DEVELOPMENT OF AN EXPOSURE FACILITY TO CONDUCT INHALATION STUDIES TO AMBIENT AEROSOLS

Final Report

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ABSTRACT

Particles in the atmosphere are a complex and heterogeneous mixture that have been difficult to reproduce in the laboratory. As a result, scientists have not been able to conduct toxicological and clinical experiments that replicate realistic conditions in the environment. Investigators have typically generated synthetic atmospheres that differ in significant ways from the true environment. This has made it difficult to address unanswered questions about the true nature and mechanisms of action of atmospheric particles (PM) on human health.

To study the health effect of PM in a realistic setting we designed, built and evaluated a Versatile Aerosol Concentration System (VACES) for testing the toxicological significance of concentrated atmospheric aerosols in animals. The system has been designed for conducting animal exposure studies, but it can be readily scaled-up for human exposures. This report describes the development and bench-testing of a VACES capable of simultaneously concentrating ambient particles of the coarse, fine and ultrafine size fractions for conducting *in vivo* and *in vitro* exposure studies to "real" ambient aerosols over a wide dynamic range of concentrations. The VACES consists of three parallel sampling lines (concentrators), each operating at an intake flow rate of 110 LPM. Coarse particles are concentrated using a single round nozzle virtual impactor. Concentration enrichment of $PM_{2.5}$ and ultrafine particles is accomplished by first drawing air samples through two parallel lines, having 2.5 and 0.18 μm cutpoint pre-impactors, respectively, to remove particles larger than these sizes from the air sample. Both of the smaller PM fractions are drawn through a saturation-condensation system that grows particles to 2-3 μm droplets, which are subsequently concentrated by virtual impaction. A diffusion dryer is used in the fine and ultrafine concentrators to remove excess vapor and return the concentrated particles to their original size, prior to supplying them for *in vivo* exposures. The VACES can also provide highly concentrated liquid suspensions of particles of these three modes for *in vitro* toxicity studies. This is accomplished by connecting the concentrated output (minor) flows of each of the VACES parallel concentrators to a liquid impinger (BioSampler), used in a modified configuration, to collect particles under near-ambient pressure.

Detailed laboratory characterization of the individual components of the VACES are presented in this paper, including evaluation of its ability to preserve particle mass, number, and chemical species during the concentration enrichment process. The experimental characterization of the VACES demonstrated that concentration enrichment is accomplished with very high efficiency, minimal particle losses and without any dependence on particle size or chemical composition.

During the field evalution of the VACES, the enrichment and preservation of ambient ultrafine, fine and coarse particles by size and chemical composition is determined by comparisons made between the VACES and a co-located multistage MOUDI impactor, used as a reference sampler. Furthermore, preservation of the ultrafine fraction is measured by the enrichment based on ultrafine particle numbers, morphological characteristics as well as their elemental carbon (EC) content. The results suggest that the concentration enrichment process of the VACES does not differentially affect the particle size or chemical composition of ambient PM. The following fractions: 1) mass (coarse and fine PM); 2) number (ultrafine PM); 3) sulfate (fine PM); 4) nitrate (fine PM, after correcting for nitrate losses within the MOUDI); 5) EC (ultrafine PM); and 6) selected trace elements and metals (coarse and fine PM), are concentrated very close to the "ideal" enrichment value of 22 – thereby indicating a near 100% concentration efficiency for the VACES. The field results also suggest that volatile species, such as ammonium nitrate, are also preserved throughout the supersaturation and concentration-enrichment processes. Furthermore, ultrafine particles are concentrated without substantial changes in their compactness or denseness, as measured by fractal dimension analysis.

EXECUTIVE SUMMARY

The goal of this investigation was to design, build and install a mobile particle concentrator for testing the toxicological significance of concentrated atmospheric aerosols. This system, which we have named Versatile Aerosol Concentration Enrichment System (VACES) is the first capable of concentrating ultrafine, fine and coarse particles. Particle concentration enrichment is accomplished by means of virtual impaction, using well characterized, and single-nozzle virtual impactors. The portable concentrators were designed for use primarily in animal inhalation studies since these systems are compact. In addition, their modular design makes them readily adaptable to accommodate higher output flow rates that are required for human exposures.

Extensive proof-of-concept testing was conducted during the period covered by this report in order to determine any influence of the process and system of concentrators on the physical or chemical properties of ambient aerosols. These proof-of-concept studies were conducted at UCLA and USC. Particle size and chemical composition of the concentrated aerosols was compared to ambient aerosol at the concentrator inlet to ensure that no substantial distortion in the physico-chemical characteristics of PM occurs during the concentration enrichment process. The characterization of the coarse, fine and ultrafine concentrators for animal exposures has been completed ahead of schedule allowing us to initiate toxicological studies, starting in late June 2000, prior to the second year contract. The particle concentrator completed under this contract can be transported to various locations to take advantage of regional variation in particulate air quality, populations of interest, and to coordinate with ongoing field studies of air quality. At these sites extensive animal toxicology and human clinical studies will be undertaken to provide further understanding of the relationship and mechanism between adverse health effects and exposure to airborne particulate matter.

We are currently ahead of schedule defining *in vitro* and *in vivo* toxicological experiments using the concentrator. The advanced schedule has been facilitated by rapid development and validation of a new generation of more portable and versatile concentrators. These new concentrators can also be scaled up to accommodate the higher output flows that are desirable in conducting human exposure studies. This scale-up is easily achieved by placing several of the single-nozzle virtual impactors in parallel. While the design and construction of these larger systems is not part of our activities covered by this contract, this will be pursued in future research.

In our original scope of work, we proposed to enclose the entire concentrator facility, including the exposure chambers, in a 4 m x 4 m x 5 m shipping container, so that it could be transported to different locations within the greater Los Angeles Basin. We substantially revised that plan and decided to build the

mobile concentrator facility in a trailer, as this design makes it even easier to transport to other locations, with minimum installation and/or dismantling time. or all of the coarse, fine and ultrafine size fractions of PM. A system was developed for particle collection for *in vitro* analysis. This collection is The Versatile Aerosol Concentration Enrichment Systems VACES that we developed are substantial technological improvements over the originally proposed Harvard Ambient Fine Particle Concentrators. The VACES portable concentrators are capable of enriching the concentration of particles in the entire range of 0-10 μm by a factor up to 40, depending on the output flow rate. These systems are very compact in size and modular in design.

