future experiments to 1) target the appropriate post-exposure times for analyses and 2) gauge the magnitude of expected changes in HRV.

The endotoxin inhalation model was used to examine the effects of ultrafine carbon/Fe particles in combination with ozone in young and old mice (see section A above). Separate studies were performed to assess the effect that systemic priming with endotoxin has on the response to inhaled ultrafine particles. (See section A above).

3. *Influenza rodent model.*

We have developed a model of influenza A virus infection in mice and rats as a priming agent for subsequent ultrafine particle exposures. The inflammatory response in the lung after intranasal instillation of influenza virus into mice and rats was measured over several post-exposure days and compared to results obtained after intratracheal instillation. The peak of the inflammatory response in terms of appearance of PMNs in lung lavage occurs at ~48 hrs. after instillation, at which timepoint a lymphocytic infiltration also occurs. Individual variability after intranasal instillation appears to be much greater compared to intratracheal instillation and subsequent studies will, therefore, be done using intratracheal instillation of influenza A. Pilot studies have been performed in influenza-primed mice and rats with additional instillation of ultrafine TiO₂ particles in order to test the concept that influenza priming increases sensitivity to a subsequent second particulate stimulus. Results showed, indeed, that ultrafine TiO₂ administered on day 2 after influenza priming caused significantly greater pulmonary inflammation than TiO₂ given to unprimed animals. This model has now successfully been used with inhaled ultrafine particles as described above in Section A.

4. *TNF transgenic mice.*

We are also testing a mouse model that demonstrates increased sensitivity to inflammatory stimuli, *i.e.*, TNF α transgenic mice. These mice express the human TNF α gene, yet baseline levels of inflammatory mediators are not different from those in control mice. However, after an inflammatory stimulus in these primed mice, responses are expected to be significantly greater, particularly for TNF α production, compared to wild type mice. Initial studies to evaluate the usefulness of this mouse model for inhaled particle exposures compared the responses to ultrafine *vs.* fine TiO₂ particles. Contrary to what we expected, the TNF α transgenes showed a lower response to the TiO₂ test particles than the wild type mice. Uncovering the reason for this unexpected outcome needs further studies, however, we have no further plans at this time to use this model with inhaled ultrafine particles.

C. DOSIMETRY STUDIES.

Our present studies evaluating the fate of inhaled ultrafine particles in the lung are focused on their translocation to extrapulmonary tissues. For this purpose, we have developed a method to produce ^{13}C graphite electrodes using ^{13}C amorphous carbon powder and ^{13}C glucose as binder. Our initial results following inhalation of these ultrafine ^{13}C particles in rats and mice had indicated that there seems to be a rapid transport of the ultrafine carbon particles to extrapulmonary sites shortly after inhalation exposure. Subsequent analyses of the data have shown that there is significant variation in the results upon repeated measurements. We spent much of our efforts on resolving this issue of variability. Variability depends largely on the size of the tissue sample used for ^{13}C analysis. Small differences in baseline levels of ^{13}C in organs can also occur. A major improvement, therefore, was achieved by increasing the amount of lyophilized tissue for analysis from 50 μg to 1 mg which reduced variability significantly.

After several methodological improvements, we performed a study with the objective to determine whether ultrafine elemental carbon particles translocate to the liver and other extrapulmonary organs following inhalation as singlet particles by rats (Oberdörster *et al.*, 2002). We generated ultrafine ^{13}C particles as an aerosol with CMD's of 20-29 nm (GSD 1.7) using electric spark discharge of ^{13}C graphite electrodes in argon. Nine Fischer-344 rats were exposed to these particles for 6 hrs. in whole-body inhalation chambers at concentrations of 180 and 80 $\mu\text{g}/\text{m}^3$; three animals each were killed at 0.5, 18 and 24 hrs. post-exposure. Six unexposed rats served as controls. Lung lobes, liver as well as heart, brain, olfactory bulb, and kidney were excised, homogenized and freeze-dried for analysis of the added ^{13}C by isotope ratio mass spectrometry. Organic ^{13}C was not detected in the ^{13}C particles. The ^{13}C retained in the lung at 0.5 hrs. post-exposure was about 70% less than predicted by rat deposition models for ultrafine particles, and did not change significantly during the 24 hr. post-exposure period. Normalized to exposure concentration, the added ^{13}C per gram of lung on average in the post-exposure period was ~ 9 ng/g organ/ $\mu\text{g}/\text{m}^3$. Significant amounts of ^{13}C had accumulated in the liver by 0.5 hr. post-inhalation only at the high exposure concentration, whereas by 18 and 24 hrs. post-exposure the ^{13}C concentration of the livers of all exposed rats was almost half the ^{13}C concentration found in the lung (Fig. 2). Considering the ~ 10 -fold greater weight of the liver compared to the lung, the ^{13}C amount in the liver was ~ 5 -fold greater than in the lung by 18 and 24 hrs. after exposure. No significant increase in ^{13}C was detected in the other organs which were examined. These results demonstrate effective translocation of ultrafine elemental carbon particles to the liver by one day after inhalation exposure. Potential translocation pathways include direct input into the blood compartment from ultrafine carbon particles deposited throughout the respiratory tract.

Normalized Lung and Liver Excess ^{13}C Concentration Following Ultrafine ^{13}C Particle Exposure in Rats (n=3)

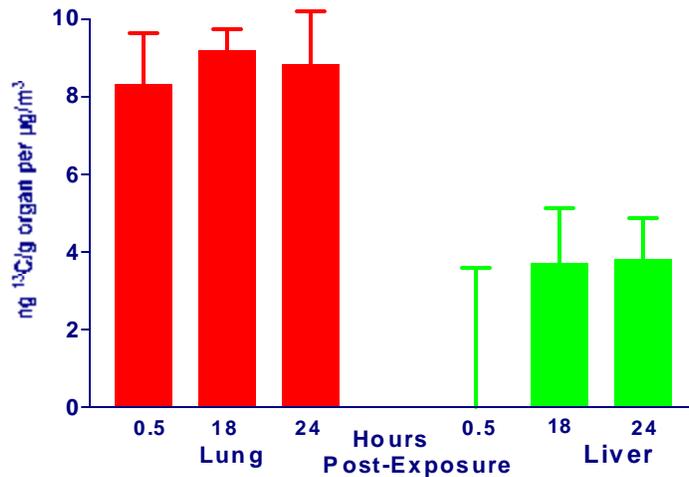


Figure 2: Lung and liver ^{13}C concentration in rats at different times after exposure to ultrafine ^{13}C particles, normalized to the exposure concentration

In a pilot study, ^{13}C analysis of lung and extrapulmonary organs was analyzed on day 1 and day 7 post ultrafine ^{13}C particle exposure. Results showed again significant amounts of added ^{13}C in the liver on day 1, but no longer on day 7. However, on day 7, significant increases in added ^{13}C in heart, brain and olfactory bulb were found. This prompted us to perform a time-course study with measurement of ^{13}C organ content on days 1, 3, 5 and 7 after a 6-hr. ultrafine ^{13}C particle exposure. Based on earlier studies reporting translocation of ultrafine organic particles along sensory nerve endings in the lung (Huner and Undem, *Am. J. Respir. Crit. Care Med.* 159: 1943-1948, 1999), we hypothesized that translocation of inhaled ultrafine particles occurs in the nasal compartment along the olfactory nerve into the olfactory bulb. This would explain findings of our pilot study. Results of our time-course study, indeed, showed that olfactory bulb ^{13}C content was significantly increased on all post-exposure days and that cerebrum and cerebellum also had increased ^{13}C levels which were significant on days 1 and 7 and on days 1 and 5, respectively (Fig. 3).

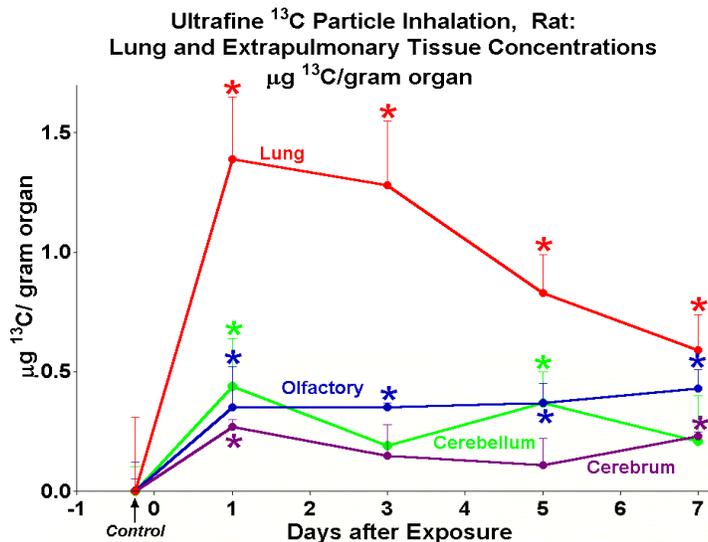


Figure 3: Translocation of inhaled ultrafine ^{13}C particles to tissues of the central nervous system over 7 days post-exposure. *Significantly increased ^{13}C compared to control levels ($p < 0.05$, Dunnett's test)

These are intriguing findings, and further studies are underway to determine ultrafine carbon particle translocation pathways (gavage *vs.* inhalation of ultrafine ^{13}C) and in old SHR rats with increased pulmonary epithelial permeability. It is of interest to see that the time pattern in Fig. 3 is similar to that seen in the epidemiology in Erfurt, where UFP effects on cardiovascular deaths showed a first (smaller) peak on day 1 and a later (more pronounced) peak later on days 4-5, whereas the effects in the lung (respiratory deaths) were seen immediately on days 0-1 (Wichmann *et al.*, 2000, Core 3).

Our studies performed within our PM Center's Pilot Programs used ultrafine ^{192}Ir particles. Iridium is the least soluble of metals in the lung and is, therefore, best suited to study its disposition after inhalation. Dr. Kreyling of the GSF München, is the PI of this pilot study, and he found in contrast to our results with ultrafine ^{13}C particles that after intratracheal inhalation exposure ultrafine iridium particles are not translocated to a significant degree to extrapulmonary organs (Kreyling *et al.*, 2002) suggesting that not only the particle size but also the material and surface properties like structure and composition may influence the amount of transportation. Results of his detailed studies are given in his attached progress report. Differences in translocation of inhaled ultrafine particles based on their chemistry will be further investigated in a collaborative approach.

Future plans for exposures with concentrated ambient ultrafine particles:

We have initiated collaborative studies with the Harvard PM Center (P.Koutrakis) to use their ultrafine particle concentrator for exposures of our aged SH rats prepared for telemetry recordings of ECG, blood pressure and body temperature. These studies will be performed at Rochester, with the Harvard ultrafine concentrator in a location where air from an adjacent busy road can be drawn in and concentrated for animal exposures. The concentrator has been installed and we are presently testing it. We will in these studies also use our LPS priming model. We also plan to use our other animal models described above. We focus on the use of models of compromised animals because the epidemiological studies have demonstrated that adverse responses to ambient particles are only seen in compromised hosts but do not occur in the healthy organism. Animal studies seem to confirm this unless very high concentrations or very long exposure times are used. Concentrating the ambient ultrafine particles in our planned studies should mimic episodic increases of those particles which have been reported in the literature.

Publications by Core Investigators :

- Elder, A.C.P., C. Johnston, J. Finkelstein, and G. Oberdörster. Induction of Adaptation to Inhaled Lipopolysaccharide in Young and Old Rats and Mice. *Inhal. Toxicol.* 12:225-243, 2000a.
- Elder, A.C.P., R. Gelein, J. Finkelstein, C. Cox, and G. Oberdörster. Endotoxin Priming Affects the Lung Response to Ultrafine Particles and Ozone in Young and Old Rats. *Inhal. Toxicol.* Inhal. Toxicol. 12, Suppl. 1,85-98, 2000b.
- Oberdörster, G. Pulmonary effects of inhaled ultrafine particles. *Intl. Archives of Occup. & Environ. Health* 74 (Issue 1): 1-8, 2001.

- Johnston, C.J., J.N. Finkelstein, P. Mercer, N. Corson, R. Gelein and G. Oberdörster. Pulmonary Effects Induced by Ultrafine (Uf) PTFE Particles. *Toxicol. and Appl. Pharmacol.*, 168, 208-215, 2000.
- Oberdörster, G. Toxicology of ultrafine particles: *in vivo* studies. *Phil.Trans.R.Soc.Lond. A* (2000) **358**: 2719-2740.
- Riesefeld, E., Chalupa, D., Gibb, F.R., Oberdörster, G., Gelein, R., Morrow, P.E., Utell, M.J., Frampton, M.W. Ultrafine particle concentrations in a hospital. *Inhal. Tox.* **12** (Suppl. 2): 83-94, 2000.
- Chalupa, D., Gibb, F. R., Morrow, P.E., Oberdörster, G., Riesefeld, E., Gelein, R., Utell, M. J., and Frampton, M.W. A facility for controlled human exposures to ultrafine particles. In: Proceedings of the Third Colloquium on Particulate Air Pollution and Human Health (Submitted, 2001).
- Couderc, J-P, ACP Elder, C Cox, W Zareba, G Oberdörster. Power Spectrum Analysis and Time-domain Analysis of Heart Rate Variability in Short-term ECGs Recorded using Telemetry in Unrestrained Rats. *Ann. Biomed. Eng.* (in submission).
- Couderc, J-P, ACP Elder, C Cox, W Zareba, G Oberdörster. Limitation of Power Spectrum and Time-domain Analysis of Heart Rate Variability in Short-term ECG Recorded using Telemetry in Unrestrained Rats. *Computers in Cardiology*, IEEE Computer Society Press. Vol. 28, 2001, in press.
- Elder, A.C.P., Gelein, R., Azadniv, M., Frampton, M., Finkelstein, J.N. and Oberdörster, G. Systemic interactions between inhaled ultrafine particles and endotoxin. *Annals of Occup. Hygiene*, 2001, in press.
- Johnston, C.J., Oberdörster, G., and Finkelstein, J.N. Recovery from oxidant-mediated lung injury: Response of metallothionein, MIP-2, and MCP-1 to nitrogen dioxide, oxygen, and ozone exposures. *Inhalation Toxicology* 13(8): 689-702, 2001.
- Kreyling, W.G., Semmler, M., Erbe, F., Mayer, P., Takenaka, S., Schulz, H., Oberdörster, G. and Ziesenis, A. Ultrafine insoluble iridium particles are negligibly translocated from lung epithelium to extrapulmonary organs. Submitted to: *J. Tox. & Environ. Health*, 2002.
- Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Lunts, A., Kreyling, W. and Cox, C. Extrapulmonary translocation of ultrafine carbon particles following inhalation exposure. Submitted to: *J. Tox. & Environ. Health*, 2002
- Zareba W, Couderc JP, Nomura A, Frampton M, Utell MJ, Peters A, Oberdörster G. Cardiac effects of air pollution: what to measure in ECG? *Toxicological Sciences* 60: 16, 2001.