There are several advantages to using the new, portable concentrators compared to the older version of Harvard concentrators. First, Harvard concentrators focus mainly on concentrating the accumulation mode of ambient PM, e.g., $PM_{2.5}$ without its ultrafine or coarse PM component. These concentrators are bulky and not easily transportable as they require placement inside a large trailer. They require a considerable amount of electric power; and the blower that drives the major flows of the virtual impactors requires a three-phase, 30-amp current. The concentration enrichment depends on particle size, with larger particles in the accumulation mode being concentrated in general more effectively than smaller particles. Under certain conditions, the performance of the Harvard concentrators becomes unstable during operation. Typical indications of instabilities are abrupt increases in pressure drop across the slit nozzles of the virtual impactors, followed by a sharp decrease in the concentration enrichment factor. These problems have been observed under conditions of high particle concentration and/or when operating these systems in days with high humidity and temperature.

The VACES consists of three parallel sampling lines (concentrators) that separately sample ambient coarse, fine and ultrafine aerosols, each at 110 LPM. The fine and ultrafine fractions are separated from the air sample and drawn through a supersaturation and condensational growth system. All fractions (i.e., size-selected and enlarged fine and ultrafine, and ambient coarse) are subsequently concentrated with a virtual impactor. The number/mass concentration may be enriched by a factor as great as 33, which is, ideally, determined as a function of the ambient inlet flow rate to the minor flow-rate of the virtual impactor (typically between 3.3 and 10 LPM, depending on the desirable configuration). In the experiments described in this field study, the minor flow of each concentration-enrichment sampling line of the VACES was set at 5 LPM, thereby resulting in an ideal concentration enrichment factor of 22for coarse, fine and ultrafine aerosols.

The VACES has been designed to simultaneously conduct *in vivo* and *in vitro* exposures to concentration-enriched ambient particles of either one, accomplished by connecting a modified liquid impinger (BioSampler™) to each

of the minor flows of the coarse, fine and ultrafine portable concentrators, respectively. Highly concentrated aqueous suspensions can thus be obtained, which can be readily used for exposing cell cultures to ambient particles of all three modes. This direct particle collection also eliminates uncertainties related to incomplete extraction from filter media and preserves the biologically active components of the collected PM.

The ability of the VACES to concentrate particles was first tested in laboratory experiments using different type of particles in the size range of 0.05-1.9 μm and at three minor flow rates of two 7, 10, and 20 LPM with the total intake flow rate of 220 LPM. The enrichment factors based on number concentrations were close to the ideal values. Hygroscopic aerosols, such as ammonium sulfate and ammonium nitrate were concentrated as effectively as hydrophobic PSL particles.

The experimental characterization of the VACES demonstrated the concentration enrichment does not depend on particle size or chemical composition. Volatile species such as ammonium nitrate are preserved through the concentration enrichment process under the laboratory conditions used in this study.

Field characterization of the VACES was conducted outdoors in the facilities of Rancho Los Amigos National Rehabilitation Center in south-central Los Angeles. The coarse, fine and ultrafine particle concentrations of the VACES were compared to direct concurrent measurements made with a co-located MOUDI. Comparisons between the VACES and MOUDI for coarse and fine PM are based on particle mass, sulfate, nitrate and selected trace element and metal concentrations. For ultrafine PM (aerodynamic diameter smaller than a 0.18 μm), the VACES number concentrations is compared to those of a co-located Condensation Particle Counter, whereas the preservation and concentration enrichment of the elemental carbon (EC) content is determined by comparing VACES concentrations to those of the MOUDI within this size-fraction.

Results from the field study indicated that concentration enrichment is differentially affected by particle size or chemical composition. For either coarse or fine particles, the concentration enrichment factors based on mass, sulfate, nitrate after correcting for nitrate losses of the MOUDI, and selected trace elements and metals were very close to the ideal enrichment value of 22. The experiments, additionally, indicated that volatile species such as ammonium nitrate are preserved throughout the concentration enrichment process. Furthermore, concentration enrichment obtained for ultrafine particle counts suggests that no particle coagulation occurs during the enrichment process. Finally, ultrafine EC concentrations obtained with the VACES were about 22 times higher than those obtained with the MOUDI, thereby indicating that ultrafine PM are concentrated without loss, with a nearly 100% collection efficiency by this system. In addition, detailed morphological examination of

ambient and concentrated ultrafine particles indicated that ultrafine particles are concentrated without substantial changes in their compactness or denseness.

The ability of the VACES to enrich the concentrations of all particles in the fine mode including its ultrafine particle component enables inhalation toxicologists to conduct exposures to any selected sub-range of PM2.5. For example, previous studies in California showed the presence of two sub-modes within the accumulation mode of ambient PM; one mode peaks at around 0.2 μm consisting mainly of gas-to-particle reaction products, such as carbonaceous PM and the other peaks at about 0.7 μm mainly associated with hygroscopic PM such as ammonium sulfate and nitrate. These observations have been confirmed by our recent yearlong measurements at the facility of Rancho Los Amigos in Downey. By placing a conventional impactor (with a 0.4 μm cutpoint) upstream of the fine concentrator of the VACES, inhalation studies could be conducted to ultrafine PM plus the elemental and organic carbon content of the accumulation mode, but without the majority of the larger sulfate and nitrate constituents.

In addition to the animal and human exposures, we will also use the newly developed versatile concentrators for direct PM collection for *in vitro* tests. Collection and chemical characterization of coarse, fine and ultrafine particles for *in vitro* tests using the combined concentrator/BioSampler method will be conducted at the PIU locations as well as at the sites where animal exposures to freeway-originated aerosols are planned. We have already initiated these studies, by collecting PM samples using the VACES at UCLA and at Rancho Los Amigos. In our current sampling scheme, outdoor particles are collected concurrently with human exposure studies. In addition to the biological content of ambient or indoor PM, we also monitor the following parameters over the 6 hours of the experiment: particle number concentration (continuously) and particle mass concentration (time-integrated).

We have made considerable progress in using the VACES for animal inhalation studies in two different locations in the Los Angeles Basin. These studies were not part of the originally proposed scope of work but have been made possible by the rapid development of VACES. The studies were conducted jointly by investigators from UCLA, University of Southern California, UC Irvine and UC Davis. Healthy rats (and in a later summer study, sensitized mice) were exposed to fine and ultrafine PM, concentrated by a factor of 22, harvested at UCI in June 2000 and UCLA in July 2000 in west Los Angeles. Preliminary measurements in the later location have indicated an unusually high number concentration of both ambient as well as indoor PM on the order of 10,000-50,000 particles/cm^3, roughly 5-10 times higher than levels typically encountered in urban areas of the East Coast of the U.S., which makes these experiments of particular interest. The same particle sampling protocol used for the *in vitro* tests was also used to monitor the physico-chemical PM characteristics during the animal exposure studies: monitoring of particle mass, number concentration, elemental

composition and selected PAH. Biological analysis of the animals exposed in these studies is currently under way.