RESEARCH CORE 5: Ultrafine Particle Cell Interactions: Molecular Mechanisms Leading to Altered Gene Expression

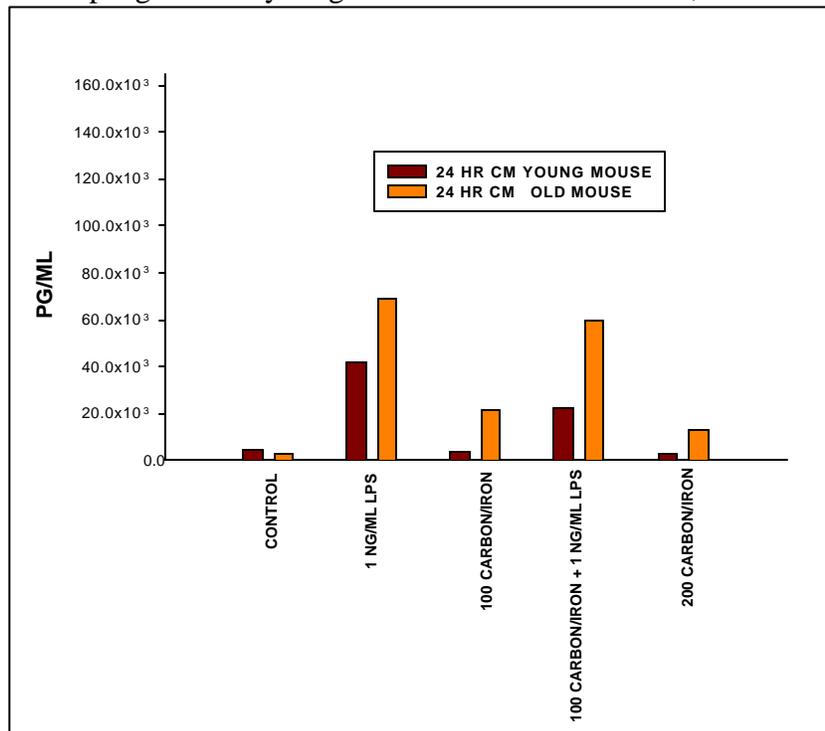
Principle Investigator: Jacob Finkelstein

Co-Investigators: Richard Phipps, Barry Stripp, Michael O'Reilly, Günter Oberdörster, R. Gelein, (Particle Generation Core)

Summary of Progress to Date

Research during the current year continues to develop *in vitro* models of particle cell interactions with the goal to define mechanisms of cellular activation, the effects of age or prior activation on cytokine gene activation and differential responses of epithelial cells and macrophages to particles of different sizes. While a number of experimental difficulties have altered some of the approaches, we continue to make significant progress in our experimental goals.

One of our main goals for this year was to evaluate the effect of age on the response of cells to particles. In our initial studies we compared macrophage production of cytokines following LPS and particles from 22-27 month old rats to cells from 10-12 week old rats. When macrophages from young rats are treated with LPS, a clear dose response, with MIP-2 as the



**Figure 1 Expression of MIP-2 by Macrophages
Effect of Age**

endpoint, is obtained. A similar dose response relationship was observed with carbon particles alone. When the two stimuli are combined, no enhanced effect is observed except at the highest dose of particles. When a similar study was performed with macrophages from “old rats” a number of clear differences were observed. Interestingly, baseline (unstimulated) production of MIP-2 (and TNF) was elevated 30-50% in these cells. In addition, response to LPS was enhanced at every dose. Response to particles alone

was similar to that observed in young cells. 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 lead 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. Figure 1 shows the results of our most recent studies in mice, comparing the effects of age on macrophage responses to particles and LPS. Similar to our data in rats, mouse macrophages also showed an age dependent difference in cytokine production following stimulation with particles or LPS. In these studies we chose to make use of a particle that was composed of both Carbon and Iron. This was chosen, in part based on the data provided by our Analysis Project (Core1) which has suggested that Iron is among the most abundant

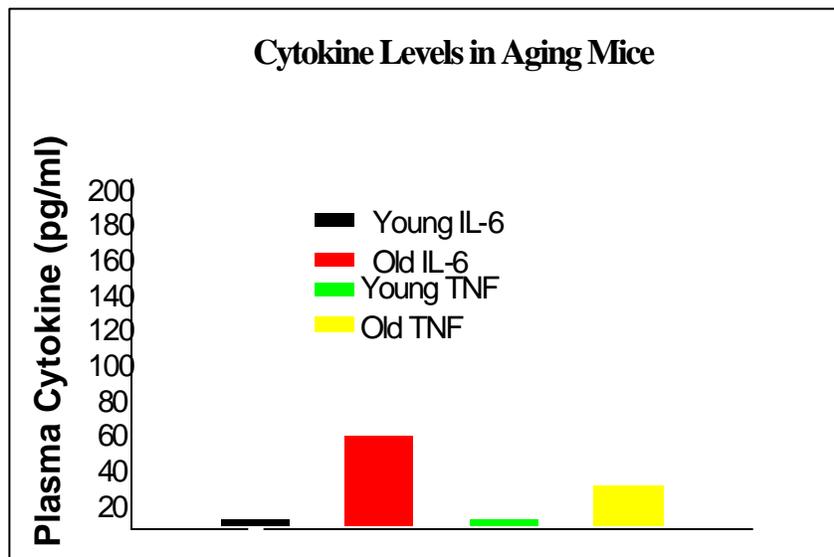


Figure 2 Cytokine Levels in Plasma of Young and Old Mice

metal constituents. Additionally, our studies with this particle would be used to assess the likelihood of using this particle in both the animal studies (Core 4) as well as potentially in the Human Clinical Studies (Core3)

In an effort to better characterize the aged mice and begin to evaluate their utility as a model of human response we measured baseline cytokine levels in their plasma. The cytokines

we chose to measure were based on the recommendations of our advisory committee that we use similar markers that are used, or

suggested for use in the *in vivo* animal experiments and the human clinical studies. As shown in **Figure 2** both TNF α and IL-6 were significantly elevated in the aged mice. Although our initial studies with mouse macrophages have show a somewhat blunted response in these cells in comparison to rats, we nevertheless have reproduced the age effect.

An important question regarding the *in vitro* studies is the choice of the appropriate end point to measure. While production of TNF α or MIP-2 following interaction with particles is well described, their role in environmental particle induced systemic disease is less clear. Thus, some studies were carried out looking at additional endpoints. These were chosen on the basis of data obtained from the clinical studies and the possibility that measurements could be made in the *in vivo* studies. Among the cytokines measured the only one that showed some promise was IL-6. Production of IL-6 was observed when epithelial cells were cultured in the presence of silica, used as a positive particle control, and LPS. However no effect of the addition of Carbon particle on IL-6 protein or mRNA was observed in our mouse experiments. We will continue to consult with the *in vivo* animal studies to attempt to develop additional *in vitro* markers that could accurately predict effects of the inhalation studies.

We have also begun to develop reagents and approaches that would allow extension of our *in vitro* studies to human cells while also developing a test of our oxidant stress hypothesis.

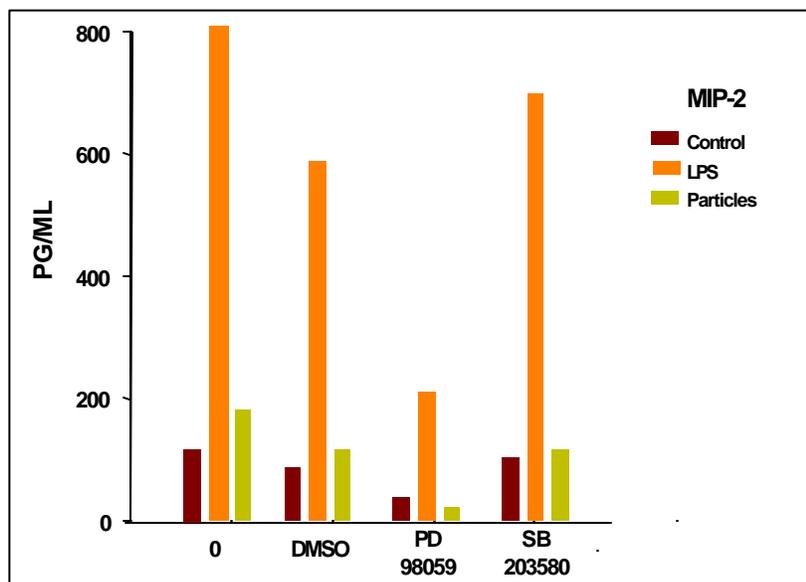


Figure 3 Effect of Kinase Inhibitors on MIP-2 Expression by Epithelial Cells after Particle Stimulation

We have developed a human lung cell line, A549, that was stably transfected with a reporter gene that other studies have shown was responsive to oxidant stress. Preliminary experiments confirm particle-induced increase in reporter gene activity with a peak at 6 hours post treatment.

We have used this cell line along with specific inhibitors of signaling pathways to begin to investigate the mechanism of particle induced gene expression. As shown in Figure 3 addition of the p38 kinase inhibitor PD98059 effectively inhibited both LPS and particle

induced MIP-2 expression. In contrast SB203580, which blocks p44 MAP kinase had little effect. We plan to continue to use this cell line as well as others to test the response of cells to particles and develop an understanding of the potential interactions between particles and endotoxin.

Future Plans :

In the coming year we plan to continue to characterize the difference in response to stimuli, alone and in combination, as a function of age. We also expect to extend these studies from macrophages to parenchymal cells, fibroblasts and epithelial cells, as well. We will also investigate other markers of response. Measurement of prostaglandin production, and COX-2 activation will be evaluated with respect to its usefulness as a marker. Studies have shown COX-2 to be important in the induction of the inflammatory response and systemic responses.

Also, in support of the *in vivo* projects, we will evaluate *in vitro* effects of particles of differing composition. We will continue to examine the cytokine response to particles containing carbon and iron and begin studies of concentrated real world particles.

Publications by Core Investigators :

Elder ACP. Gelein R. Finkelstein JN. Cox C. Oberdörster G. Pulmonary inflammatory response to inhaled ultrafine particles is modified by age, ozone exposure, and bacterial toxin 2000. *Inhal Toxicol.* 12(4):227-246

Avissar NE. Reed CK. Cox C. Frampton MW. Finkelstein JN. Ozone, but not nitrogen dioxide, exposure decreases glutathione peroxidases in epithelial lining fluid of human lung. 2000 *Am J Resp Crit Care Med.* 162(4): 1342-1347

Johnston CJ. Driscoll KE. Finkelstein JN. Baggs R. O'Reilly MA. Carter J. Gelein R. Oberdörster G. Pulmonary chemokine and mutagenic responses in rats after subchronic inhalation of amorphous and crystalline silica. 2000 *Tox Sci.* 56(2): 405-413

PILOT PROJECT: Development of an Electrodynamic Quadrupole Aerosol Concentrator

Principal Investigator: David S. Ensor, Ph. D.

Background:

The objective of this project is to develop a new approach for concentrating ultrafine particles for animal inhalation studies. The electrodynamic aerosol concentrator invented by Periasamy, Ensor and Donovan (*Device for Focussing Particles Suspended in a Gas Stream*. US Patent 5,439,513, 1995) uses an ac field quadrupole to deflect the particles to the center of a flowing gas stream between the electrodes. RTI showed in earlier experiments that an electrodynamic quadrupole demonstrated significant focussing when using a monodisperse 14 μm oleic acid aerosols tagged with uranine dye. Two key questions are being addressed in the current research:

1. Can the performance be extended to sub 0.1 μm particles?
2. Can the device be scaled up to provide sufficient flow for laboratory studies?

Progress Report:

During the second year, RTI has focused on theoretical analysis and laboratory experiments. The theoretical analysis performed by Dr. Seok Joo Park during a post doctoral appointment at University of Minnesota (Park *et al.*, "Electrodynamic Focusing of Charged Fine Particle Using Alternating 2-dimensional Quadrupole Electric Field", 2000) was extended by Dr. John Franke of North Carolina State University, Department of Mathematics. Additional theoretical analysis confirms our finding that focusing and concentrating of particles smaller than 0.1 μm at atmospheric pressure will be difficult. The key parameter is the level of charge on the particles.

A series of experiments was conducted with monodisperse oleic acid uranine dye aerosols (1 to 15 μm) generated with a vibrating orifice aerosol generator using a ring electrode charger. The focusing was determined by using the dye image formed on a filter placed after the quadrupole section. These experiments demonstrated significant focusing of particles at 1 μm . The quadrupole used in these experiments was 20 cm long with 1.3 cm diameter electrodes in a 3.8 cm diameter tube. This experimental approach was limited to 1 μm aerosol by the ability to detect the dye patterns on the surface of the filter.

Since the eventual application of the device will be to concentrate atmospheric aerosol, we are looking at aerosol charging and equipment scale-up. The experiments conducted over the last 6 months have taken a different approach of building a larger quadrupole with 1.9 cm diameter electrodes in a 10 cm diameter tube with length up to 210 cm. A PMS LAS-X particle counter was used to traverse the exhaust of the quadrupole. The results to date have been inconclusive because of experimental limitations from aerosol charging. The major difficulty has been to obtain adequate charge without precipitating the particles in the charger.

A novel aerosol charger based on the design reported by Cheng *et al.* (*AS&T* 26:433-446, 1997) has been constructed and tested. This charger has a porous outer wall with central corona discharge. Transpiration flow through the porous wall is used to prevent deposition of charged particles on the wall.

Future Plans:

In the final months of the second year, the priority will be to document the research conducted under this Pilot Project for a peer reviewed paper. Two-dimensional quadrupoles may be an important aerosol research tool in appropriate applications requiring transporting and focusing of aerosols. The paper will include numerical analysis of the particle equations of motion by two different solvers and experiments under atmospheric pressure. The numerical solutions for the two-dimensional geometry has previously only been approximated by Masuda *et. al.* (*Denki Gakkai Zasshi*, **90**: 861-869, 1970) and there are important differences from the solutions for the three-dimensional case reported by Davis (*Langmuir*, **1**:379-387, 1985). The experimental results will be limited to the successful test setups and will stress the mating of the aerosol charger and the concentrator. A preliminary title for the paper is as follows:

ELECTRODYNAMIC FOCUSING OF CHARGED FINE PARTICLES USING ALTERNATING 2-DIMENSIONAL QUADRUPOLE ELECTRIC FIELDS. David S. Ensor, Debbie Franke, Jim Hanley and Clint Clayton, Research Triangle Institute; John Franke, North Carolina State University; Seok Joo Park, Korea Institute of Energy Research; Peter H. McMurry, Hiromu Sakurai and Shintaro Sato, University of Minnesota..

Pilot Project: “Kinetics of clearance and relocation of insoluble ultrafine iridium particles from the rat lung epithelium to extrapulmonary organs and tissues

Principal Investigator: Wolfgang G. Kreyling

Co Investigators: M Semmler, F Erbe, P Mayer, S. Takenaka, *G Oberdörster & A Ziesenis
GSF-Institute for Inhalation Biology, D-85758 Neuherberg/Munich, Germany
and *University of Rochester, Medical Center, Rochester, NY 14642, USA

Recently it was speculated that ultrafine particles may translocate from deposition sites in the lungs to systemic circulation. This could lead to accumulation and potentially adverse reactions in critical organs such as liver, heart and even brain, being consistent with the hypothesis that ultrafine insoluble particles may play a role in the onset of cardiovascular diseases as growing evidence from epidemiological studies suggests.

To study ultrafine particle translocation into secondary organs, aerosols of ultrafine iridium particles radio-labelled with Ir-192 (count median diameter 15 nm and 80 nm; geometric standard deviation 1.6 each) were generated with a spark generator using neutron-activated pure iridium electrodes. For inhalation, young adult, healthy, male WKY rats were ventilated for one hour via an endotracheal tube. After exposure rats were maintained in metabolic cages and excreta were collected separately and quantitatively. At time points ranging from 6 h to 21 days, rats were sacrificed, and a complete balance of Ir-192 activity retained in various organs, tissues and the remaining carcass and cleared by excretion was determined gamma spectroscopically. In additional studies the biokinetic of ultrafine particles and soluble Ir-192 was studied after administration by either gavage or lung instillation or intravenous injection.

Seven days after soluble Ir-192 administration, 60% was excreted vastly via urine, 8% stayed in the lungs and 10% fractions were retained in soft tissue and bone each; little was left in other organs. Auxiliary in vivo studies confirmed in vitro studies on the very low solubility of ultrafine iridium particles (< 1% per 7 days). Both, inhaled 15 nm and 80 nm ultrafine iridium particles continued to be exclusively retained in the lungs for one week after fast tracheobronchial clearance was complete. Only about 0.5% of the particles were retained in bone and soft tissue, <0.1% in liver and even less in heart and brain.

Balancing the entire deposited Ir-192 activity and the negligible solubility of the particles allowed a sensitive and quantitative analysis of the fate of retained particles in the lungs versus that of translocated particles to either the gastro-intestinal tract and feces or to other organs after systemic translocation. This study indicates, only tiny fractions of ultrafine iridium particles have access to systemic circulation and extrapulmonary organs. However, chemical particle composition and the properties of the particle surface may be other important determinants.