1. Introduction

The goal of this investigation was to design, build and install a mobile particle concentrator exposure facility at UCLA for testing the toxicological significance of concentrated atmospheric aerosols. This facility is the first capable of concentrating ultrafine, fine and coarse particles. Extensive proof of concept testing was conducted during the period covered in this report to determine any influence of the process and system of concentrators on the physical or chemical properties of ambient aerosol. Particle size and chemical composition of the concentrated aerosols was compared to ambient aerosols at the inlet to ensure that no substantial distortion in the physico-chemical characteristics of PM occurs during the concentration enrichment process. The characterization of the coarse, fine and ultrafine concentrators for animal exposures has been completed ahead of schedule and we have initiated toxicological studies, which began in June 2000. The completed particle concentrator is transportable to take advantage of regional variation in particulate air quality, populations of interest, and to coordinate with ongoing field studies of air quality. Extensive animal toxicology and human clinical studies will be undertaken to provide further understanding of the relationship and mechanisms of adverse health effects associated with exposure to PM. We are ahead of schedule defining *in vitro* and *in vivo* toxicological experiments using the concentrator. The advanced schedule has been facilitated by rapid development and validation of a new generation of more portable and versatile concentrators.

This report addresses the period between June 1, 1999 and August 31, 2000. During this period we were awarded two additional grants from EPA which led to creation of the Southern California Particle Center and Supersite (SCPCS). The support from the California Air Resources Board has been crucial in our successful competition for federal funds to develop a major particle center in Southern California, and we are wholly dependent on ARB support for the successful development of the range of activities in SCPCS.

2. Construction of Concentrator Trailer and Set-Up of Related Aerosol Instrumentation

In our original scope of work, we proposed to enclose the entire concentrator facility, including the exposure chambers, in a 4 m x 4 m x 5 m shipping container, so that it can be transported to different locations within the greater Los Angeles Basin. We revised that plan to house the mobile concentrator facility housed in a trailer, since this design would make it easier to transport to other locations with minimum installation and/or dismantling time. A trailer would

not require special transportation permits that are required for shipping and transporting containers.

Two trailer laboratories were constructed that can be towed with a 3/4-ton pickup truck to various sites. The two trailers were ordered from Wells-Cargo, Inc. for February 1, 2000 delivery. One trailer is 32' X 8' for the concentrator and the other is 20' X 8' for a Particle Instrumentation Unit (PIU) to be used for PM physicochemical characterization. The latter trailer has been purchased and equipped through funding from the SCPCS.

The concentrator trailer has two separate compartments, partitioned by a plywood bulkhead; one compartment is 12' X 8' and will be used for all concentrator-related apparatus. The other compartment will be used for equipment related to the animal studies, including nose only and whole-body animal exposure chambers, as well as for an animal vivarium to store the animals for the exposures that will be conducted at locations other than UCLA. Both compartments of the exposure trailer are air-conditioned.

We have received and calibrated all major pieces of sampling equipment and direct reading instruments being used to characterize the concentrated aerosols. These include a Tisch Environmental Hi-Vol sampler with a PM-10 inlet, a TSI Aerodynamic Particle Sizer (APS), three rotating-versions of the MSP ten-stage Microorifice cascade impactors (MOUDI), and a MIE DataRAM. In addition, the TSI Scanning Mobility Particle Spectrometer (SMPS) has been received. These instruments were purchased through the SCPCS. They have been used to evaluate the performance of the concentrators and will be also used to provide data on ambient and concentrated aerosol characteristics during human and animal exposures.

We have also completed the construction of the whole-body exposure chambers for human inhalation studies. The single-person exposure chamber is a plywood-and-Plexiglas whole-body plethysmograph modified by extending the lower front wall to form a foot well, in which a small cycle ergometer can be placed. The straight 7.5-cm stainless steel outlet pipe from the particle concentrator enters the chamber at the chest height of a seated subject. The inlet pipe is interrupted by a demountable butt joint to permit disassembly of the system for cleaning. The inlet and outlet ports of the concentrated aerosol are designed such that the exposure atmosphere exits the chamber through multiple ports above and behind the subject's head. Further details of the whole-body human exposure chamber are given in Gong et al. (1999).

We have also received sampling equipment and direct reading instruments for gaseous pollutants. These instruments were provided in-kind by the Biological Effects Research Section of the California Air Resources Board. These monitors include a Continuous Chemiluminescence Analyzer (Monitor Labs Model 8840)

for nitrogen oxides measurement, Thermo Environmental Inc. Model 48C trace level carbon monoxide analyzer and a UV photometer (Dasibi Model 1003 AH) for measurement of ozone. The instruments were installed in the PIU trailer and calibrated.

3. Development and Characterization of the Coarse, Fine and Ultrafine Particle Concentrator for Animal Exposure and *In Vitro* Studies

3.1. Operating Principle of Particle Concentrators

Concentration enrichment of particles larger than a critical size (herein referred to as the cutpoint of a concentrator) is accomplished by means of virtual impaction. Particles are drawn through a nozzle gradually decreasing diameter and become accelerated to a high velocity, the magnitude if which depends on the cutpoint of the virtual impactor (higher velocities are required for smaller cutpoints). Immediately downstream of the acceleration nozzle, the majority of the airflow (herein referred to as the "major" flow) is deflected around a probe placed within few mm from the exit of the acceleration nozzle and in perfect alignment with the acceleration nozzle. A small portion of the original total air volume (typically 3-10%, also called "minor" flow) is diverted into the collection probe, and along with it particles that have acquired sufficient momentum to cross the deflected air streamlines. These particles are concentrated ideally by the ratio of the total-to-minor flow rates. Thus diverting the particles into a minor flow 5% of the total flow entering the virtual impactor would ideally concentrate particles larger than the cutpoint by a factor of 20. The concentration enrichment of a virtual impactor can be adjusted by adjusting the minor-to-total flow ratio.

A major advantage of virtual impactors is that they accomplish particle concentration enrichment while keeping the particles airborne, i.e. without collecting the particle on a filter or any other substrate. These concentrated and airborne particles could subsequently be supplied to exposure chambers for human or animal inhalation studies with minimum distortions in their physical, chemical and morphological characteristics and their gaseous copollutants equilibrium.