Report on Visiting Scientist Project
Ultrafine Oil Aerosol Generation for Inhalation Studies
Submitted by John Veranth
January 8, 2001

P.I.: John Veranth, University of Utah

Co-investigators: Günter Oberdörster, Robert Gelein, University of Rochester

Abstract

A particle generator was assembled and used to do a controlled exposure of rats to an organic carbon aerosol that is a surrogate for the organic component of internal combustion engine tailpipe emissions. The inhalation experiments were based on the recommendation to conduct a screening experiment to look for responses to ultrafine aerosol derived from used motor oil. A vaporization-condensation aerosol generator was assembled and characterized. A 6-hr inhalation experiment with oil aerosol was conducted on December 3, 2001. The particle sampling data during the run show that the animal exposure study met the aerosol criteria of having greater than 1×10^6 particles / cm^3 with a count median diameter between 30-50 nm..

Introduction

Dr. John Veranth spent three months at Rochester as a visiting scientist working with Drs. Günter Oberdörster and Robert Gelein as part of the University of Rochester EPA Airborne Particulate Matter Center's enrichment programs. The work was performed during three visits to Rochester between February and December. This report documents Dr. Veranth's activities related to ultrafine particle generation.

Diesel engine exhaust is thought to be a major source of ultrafine particulate matter (PM) in urban environments. [1-6] Diesel engines produce a multimodal particle size distribution that varies with engine speed, load, fuel sulfur content, and exhaust dilution conditions. [7-10] Soot agglomerates form the largest diameter particle mode from diesel engines. Newer low-emissions engines do not produce clouds of black smoke but can produce high number concentration of particles smaller than 100 nm and often show a separate nucleation mode between 20 nm and the lower detection limit of the instruments. [7] The current opinion is that the particle mode below 100 nm is composed of high boiling point hydrocarbons condensed on soot, sulfuric acid, or metal oxide nuclei. [11, 12] These hydrocarbons are derived from lubricating oil and unburned fuel.

The EPA Airborne Particulate Matter Center's external Science Advisory Committee, at the May 2001 meeting, recommended animal inhalation studies an ultrafine organic aerosol to screen for evidence of effects. Previous studies have shown that inhalation of ultrafine particles can produce inflammation and other responses even when the ultrafine aerosol is a substance that is considered inert when inhaled as larger particles.[12, 13] Based on the committee recommendation, an exposure study using ultrafine aerosol derived from the complex mixture in used motor oil was planned. Dr. Veranth's work was focused on design and validation of an ultrafine aerosol generator for hydrocarbon aerosols and support of the inhalation studies using LPS-primed rats.

Technical Background

Physical processes constrain the formation of an ultrafine organic aerosol for inhalation. The maximum mass concentration that can be achieved is limited by the coagulation growth of particles over the duration of the residence time in the source, transport lines, and inhalation chamber. For example, if the characteristic time for coagulation is to be greater than 5 minutes the 25 nm particles are limited to a maximum concentration of 1×10^6 particles / cm^3 which is equivalent to a mass concentration of about $10 \mu\text{g}/\text{m}^3$. For the organic particles to form the supersaturation must either be sufficiently high to allow heterogeneous nucleation to take place or there must be sufficient nuclei present to allow condensation deposition of the organic material. Particles are not stable unless the supersaturation and diameter are above the Kelvin limit, which depends on the molecular weight and surface tension of the condensing species. [14] High saturation of the condensing species is necessary to form the ultrafine aerosol, but once the particle exceeds the Kelvin diameter the remaining gas-phase material continues to condense, which results in aerosol growth. The condensing species is brought to supersaturation by a combination of cooling due to heat transfer and cooling due to dilution. The slope of the vapor pressure versus temperature curve is determined by thermodynamics. [15, 16] Briefly, the higher the boiling point the easier it is to achieve high supersaturation by cooling.

The goal for the aerosol generation was based on a combination of prior experience with particle inhalation studies, relevance to real-world aerosols, preliminary particle generation experiments in February-April 2001, and the physical / thermodynamic constraints discussed above. The stated objective became to produce a number concentration greater than 1×10^6 particles / cm^3 of hydrocarbon mixture particles, derived from used motor oil, with a number mode between 20 and 100 nm, with the majority of the particle volume (mass) distribution in the submicron range. In addition, the gas phase had to be $21 \pm 2\%$ oxygen and dilution solvents needed to be well below toxic levels.

Work started in February 2001, and the initial effort was to perform a review of relevant prior aerosol generation efforts. Much of the previous work in laboratory aerosol generation was directed toward generation of either monodisperse supermicron aerosols for instrument calibration [17-19] or the generation of ultrafine aerosols of metal or salt for nucleation and coagulation studies. [20, 21] No references were found that reported laboratory generation of organic aerosols in the ultrafine size range. [22] Use of a small diesel engine as an aerosol source was considered but rejected as infeasible for the current project. Difficulties included the heat, noise and vibration of an engine in a biomedical laboratory setting, and the need to remove copollutants such as soot, CO, and NO_x if the aim was to study the effects of ultrafine organic aerosol alone. Concurrent efforts to generate a suitable aerosol using an electrospray generator were attempted by TSI, Inc. but were not successful as the electrospray requires a conducting fluid. [23] Attempts to operate the electrospray with water-oil emulsions [24] were unsuccessful.

The literature search suggested that a vaporization-condensation particle generator based on the classical Sinclair-LeMer generator [17, 25] should be capable of generating a suitable organic aerosol if operating conditions were optimized. This type of particle generator is a two-step process where nuclei of a very high boiling point compound are produced then the lower boiling aqueous or organic compound is condensed on the nuclei. Several weeks were spent testing nuclei generation methods and reviewing the vaporization-condensation aerosol generation

literature to develop a quantitative understanding of the factors affecting nucleation rate and subsequent particle growth. [26, 27] The initial plan was to generate a 5-10 nm NaCl nuclei aerosol based on the operating conditions used by Scheibel and Porstendörfer [28] followed by condensation of sufficient hydrocarbon to produce a 30 nm final particle, which would be 90% hydrocarbon by volume. Initial work was conducted with pure hydrocarbons and various configurations were tried to introduce into the gas stream the proper amount of salt nuclei and hydrocarbon to achieve the target aerosol number concentration and size. By the end of April an ultrafine aerosol composed of triacontane ($C_{30}H_{62}$ n-alkane) condensed on NaCl nuclei had been produced in demonstration experiments. However, during November testing to determine reproducibility of the system when running used motor oil showed that the NaCl nuclei generation was unnecessary when running at the high aerosol concentrations desired for the inhalation studies. The work then focused on demonstrating the reproducibility and stability of a simplified aerosol generation system as described in “Methods” and “Results” below.

The efforts were successful and an aerosol that met the original specification was generated for a 6-hour inhalation study of saline-treated and LPS-treated rats. A aerosol engineering publication is planned to document the particle generation technology.

Methods

The number and size of the aerosol was measured with a TSI, Inc. (St. Paul MN) Model 3071A Scanning Mobility Particle Sizer (SMPS) and Model 3022A Condensation Particle Counter (CPS). At various times in the study the SMPS was operated at 2 lpm aerosol with 20 lpm sheath flow to obtain resolution below 10 nm. Alternatively, the SMPS was operated at 0.3 lpm aerosol and 3 lpm sheath to resolve both the nucleation and accumulation modes. The instrument was set up according to the TSI operating manual and flows were checked with a bubble flow meter. The mass distribution of particles larger than the SMPS cutoff was determined using a cascade impactor (In-Tox Products, Albuquerque, NM).

The test hydrocarbon was “used commercial motor oil” collected from a Honda automobile. The used motor oil was dark and opaque. Solids settled out from the mixture and only the supernatant was used. Preliminary testing also used C25 (pentacosane), C30 (triacontane), C40 (tetracontane) straight-chain alkanes (Aldrich, Milwaukee WI) and light paraffin oil (EM Science)

The aerosol generation system used for the rat inhalation studies is shown in Figure 1. The flow rates, system geometry and line sizes, operating temperatures, and organic feed were arrived at by a combination of design calculations and empirical experimentation which iteratively lead to aerosol that met the specifications. Alternative designs are possible. Preliminary testing indicated that NaCl nuclei were needed to form an ultrafine aerosol when operating at low organic feed rates but nuclei were not needed when the organic feed rate was increased. Consequently, the upstream furnace (not shown in Figure 1) which had been used for NaCl nuclei generation was shut off and served only as a connecting pipe leading to Furnace 2. The carrier gas for organic vaporization was argon since preliminary experiments with long-chain n-alkanes showed partial oxidation of the hydrocarbon leading to deposits and changes in aerosol size with time.

The feed rate of oil proved to be the most critical variable controlling aerosol size and number, as discussed in results. Material balance calculations indicated that a very small feed rate was required. Complete evaporation of a spray was selected since evaporation from the surface of a stationary pool of oil in the furnace would result in time-varying aerosol composition due to preferential distillation of the low-boiling components. At low flows the nebulizers tested were unable to generate a fine mist or even maintain a steady flow due to surface tension effects at the mixing point. The solution proved to be a combination of high dilution of the oil in a volatile carrier solvent plus an inefficient nebulizer that deposited most of the oil as large droplets that impacted in the knockout flask. Two different Sherba (New Port Richey, FL) Model 55007 ceramic nebulizers, sold for use on ICP instruments was used. The performance of the two nebulizers was not identical but similar aerosol generation was achieved by adjusting the motive argon flow in the range from 1-2 lpm. The minimum liquid flow for good operation was about 100 microliters/minute, and this value was used thereafter. Oil accumulated in the nebulizer leading to loss of the fine mist but periodic cleaning in hexane controlled this problem. To achieve the desired oil flow rate while meeting nebulizer minimum liquid flow the oil was dissolved 1:400 in carrier solvent. Hexane was used for initial testing but material balance indicated that the resulting hexane in the inhalation chamber would be higher than the occupational exposure limit for humans. Ethyl alcohol was considered preferable since it can be water scrubbed and is metabolized but motor oil does not dissolve in ethyl alcohol. A mixture of 1 part used motor oil, 9 parts hexane, and 390 parts ethyl alcohol was found to form a stable solution and was used for generating the inhalation study aerosol.

The large droplets formed by the nebulizer were inertially deposited in the knockout flask but 1-4% of the aerosol volume in the form of fine mist exited the flask and mixed with the main carrier gas flow at the inlet to the Lindberg/Blue M (Ashville, NC) tube furnace. Calculations showed that the residence time and wall temperature were sufficient to completely vaporize the oil - solvent mixture. Aerosol begins to form as the gas cools downstream of the center of the furnace and an ultrafine aerosol could be detected when sampling the exit of the tube furnace. However, at the low vapor concentrations needed to form ultrafine oil aerosol the characteristic time for particle growth by molecular condensation is significant. Addition of an aging flask downstream of the furnace showed that both the number of particles larger than the detection limit of the SMPS and the total volume of aerosol increased on the scale of 30-60 seconds.

The system was designed to allow addition of medical air dilution of the mixture immediately downstream of the furnace. The 390 parts of ethyl alcohol resulted in a concentration in the inhalation chamber that would have physiological effects so a glass impinger was added to scrub the alcohol. An ice bath was used to both decrease the equilibrium partial pressure of alcohol over the scrubbing water and to minimize moisture entering the inhalation chamber. A second dry impinger provided rewarming of the gas which avoided downstream condensation. The oxygen content in the inhalation chamber was maintained at 21% by addition of oxygen in the tubing tee upstream of the inhalation chamber. The inhalation chamber was maintained under slight (1-2 mm H₂O) negative pressure by adjusting a valve leading to a vacuum vent.

During the inhalation exposure the 5 ml syringe was refilled, the scrubber water (250 ml) was replaced, and the nebulizer was cleaned at 45 minute intervals. Practice runs had demonstrated that this periodic servicing produced a consistent aerosol and gas-phase

composition. The inhalation exposure used the same equipment and procedures as used for previous studies in Dr. Oberdörster's laboratory. The inhalation chamber was a 30 liter Plexiglas gasketed box with distribution manifolds on the inlet and exit. The rats were in an eight-compartment cage which was placed inside the 30 liter box. The procedures and results of the inhalation experiment are reported separately.

Results

Hundreds of SMPS scans were collected during the iterative development of the aerosol generator. These data have been archived and will be reviewed when writing the planned aerosol engineering paper on ultrafine organic aerosol generation. This report focuses on the final aerosol generator configuration used for the inhalation studies.

During preliminary demonstration runs the final aerosol generator configuration was shown to be able to produce a well-defined particle number distribution between 30-100 nm with a count median diameter (CMD) between 40-50 nm. The concentration in the 30 l inhalation chamber was $1-2 \times 10^6 / \text{cm}^3$ and the integrated volume from 13 to 789 nm was $2.8 \text{ nm}^3/\text{cm}^3$ which is about $200 \mu\text{g}/\text{m}^3$.

A testing program quantified the effect on particle size and concentration of changes in each of the operating variables. The key variable for system performance was the amount of oil entering Furnace #2. This is controlled by 1) The nebulizer cleanliness, 2) The nebulizer gas flow, Q2, 3) The concentration of the working solution of oil and dilutant. I gave up adjusting the syringe flow since there is a minimum liquid flow needed to avoid rapidly plugging the nebulizer. The two Sherba ceramic nebulizers used were not identical and gas flow Q2 had to be adjusted if the nebulizer is switched.

Experiments showed that the system performance was moderately sensitive to: Q1-main argon flow, Q3-downstream air dilution, system back pressure, and aerosol age. Changing the Q1-main argon flow over the range from 1 to 9 lpm showed that aerosol count median diameter increased with both increases and decreases in Q1 from the nominal 4 lpm setpoint. This was tested at both constant total flow Q1 + Q3 downstream dilution and at constant ratio of oil per volume of main flow. Adding dilution air downstream of Furnace 2 decreased both aerosol number concentration and volume concentration, however the decrease was more than expected by material balance suggesting that the particles are volatile and that changes in gas conditions can cause particle loss by evaporation. Similarly addition of a cascade impactor between Furnace 2 and the inhalation chamber caused a reproducible decrease in aerosol which is suspected to be due to the pressure change. As expected from theory, the aerosol was sufficiently concentrated that particle coagulation was significant on the scale of 1-5 minutes. This resulted in the mixed chamber aerosol being slightly larger and at lower number concentration than the aerosol measured at the chamber inlet.

Experiments showed that the system performance was relatively insensitive to: choice of dilutant, presence of nuclei at high oil concentration, T2- furnace temperature, and type of oil. Both hexane and ethanol were used as dilutants and the aerosol change was within normal run-to-run variation. Addition of NaCl nuclei had no effect on the aerosol size at the high oil feed rates and high furnace temperatures used to generate the diesel surrogate aerosol. However at

conditions where less oil was vaporized (furnace temperature below 300 C, diluted oil/hexane) no ultrafine aerosol was detected until NaCl nuclei were added. The combination of the low concentration oil vapor and nuclei resulted in a size and volume increase above that measured for the nuclei alone. At the nominal setpoints used, changes in Furnace 2 temperature over the range from 300 – 400 C had no effect on aerosol size distribution beyond run-to-run variation. This was attributed to all oil being vaporized at the operating conditions.

At times, the particle volume distribution showed a large-particle tail. However impactor sampling showed that most of the mass was below 400 nm, consistent the SMPS data plotted as volume. Figure 2 shows the differential and cumulative size distribution from a typical impactor sample. Incidentally, the impinger used for alcohol scrubbing also was able to remove particles $D > 2.5 \mu\text{m}$ by inertial impaction. For a 3-hour demonstration run on November 24, the aerosol mass computed from the average of the SMPS measurements using specific gravity 1.0 spheres was 307 microgram/ m^3 compared to 281 microgram/ m^3 measured by weight change on Stages 6, 7 and filter of the Intox impactor run during the same time. This is excellent agreement considering the many assumptions in the calculations.