3.2 Justification for Changing the Design of the Concentrators

We had originally proposed to install Harvard Ambient Fine Particle Concentrators in the first year of this program (Sioutas et al., 1995; Sioutas et al., 1997). Our original plan was to develop improved coarse and ultrafine particle concentrators in subsequent years. We had initially projected installation of the fine particle concentrator around the second week of December 1999. That installation was postponed to the second or third week of March 2000. In a subsequent communication with Dr. Petros Koutrakis (Harvard School of Public Health), we were informed because of construction problems relating to quality

control difficulties in the machining and alignment processes of the slit-nozzle virtual impactors, Harvard could not commit to any delivery time prior to late June 2000. Harvard would not provide any assurances that even this late delivery would be met.

This delivery time was unacceptable, since it substantially delayed our proposed health studies to concentrated PM, which are major foci of our ARB as well as our PM Center programs. We therefore requested ARB's approval to a change in our research direction. We decided not to proceed with the Harvard fine particle concentrator for this program. Instead, we used the new and improved portable concentrators (described in section 3.2) that we have developed over the past two years. These portable concentrators are based on technologies already developed and published (Sioutas et al., 1999; Kim et al., 2000a) by the Aerosol Laboratory of the University of Southern California, and are capable of enriching the concentration of particles in the entire range of 0-10 μm by a factor up to 40, depending on the output flow rate. These systems are very compact in size and modular in design. They can thus be readily adaptable to accommodate higher output flow rates that are desirable in conducting human exposure studies. Over the past year, scaled-up versions of the coarse, fine and ultrafine concentrators were developed through this program and their laboratory and field evaluation is described in greater detail by Kim et al (2000b; 2000c).

Unique features of the new generation of portable concentrators:

1. The virtual impactors of these systems employ round acceleration and collection nozzles, compared to the rectangular geometry designs of older version of concentrators (described by Sioutas, C., Koutrakis, P., and Burton, R.M. "A technique to expose animals to concentrated fine ambient aerosols." *Environmental Health Perspectives,* 103:172-177, 1995). Due to intrinsic design characteristics associated with the three-dimensional flow of round nozzles (compared to the axisymmetric flow of slit-nozzle impactors), higher particle efficiency and lower losses are achieved. Thus, a single-stage system can concentrate particles up to a factor of 40, without altering the size distribution and chemical composition of the sampled and concentrated aerosols.

2. The high-efficiency, single-stage design makes the entire system very small and portable. This is exceedingly important, as it makes it possible to place these concentrators in light-duty trailers and transport them at various sites with distinctly different chemical and physical characteristics of PM.

3. These concentrators are capable of concentrating particles of all three discrete size groups concurrently. These groups are:

- Ultrafine Particles (<0.1μm), which are freshly generated particles, such as those generated by combustion,
- Fine Particles (including their ultrafine mode) of any size sub-range between 0-2 μm and;
- Coarse (>2.5 μm) particles.

4. Concurrent concentration of all of three PM modes allows specific size ranges and chemical characteristics of concentrated ambient PM to serve as test aerosol to conduct specific hypotheses-driven toxicity studies.

5. Short-term health impacts of real-life PM associated with different size ranges and sources can be evaluated.

6. Because of their high concentration efficiency, operation of these systems requires very low power. For example, for a 9-nozzle system that provides 100 LPM of concentrated PM for human exposures, all flows can be driven by three Gast 2067 pumps. Each of these pumps consumes 0.7 kW (total of 2.1 kW). These pumps are single-phase, 110 Volts, and can be readily plugged into any standard power outlet. Compared to these systems, the previously developed Harvard concentrators employ a three-phase blower, consuming 9 kW. This blower requires three-phase power installation, generates 120 dB of noise, and hence requires some type of enclosure for noise reduction, which in turn requires some means of ventilating the generated heat by the blower. The volume and power requirement of the Harvard concentrators makes them impractical for transportation and field use. None of these problems are encountered in the use of the new, portable particle concentrators.

7. A unique feature of these systems is also the ability to provide concentrated ultrafine particle to an exposure chamber with a very low-pressure drop (less than 5 inches of H_2O). Concentrated coarse and fine particles with their ultrafine component can be provided to an inhalation chamber under a negative pressure of less than 1 inch of H_2O, almost atmospheric pressure. By comparison, the older version of Harvard Concentrators delivers the aerosol under 15-20 inches of water negative pressure.

8. Due to the larger size of the round nozzles (0.4-0.6 cm) compared to the width of the previously developed Harvard concentrators (0.03 cm), the new systems do not suffer from clogging and performance instabilities associated with the rapid increase in pressure drop, followed by a sharp decrease in the concentration enrichment factor. These problems have been observed under conditions of high particle concentration or when operating these systems in days with high humidity and temperature (personal communications; F.R. Cassee, RIVM, Netherlands, C.S. Kim,

U.S. EPA, J.J. Godleski, Harvard University, D. Costa, U.S. EPA, J.R. Brook, Health Canada). A paper investigating the effects of parameters such as ambient relative humidity, dew point temperature, ambient $PM_{2.5}$ mass concentration, ambient $PM_{2.5}$ mass median diameter (MMD), and total pressure drop per unit time across the Concentrator on the overall concentration enrichment achieved by the Harvard Fine Particle Concentrator has been just accepted for publication (Kim et al., 2000).

9. Another unique feature of the portable concentrators is their ability to be used in conjunction with a liquid impinger (BioSampler™, SKC West Inc., Fullerton, CA) to collect directly large volumes of outdoor and indoor particles on a cell culture solution or any other liquid solution. Traditionally, particle collection for *in vitro* tests has been conducted by using collection substrates such as filters or impactors. The collected particles are subsequently extracted from the substrates and administered into the *in vitro* culture either directly or after lyophylization of the solvent. This process suffers from several shortcomings, including imperfect particle extraction from the substrate but most importantly, this mechanism for particle collection does not preserve biologically active agents of airborne PM. Direct impingement of these particles onto the cell culture solution will substantially improve the *in vitro* evaluation of toxic effects of PM. The collection efficiency of the BioSampler is close to 100% for particles larger than about 1.5 μm, and operating at a flow rate of 12.5 l/min. For particles less than 1.0-micron diameter the collection efficiency decreases sharply to less than 50% for particles at 0.5 μm. Operating in conjunction with our prototype ultrafine, fine or coarse particle concentrators, the BioSampler can collect any of the PM size ranges with 100% efficiency and at sampling flow rates that are 20-30 times higher than its nominal operating flow rate. Thus, the condensation growth of even ultrafine PM to super-micrometer particles enables effective trapping of these particles by the impinger and allows us to “concentrate” large volumes of ambient PM into a very small solution on the order of 5-10 ml. The resulting particle concentration in the *in vitro* solution is on the order of 50-400 μg/ml, depending on ambient PM number and mass concentrations.

10. The ability to collect large volumes of particles directly into a small volume of any solution is a particularly attractive feature when intratracheal instillation is used as the method to conduct particle toxicity tests.

3.3 Description of the Portable Coarse, Fine and Ultrafine PM Concentrators

Figures 1a and 1 b show a schematic of two different configurations of the new concentrators, which we have named Versatile Aerosol Concentration Enrichment Systems (VACES). The VACES incorporate the following features:

1. The ability of concentrating ultrafine particles only, and supplying them to an exposure chamber at virtually atmospheric pressure (0.99 atmospheres).

2. The ability to allow concurrent animal exposures to coarse, fine and ultrafine particles.

3. When exposures to one PM mode are desirable, this technology can concentrate up to 330 LPM of ambient PM to a flow rate as low as 10 LPM. This feature makes it possible to use more animals in an inhalation study, hence increase the confidence level in the observed outcomes.

4. The capability of collecting concurrently very high quantities of coarse, fine and ultrafine PM in a small liquid volume (4-10 ml). The resulting highly concentrated suspensions can be used for *in vitro* tests to evaluate the relative toxicity of ambient PM, collected simultaneously in a given location.

Figure 1a shows the configuration used for *in vivo* inhalation exposures, whereas Figure 1b shows the version of the same system for *in vitro* toxicity studies.

The VACES consists of three parallel sampling lines. In each line, ambient coarse, fine and ultrafine aerosols are drawn at 110 LPM. Coarse PM is drawn through a round nozzle, single-stage virtual impactor, having a 50% cutpoint at 1.5 μm. The performance of these virtual impactors is described in greater detail by Kim et al (2000a). Coarse particle in this sampling line can be concentrated by as much as a factor of 35, and supplied to the exposure chamber at a flow rate ranging from 3.3-11 LPM.

The other two sampling lines of the VACES consist of identical components, with the only exception of the cutpoints of the impactors through which the samples are drawn prior to passing through the saturator. In the line concentrating fine plus ultrafine PM, air samples are first drawn through a single slit nozzle impactor, having a 50% cutpoint at 2.5 μm at a flow rate of 110 LPM. The impactor's acceleration nozzle is 0.2 cm wide and 5 cm long. At a sampling flow rate of 110 LPM, particles are accelerated to a velocity of 1834 cm/s, and the corresponding pressure drop across the impactor is 1.5 inches of H_2O.

In order to remove all but the ultrafine PM, particles in the third sampling line of the VACES are drawn through a multi-nozzle, high volume conventional impactor with a design 0.15 μm cut-off size at a flow rate of 110 LPM. Separation of these

particles is accomplished under a very low-pressure drop (i.e., 7-8 inches of H_2O). This is a very important feature of these new concentrators, since inhalation studies cannot be conducted under a substantial vacuum. The impactor consists of 5 slit-shaped nozzles in parallel, each 5 cm long and 0.015 cm wide. At a flow rate of 110 LPM, the resulting velocity through each rectangular jet is approximately 4200 cm/s and the corresponding pressure drop across the impactor is 7.5 inches of water (or 0.019 bar). A 5 x 0.2 cm quartz fiber strip is placed underneath each acceleration nozzle, at a distance of 0.04 cm. The strips are coated with mineral oil and serve as bounce-free impaction substrates for collecting particles above 0.16 μm in aerodynamic diameter. It should be noted that concentration of ultrafine particles is optional. Without the use of the 0.15 μm impactor, the VACES can also deliver fine and ultrafine PM at 10 LPM, enriched in concentration by a theoretical factor of 22.

After the impactor pre-separators, the aerosol in both the fine and ultrafine lines is drawn through a stainless steel container used as the aerosol saturator. The container has a capacity of 10 liters and is used to mix the aerosol with warm, distilled deionized vapor at a temperature of about 30 (± 2) degrees C. The stainless steel container is placed inside a heating bath (VWR Scientific, Model 1024), with a heating power of 0.5 kW.

The saturated aerosol is drawn through a cooler, which is an icebath with two aluminum tubes (2.2 cm in diameter and 80 cm long) through it. In each cooler, the saturated and warm air is cooled by about 9-10 degrees C. The produced supersaturation in the cooling causes all particles to grow to about 2.5-2.6 μm droplets.

The grown droplets are subsequently drawn through two identical virtual impactors. Each virtual impactor separates particles into two different size ranges, approximately above and below 1.5 μm. These virtual impactors are also identical in design to those used for concentration of coarse ambient particles. The virtual impactors are made of anodized aluminum. The grown fine and ultrafine particles are drawn into the minor flow of virtual impactor (which can be as small as 3 LPM), and thereby become concentrated by a factor up to 40.

Concentrated droplets are drawn through a Diffusion Dryer (TSI Model 3062, TSI Inc., St. Paul, MN), placed immediately downstream of the collection nozzle of each virtual impactor. The diffusion dryer is used to remove the excess moisture around the particles and return these grown particles to their original size. Operating at a maximum flow rate of 10 LPM, each diffusion dryer reduces the relative humidity of the incoming aerosol from 100% to 50%, thereby returning the grown particles to their original size.

All three major flows of the parallel virtual impactors are drawn by a single rotary vane pump (Gast model 2067, Gast Manufacturing, Cerritos, CA). This pump is

capable of drawing up to 360 LPM under a vacuum of 150 inches of water, while consuming only 0.5 kW at 110 V. The pump is light (20 lb), takes up very little space, and does not require any special power installation.

In vitro sampling:

Figure 1b shows the alternative configuration of the VACES, used for simultaneous coarse, fine and ultrafine PM collection for *in vitro* toxicology experiments. For *in vitro* collections the concentrated coarse, fine and ultrafine particles in each parallel sampling line are drawn through a liquid impinger instead of passing through a diffusion dryer (BioSampler, SKC West Inc., Fullerton, CA). The performance of this device is described in greater detail by Willeke *et al*. (1998). Unlike conventional impingers in which the aerosol is impacted into a reservoir filled with liquid, particles in the BioSampler are injected into a swirling flow for collection by a combination of inertial and centrifugal forces onto the surface over which the air flow swirls.