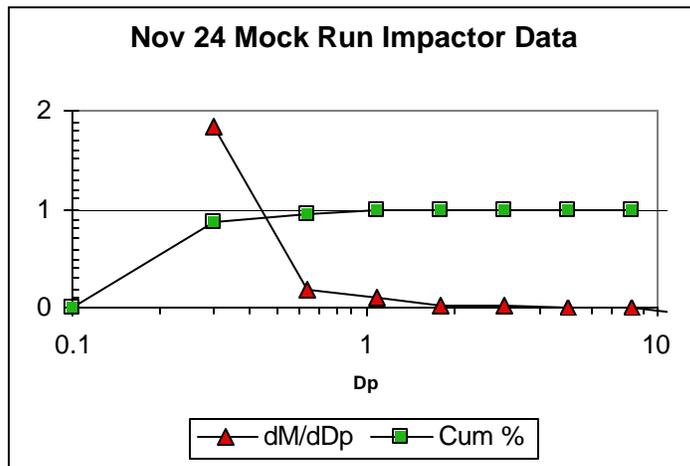


Figure 1. Cascade impactor results show the most of the aerosol in the inhalation chamber was in submicron particles smaller than 400 nm.

The addition of the impinger to remove ethyl alcohol was shown by gas chromatography analysis to result in 98-99% scrubbing which reduces the alcohol concentration from over 6000 ppm to under 100 ppm. The occupational exposure limit is 1000 ppm. The impinger was kept on ice since the equilibrium vapor pressure of ethyl alcohol over water increases rapidly with temperature decreasing scrubbing efficiency. Ultrafine aerosol is lost but measurements with and without the impinger showed this loss to be acceptable while still meeting the target aerosol concentration in the chamber.

The testing results were used to finalize the operating conditions for the inhalation experiments and to develop an operating and troubleshooting procedure. Repeated practice runs verified that the generator produced a consistent aerosol from day to day. Figure 3 shows day to day reproducibility. Operating parameters were set to the same nominal values each day and a SMPS scan was taken with no further adjustments.

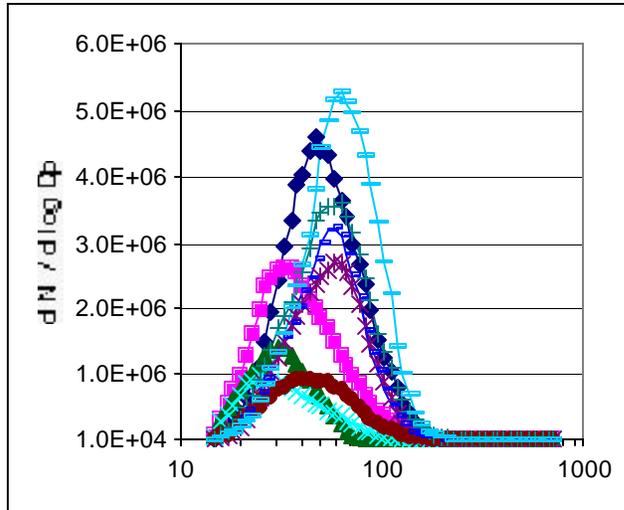


Figure 3. Reproducibility of aerosol generation comparing data from November 12, 16, 17, 18, and 19, 2001.

The preliminary runs showed that an acceptable aerosol could be reliably generated and the 6-hr inhalation experiment with oil aerosol was scheduled for December 3, 2001. The data show that the animal exposure study met the aerosol criteria that had been set based on the Science Advisory Committee priority recommendation. The exposure went as planned starting at 9:55 AM and ending at 3:55 PM. The number and volume distributions are shown graphically in Figures 4 and 5. The SMPS statistics for the exposure period from 10:10 to 3:55 (excluding transient after opening the chamber to insert the rats) were as follows:

	Average	Standard Dev	Units
Count Median	39.9	3.5	nm
Geometric Standard Deviation	1.46	.02	
Integrated Number Conc.	1.13 E6	0.3 E 6	N / cm ³
CPC Direct Number Count	2.84 E6	0.5 E6	N / cm ³
Volume Median	100.9	10	nm
Integrated Volume	1.36 E 11	0.5 E11	nm ³ / cm ³

The systematic difference in total number concentration between the number computed by TSI software from the SMPS readings and the total measured by a CPC directly has also been noted in previous work, but the issue remains unresolved.

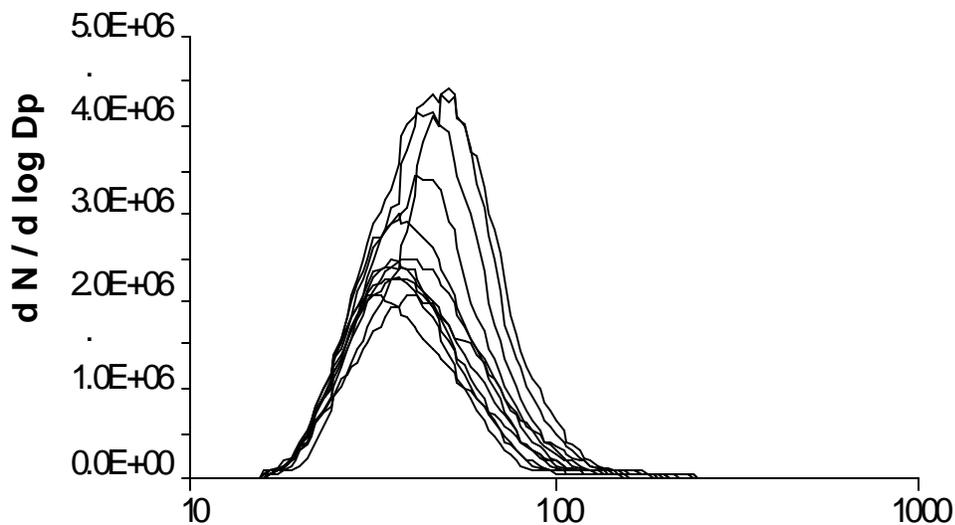


Figure 4. Number distribution at 20 minute intervals during inhalation exposure.

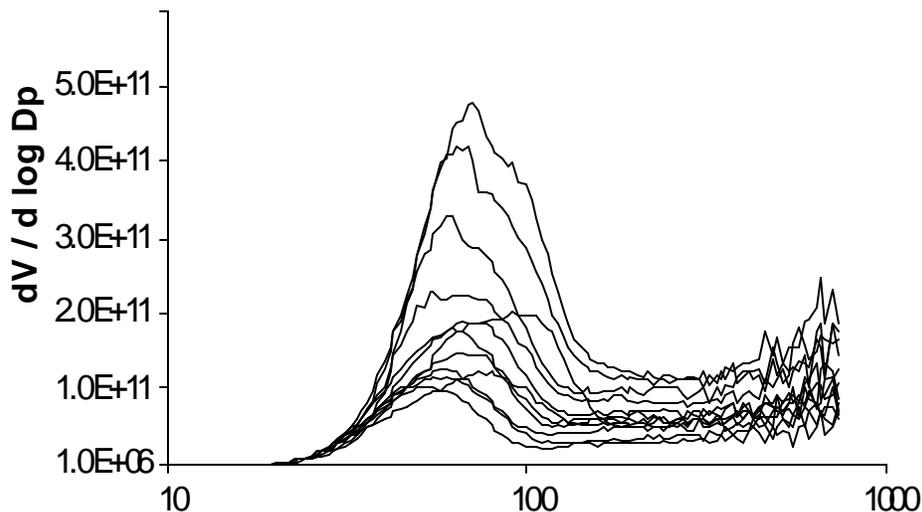


Figure 5. Volume distribution at 20 minute intervals during inhalation exposure.

In addition to the preliminary runs leading to the inhalation studies, experiments were conducted to obtain ultrafine organic particle generator characterization data for use in an aerosol engineering publication and for planning future exposure studies.