Traditionally, particle collection for *in vitro* tests has been conducted by using collection substrates such as filters or impactors. Particles collected on filters are subsequently extracted from the substrates and administered into *in vitro* culture media, either directly or after lyophylization of the solvent. This process suffers from several shortcomings, including inefficient particle extraction from the substrate, and variable losses of potentially toxic semi-volatile PM constituents, and of biologically active components of airborne PM. In addition, a recent study by Dick *et al*. (2000) showed that components of filters used to collect particles could contaminate the preparation and interfere with biological investigations.

Particle collection using liquid impingers has been shown to be advantageous over the traditional filtration or impaction methods for collection of airborne particles, because impingers are not easily overloaded (Willeke *et al*., 1998), and impingement eliminates the need for elaborate extraction procedures (Zucker *et al*., 2000). Under normal operating conditions at its nominal flow rate of 12.5 LPM, the BioSampler has collection efficiency close to 100% for particles larger than about 1.5 μm. For particles smaller than 1.0 μm in aerodynamic diameter, the collection efficiency decreases sharply to less than 50% (Willeke *et al*., 1998). Operating in conjunction with the VACES, however, the BioSampler can collect any of the PM size ranges with 100% efficiency and at sampling flow rate that is at least 10-fold higher than its nominal operating flow rate. Thus, the supersaturational growth of even ultrafine PM to super-micrometer particles enables effective trapping of these particles by the impinger and allows us to "concentrate" large volumes of ambient PM into a very small solution on the order of 5-10 ml. The ability to collect large volumes of particles directly into a small volume of any solution is a particularly attractive feature when intratracheal instillation is used as the method to conduct particle toxicity tests.

3.4 Experimental Characterization of the VACES Components

3.4.1 Characterization of the 2.5 μm and 0.15 μm Low Pressure-Drop Slit Impactors

The collection efficiency of the 2.5 μm cutpoint slit impactor was determined using monodisperse aerosols generated by atomizing suspensions of PSL particles (size range: 0.5-10 μm; Bangs Laboratories Inc.,) in a constant output Nebulizer (HEART, VORTRAN Medical Technology, Inc., Sacramento, CA). The generated aerosols passed through Po-210 static charge neutralizers and were mixed with filtered air prior to passing through the slit impactor. The mass concentrations of the monodisperse aerosols upstream and downstream of the impactor were measured by means of a nephelometer (DataRAM, MIE, Inc., Billerica, MA). For each test, repeated measurements of the concentrations upstream and downstream of the impactor were taken. The concentrations of the generated aerosols were in the range of 100-400 $\mu g/m^3$, thus several orders of magnitude higher than the limit of detection of the DataRAM which is about 1-5 $\mu g/m^3$. As a nephelometer, the response of the DataRAM is dependent on particle size (Sioutas et al., 2000). Particle collection efficiency as a function of aerodynamic diameter is shown in Figure 2. The results confirm that the cutpoint of the impactor is at about 2.5 μm in aerodynamic diameter.

The collection efficiency of the multi-slit 0.15 μm cutpoint impactor was estimated using ambient air as the test aerosol. For particles in the size range of 0.015 to 0.5 μm, penetration was determined by measuring the aerosol concentrations upstream and downstream of the impactor by means of the Scanning Mobility Particle Sizer (SMPS Model 3096,TSI Inc., St. Paul, MN). The SMPS sampled 0.2 LPM of the total flow rate of 110 LPM through the impactor. Number concentration of ambient aerosols was measured with and without the block holding the acceleration slit nozzles of the impactor to account for possible diffusional losses of ultrafine particles through the sampling lines connecting to the SMPS. Particle size was selected in the interval of 0.02-0.5 μm by adjusting manually the voltage to the Differential Mobility Analyzer of SMPS and measuring the particle counts upstream and downstream of the 0.15 μm cutpoint impactor.

In addition to the SMPS, the DataRAM was used to evaluate the collection efficiency of the multi-slit impactor for particles in the 0.2 to 1.0 μm range, using artificially generated monodisperse PSL particles as described above. The DataRAM could not be used to monitor particles less than 0.2 μm because the sensitivity of the instrument decreases sharply below this particle size.

Finally, limited field tests were conducted in which the ambient aerosol concentrations measured by the 0.15 μm cutpoint impactor was compared to that measured by means of the Microorifice Uniform Deposition Impactor (MOUDI, MSP Corp., Minneapolis, MN), which was used as a reference sampler. A 4.7 cm

Teflon filter (2 μm pore, Gelman Science, Ann Arbor, MI) was placed immediately downstream of the multi-slit impactor, which was operated at a flow rate of 110 LPM. The MOUDI was placed at a distance of 1 m from the impactor and sampled at 30 LPM Ambient particles smaller than 0.18 μm in aerodynamic diameter were collected on a 3.7 cm Teflon filter following the last impaction stage of the MOUDI. Both MOUDI and multi-slit impactor Teflon filters were weighed before and after each test on a Mettler Microbalance (MT5, Mettler-Toledo, Inc., Highstown, NJ) under the controlled relative humidity (40-45%) and temperature (22-24 °C) conditions in order to determine the mass concentrations.

Figure 3 shows the pressure drop across the multi-slit impactor as a function of flow rate. The pressure drop across the multi-slit impactor is about 7 inches of H_2O at the standard flow rate of 110 LPM. The ability of this impactor to remove all but ultrafine particles with a very low pressure drop is a very important feature of the VACES, since inhalation health studies cannot be conducted under a substantial vacuum.

The collection efficiency of multi-slit impactor, determined from the decrease of both number (SMPS) and mass (DataRAM) concentrations measured downstream of the impactor, is plotted as a function of particle aerodynamic diameter in Figure 4. Error bars represent the standard deviation of the experimental results.

The particle collection efficiency curve obtained from data using the SMPS increases sharply starting at 0.1 μm and reaches the value of about 90% at particles larger than 0.3 μm in aerodynamic diameter. The collection efficiency values obtained by means of the DataRAM are in a good agreement with those obtained by SMPS for the overlapping particle size range between 0.2 and 0.5 μm. The data shown in Figure 4 indicate that the 50% cutpoint of the multi-slit nozzle impactor has a mobility diameter of 0.18 μm.

The comparison between the mass concentrations measured by multi-slit impactor and the reference MOUDI is shown in Table 1. Despite the small number of data points, the mass concentrations of ultrafine particles obtained with the two samplers are in excellent agreement, with the average slit impactor-to-MOUDI ultrafine particle concentration being 1.07 (± 0.15). The agreement between the two samplers is remarkable because even a small cutpoint difference in the 0.1-0.2 μm range might result in substantial differences in the amounts of particles collected by two different impactors. Mass-based concentration of ambient PM-2.5 decreases sharply for particle sizes smaller than 0.2 μm (Whitby and Svendrup, 1980) and a small entrainment of accumulation mode particles into the ultrafine mode resulting from a small disparity in the impactor cutpoints would result in a significantly higher mass concentration measured by the sampler having the largest cutpoint impactor. The low cut point of the high volume multi-slit impactor with the low pressure

drop makes it possible for toxicologists to conduct health study on the ambient particles containing only ultrafine mode.

3.4.2. Characterization of the BioSampler

At the standard operation flow rate of 12.5 LPM, the pressure drop across the BioSampler is close to 0.5 atm, which has been shown to cause excessive evaporation of liquid collection media such as water. It is also expected that under these sampling conditions, excessive losses of semi-volatile components of ambient particles would occur. In order to reduce the pressure drop across the BioSampler used in conjunction with the VACES virtual impactors, a flow rate of 5 LPM was used instead. The decrease in flow rate was expected to increase the cutpoint of the BioSampler. However, as most of fine and ultrafine PM is grown to 2.5-2.7 μm via supersaturation in the VACES, our primary concern was to ensure that particles in that size range are efficiently collected by the modified BioSampler.

Another modification of the BioSamplers used in conjunction with the VACES was the amount of water used in its reservoir to collect the impinging particles. In its nominal configuration, 20 ml of liquid are required in the BioSampler reservoir. However, from the standpoint of toxicological studies, it is highly desirable to maximize the concentration of the collected ambient particles in the liquid medium of the BioSampler. We thus investigated the effect of different volumes of water on the collection efficiency of BioSampler at the reduced flow rate of 5 LPM. We specifically tested the BioSampler using water volumes of 2, 4, 10 and 20 ml, respectively. For each liquid volume, the collection efficiency for particles in the range of 0.5-5 μm was determined by measuring the upstream and downstream BioSampler monodisperse aerosol concentrations using the DataRAM, as described above. At 5 LPM, the pressure drop across the BioSampler was approximately 17 inches of H_2O. The exhaust of the DataRAM pump was returned downstream of the BioSampler in order to avoid sampling biases, which might occur when this instruments samples under a vacuum. This sampling strategy is recommended by the manufacturer.

Figure 5 shows the pressure drop across the BioSampler as a function of flow rate. The pressure drop at 5 LPM is 17 in. H_2O (0.035 atm), which is substantially lower value than the 145 in.H_2O at the standard flow rate of 12.5 LPM. As a result of this small pressure drop, less than 0.5 ml of water volatilized after 6 hours of sampling ambient concentrated air at relative humidities ranging from 45 to 65%. By comparison, 80 % or more of 20 ml of water normally evaporates within 2 hours under reduced pressure at 12.5 LPM (Willeke *et al*., 1998). The small pressure drop is essential in preserving labile semi-volatile species such as ammonium nitrate and a host of organic compounds would be more pronounced under the high pressure drop across the sampler (Zhang and McMurry, 1987).

The collection efficiency of BioSampler at 5 LPM is shown in Figure 6 as a function of particle size for various amounts of water in the BioSampler reservoir. Error bars represent the standard deviation of repeated tests. Data shown in Figure 6 indicate, for any particle size, there is no significant dependence of the collection efficiency of the BioSampler on the amount of water in its reservoir, at least for the range of 4-20 ml. Based on these results, even 4-5 ml in the BioSampler reservoir would ensure high particle collection efficiency, while maximizing the particle concentration in the aqueous suspension to be used for *in vitro* tests. Five ml is also sufficient to ensure complete wetting of the bottom of the BioSampler reservoir, a feature that ensures effective particle capture by the instrument.

The collection efficiency of the BioSampler is close to 100% for particles larger than 2 μm at a flow rate of 5 LPM, regardless of liquid volume in the reservoir. For particles less than 1 μm in aerodynamic diameter, the collection efficiency decreases sharply to about 50% at 0.5 μm. Any significant decrease in the collection efficiency due to particle bounce was not observed up to about 5 μm of aerodynamic diameter. Figure 6 also shows that the BioSampler collects fine and ultrafine particles that were grown to water droplets more efficiently than dry PSL particles of similar size.

3.4.3 Laboratory Characterization of the Fine and Ultrafine Concentrators of the VACES

The coarse particle concentrator component of the VACES had already been developed and described elsewhere (Kim et al., 2000); laboratory tests focused on the experimental characterization of the fine and ultrafine concentrators of the VACES. It should be noted that the use of the 0.15 μm impactor to remove all but ultrafine particles is optional. The VACES can also be used to concentrate fine PM including the ultrafine fraction from 220 LPM to a flow as small as 7 LPM. Thus experiments were conducted at a sampling flow of 220 LPM as a worse case scenario, since this flow rate represent the most challenging configuration for the saturator and the cooler of the VACES.

The experimental characterization of the VACES was conducted using laboratory monodisperse particles as well as real-life ambient particles as the test aerosols. Monodisperse aerosols were generated by atomizing suspensions of ultrafine and fine particles using a constant output HEART Nebulizer (VORTRAN Medical, Inc., Sacramento, CA). Different types of suspensions were used, including monodisperse PSL fluorescent latex particles (size range 0.05 – 2 μm; Polysciences, Inc., Warrington, PA) as well as monodisperse silica bead (0.36 μm; Bangs Laboratories, Inc., Carmel, IN). In addition, aqueous solutions of ammonium sulfate and ammonium nitrate were atomized. Finally, indoor aerosol was also used as test aerosol. The size distributions of the polydisperse aerosols were determined using SMPS.

The nebulizer generated aerosols were dried, neutralized and were then drawn to the saturator at 220 LPM. The aerosol was mixed and saturated with water vapor at about 30-32 °C, and drawn through two condenser tubes at 110 LPM each. The temperature of the aerosol exiting the condenser was about 23 (± 1)°C.

The grown droplets were subsequently drawn through the two virtual impactors. Three different minor flow rates were tested, 7, 10, and 21 LPM, respectively (corresponding to theoretical enrichment factors of 30, 22, and 10.5, respectively). The TSI Condensation Particle Counter (CPC 3022, TSI, Inc., St. Paul, MN) was connected immediately upstream of the saturator and downstream of the diffusion drier (as shown in Figures 1a and 1b) to measure the number concentrations of the original and concentrated aerosols. For each particle size, concentration enrichment was defined as the ratio of the concentration measured downstream of the diffusion dryer to that measured upstream of the saturator.