The generator was tested with different organic feeds and was able to produce similar aerosol size distributions using the “used motor oil,” clear paraffin oil, and triacontane (C₃₀H₆₂) dissolved in hexane. This indicates that the aerosol is not dependent on unique properties of the used motor oil, such as trace contaminants serving as nuclei. Based on both calculations from aerosol theory and these demonstration experiments it is likely that other low volatility hydrocarbons can be made generate an ultrafine aerosol with appropriate adjustment of operating setpoints.

The particle generator produced larger median diameter particles as the total amount of aerosol increased, which is consistent with aerosol formation theory. This data is useful both for planning exposure studies and for comparing actual system performance with theory. A separate series of experiments were run in triplicate to quantify the relationship between size distribution and total aerosol mass concentration.

Discussion

A particle generator was assembled and used to do a controlled exposure of rats to an organic carbon aerosol that is a surrogate for the organic component of internal combustion engine tailpipe emissions. The design was essentially the first thing that worked and further optimization is possible. A material balance calculation showed that the concentration of oil aerosol produced by the particle generator is approximately what would be found from an engine that emitted one quart of unburned oil smoke per 10,000 miles. The average mass concentration of the oil aerosol in the inhalation chamber, calculated from SMPS data assuming 0.8 S.G. spheres, was 110 $\mu\text{g}/\text{m}^3$ which is less than twice than the PM_{2.5} air quality standard. The aerosol generator seems to have a minimum size for the organic aerosol. The particles derived from the used motor oil are relevant to real-world exposures but allow testing of the effect of ultrafine organic particles free from the soot, metal oxides, and gas-phase pollutants found in engine exhaust.

The ability of the aerosol generator to work with both complex mixtures such as used motor oil and with single-component hydrocarbons suggests that future studies can be designed using a range of environmentally-relevant organic aerosols. This would include controlled mixtures of relatively benign aliphatic hydrocarbons with known biologically active species such as polycyclic aromatic hydrocarbons (PAH). Further, the condensation organic particle generator can be combined with a nuclei generator to create mixtures of organic compounds and carbon or metal oxides. This opens the way to a reductionist approach to studying the toxicology of engine emissions by hypothesis-driven inhalation studies of single components and of mixtures of components found in real engine exhaust.

An unresolved issue is the lower limit of organic particles that can be generated by a vaporization-condensation generator. The relevance is that diesel engines have been reported to generate a particle mode around 10 nm, which is smaller than was achieved in this study. [7, 9] As the organic feed rate is decreased the particle size decreases until the CMD is 25-30 nm then the size stays nearly constant but the particle number drops. The relative importance of vapor pressure (Kelvin effect) and mass transfer kinetics has not been fully evaluated. This issue will be quantitatively discussed in the aerosol engineering publication planned for later in 2002.

Citations

1. Hitchins, J., L. Morawska, R. Wolff, and D. Gilbert, Concentrations of submicrometre particles from vehicle emissions near a major road. *Atmospheric Environment*, 1999. **34**: p. 51-59.

2. Kittelson, D.B., Engines and nanoparticles: a review. Journal of Aerosol Science, 1998. **29**(5/6): p. 575-588.
3. Harrison, R.M., M. Jones, and G. Collins, Measurements of the physical properties of particles in the urban atmosphere. Atmospheric Environment, 1999. **33**: p. 309-321.
4. Harrison, R.M., P. Brimblecombe, R.G. Derwent, G.J. Dollard, S. Eggleston, R.S. Hamilton, A.J. Hickman, C. Holman, D.P.H. Laxen, and S. Moorcroft, Airborne Particulate Matter in the United Kingdom, University of Birmingham, UK, Third Report of the Quality of Urban Air Review Group, Department of the Environment, 1996
5. HEI, Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects, Health Effects Institute, 1995
6. Morawska, L., N.D. Bofinger, L. Kocis, and A. Nwankwoala, Submicrometer and supermicrometer particles from diesel vehicle emissions. Environmental Science and Technology, 1998. **32**(14): p. 2033-2042.
7. Abdul-Khalek, I.S., D.B. Kittelson, B.R. Graskow, Q. Wei, and F. Brear, Diesel Exhaust Particle Size: Measurement Issues and Trends, Society of Automotive Engineers, 980525, 1998
8. Shi, J.P. and R.M. Harrison, Investigation of ultrafine particle formation during diesel exhaust dilution. Environmental Science and Technology, 1999. **33**(21): p. 3730-3736.
9. Abdul-Khalek, I., D. Kittelson, and F. Brear, The influence of dilution conditions on diesel exhaust particle size distribution measurements, Society of Automotive Engineers, 1999-01-1142, 1999
10. Brown, J.E., M.J. Clayton, and D.B. Harris, Comparison of the particle size distribution of heavy-duty diesel exhaust using a dilution tailpipe sampler and an in-plume sampler during on-road operation. Journal of the Air & Waste Management Association, 2000. **50**: p. 1407-1416.
11. Kleeman, M.J., J.J. Schauer, and G.R. Cass, Size and composition distribution of fine particulate matter emitted from motor vehicles. Environmental Science and Technology, 2000. **34**(7): p. 1132-1142.
12. Tobias, H.J., D.E. Beving, P.J. Ziemann, H. Sakurai, M. Zuk, P.H. McMurry, D. Zarling, R. Waytulonis, and D.B. Kittelson, Chemical analysis of diesel engine nanoparticles using a nano-DMA / thermal desorption particle beam mass spectrometer. Environmental Science and Technology, 2002. **In Press**.
13. Baggs, R.B., J. Ferin, and G. Oberdörster, Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. Vet. Pathol., 1997. **34**(6): p. 592-597.
14. Hinds, W.C., Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles. 1982, New York: John Wiley & Sons.

15. Smith, J.M. and H.C. Van Ness, Introduction to Chemical Engineering Thermodynamics. 1987, New York: McGraw-Hill.
16. Reid, R.C., J.M. Prausnitz, and B.E. Poling, The Properties of Gases and Liquids. Fourth ed. 1987, New York: McGraw Hill.
17. Fuchs, N.A. and A.G. Sutugin, "The Generation and Use of Monodisperse Aerosols", in Aerosol Science, C.N. Davies, Editor. 1966, Academic Press: New York. p. 1-30.
18. Willeke, K., ed. Generation of Aerosols and Facilities for Exposure Experiments. 1980, Ann Arbor Science: Ann Arbor, MI.
19. Chen, B.T. and W. John, "Instrument Calibration", in Aerosol Measurement: Principles, Techniques, and Applications, P.A. Baron and K. Willeke, Editors. 2001, Wiley: New York.
20. Husar, R.B., Coagulation of Knudsen Aerosols, Ph.D. Thesis, University of Minnesota, 1971
21. Bartz, H., H. Fissan, and B.Y.H. Liu, A new generator for ultrafine aerosols below 10 nm. Aerosol Science and Technology, 1987. **6**: p. 163-171.
22. Ristovski, Z.D., L. Morawska, and N.D. Bofinger, Investigation of a modified Sinclair-LeMer aerosol generator in the submicrometer range. Journal of Aerosol Science, 1998. **29**(7): p. 799-809.
23. Rulison, A.J. and R.C. Flagan, Electrospray atomization of electrolytic solutions. Journal of Colloid and Interface Science, 1994. **167**: p. 135-145.
24. Gomez, A., D. Bingham, J. de la Mora, and K. Tang, Production of protein nanoparticles by electrospray drying. Journal of Aerosol Science, 1998. **29**: p. 561-574.
25. Rapaport, E. and S.E. Weinstock, A generator for homogeneous aerosols. Experientia, 1955. **11**: p. 363-364.
26. Barrett, J.C. and T.J. Baldwin, Aerosol nucleation and growth during laminar tube flow: Maximum saturations and nucleation rates. Journal of Aerosol Science, 2000. **31**(6): p. 633-650.
27. Koch, W., H. Windt, and N. Karfich, Modeling and experimental evaluation of an aerosol generator for very high number currents based on a free turbulent jet. Journal of Aerosol Science, 1993. **24**(7): p. 909-918.
28. Scheibel, H.G. and J. Porstendörfer, Generation of monodisperse Ag- and NaCl-aerosols with particle diameters between 2 and 300 nm. Journal of Aerosol Science, 1983. **14**(2): p. 113-126.