Results from the laboratory evaluation of the VACES at three different minor flow rates are summarized in Figure 7. In all three minor flow configurations, the major flow rate is adjusted to yield a total intake flow of 220 LPM. Hence, the maximum obtainable concentration enrichment factors for each configuration are 31, 22, and 10.5, respectively. The concentration enrichment factors as a function of particle size, shown in Figure 7, have been obtained using monodisperse aerosols in the size range of 0.05 – 1.9 μm, except for the data corresponding to 0.025, 0.31, and 0.32 μm particles. The number mean diameter (NMD) of polydisperse aerosols were obtained from the count-based size distributions of ammonium sulfate, ammonium nitrate and indoor aerosols using the SMPS.

The enrichment factors at minor flow rates of 7 LPM, 10 LPM, and 20 LPM are 30.1, 20.4, and 9.6, respectively, which are very close to the ideal values. In addition, hygroscopic ammonium sulfate and ammonium nitrate aerosols did not show any observable difference in the enrichment factors compared to the hydrophobic PSL particles.

3.4.4. Field Evaluation of the VACES

The performance of the VACES was evaluated in a field study, conducted outdoors in the facilities of Rancho Los Amigos National Rehabilitation Center in Downey, CA. Situated near the Los Angeles “Alameda corridor”, Downey has some of the highest inhalable particle concentrations (PM_{10}) in the US, very often exceeding the National Ambient Air Quality Standard of 150 $\mu g/m^3$. The 25-mile long Alameda corridor is named after Alameda Street, which joins the coastal

area of Long Beach (where a major port, large number of industrial plants, and oil refineries are currently operating) to downtown Los Angeles.

The main goal of the field study was to confirm that the physical or chemical properties of ambient aerosol are preserved during the process of concentration enrichment using the VACES. Measurements of concentration-enriched coarse, fine and ultrafine aerosol fractions were compared to direct ambient measurements made with a co-located MOUDI which was used as a reference sampler. In part, the MOUDI was used because of its high sampling flow rate which allows for sufficient sample collection for comparisons with the VACES in relatively short time periods. Because each of the sampling lines of the VACES sample at 110 LPM, the analytical sensitivity and quantity of particle mass by the VACES, itself, was of less concern. It should be noted that the MOUDI is not a reference sampler for labile species, such as ammonium nitrate and semi-volatile organic compounds, and losses of these compounds may occur under conditions of high temperature and low relative humidity (Chang et al, 2000).

Instead of using all of the stages of the MOUDI, only those stages having cut-points of 10, 2.5 and 0.18 μm were used. The first MOUDI stage (2.5-10 μm) was used as reference sampler for coarse ambient particles, the second stage (0.18-2.5 μm) for the ambient PM accumulation mode, and the last stage (i.e., the after-filter) to determine ambient ultrafine particle concentrations. The MOUDI and VACES coarse and fine (accumulation plus ultrafine) PM concentrations were compared by mass, nitrate, sulfate, trace elements and metals. For these analyses, concentration enriched aerosols were collected on 4.7 cm Teflon filters (Gelman Science, 2 μm pore), which were placed immediately downstream of the diffusion dryer of the VACES fine and ultrafine particle concentrators, and directly downstream of the minor flow of its coarse concentrator. For direct ambient measurements, the same type of filters was placed in each MOUDI stage and its after-filter.

Ultrafine concentrations obtained by means of the VACES and MOUDI were compared based on mass and elemental carbon (EC) concentrations, as EC has been shown to be a predominant ultrafine PM constituent at this ambient site (Sioutas et al., 2000). For this analysis, quartz filters (Pallflex Corp., Putnam, CT) were placed downstream of the diffusion dryer of the VACES ultrafine concentrator and of the co-located MOUDI after-filter. Organic carbon (OC) may also be a significant constituent of ultrafine PM mass, however positive artifacts due to adsorption of organic gases on the MOUDI's quartz after-filter (Eatough et al., 1993; McMurry and Zhang, 1987) may introduce significant bias in the MOUDI-VACES comparisons. As the minor flow rate of the VACES (containing virtually all of the ambient particles) is 5 LPM compared to 30 LPM of the MOUDI, gas-phase adsorption on the VACES filter would be theoretically 1/6 of the MOUDI, thus less severe.

In this study, comparisons were based only on the EC fraction of particle-associated carbon, as the organic carbon fraction may consist of several volatile or semi volatile compounds. Data based on the EC fraction better reflect performance of the concentrators. In our first pilot study, the performance of our smaller scale ultrafine and fine concentrators (Kim et al., 2000), the OC comparisons conducted indoors between the concentrators and the MOUDI showed excellent agreement (within ±10%) between the two systems.

In order to evaluate whether the chemical composition of concentration-enriched ambient particles are effected by using the *in vitro/* BioSampler version of the VACES (Kim, et. al., 2000b), measurements were compared to those made directly onto filters. For the samples collected by means of the BioSampler, only the inorganic ion (i.e., sulfate and nitrate) content of the concentrated aerosols were determined, because of the logistical difficulties associated with weighing (for mass) the BioSampler or analyzing its aqueous extract for EC or OC.

For mass measurements, Teflon filters were weighed before and after each field tests using a Mettler 5 Microbalance (MT 5, Mettler-Toledo Inc., Highstown, NJ), under controlled relative humidity and temperature conditions. Filters were weighed immediately at the end of each experiment as well as after a 24-hour equilibration time period. Laboratory and field blanks were used for quality assurance. Filters and filter blanks were weighed twice in order to increase precision. In case of a difference of more than 3 μg between consecutive weightings, a filter was weighed a third time. The Teflon filters were then analyzed by means of ion chromatography to determine the concentrations of particulate sulfate and nitrate. Trace element and metal concentrations for ambient and concentrated PM were determined by analyzing the MOUDI ad VACES Teflon filters by means of inductively coupled plasma mass spectroscopy (ICP/MS). This analysis was conducted by the Monitoring and Laboratory Division of the California Air Resources Board.

The EC concentrations were determined by thermo-analysis. A slice of approximately 0.2 cm^2 from each filter was placed in a platinum boat containing MnO_2. The sample was acidified with an aliquot of HCl and heated to 115 °C to dehydrate the sample, and form CO_2 as an index of particle-associated carbon. The boat was then inserted into a dual zone furnace, where MnO_2 oxidized Organic Carbon at 550 °C and Elemental Carbon at 850 °C. A Flame Ionization Detector (FID) converted the CO_2 combustion product to CH_4 for detection. This analytical method is more elaborately described by Fung (1990).

Effect of Condensation and Evaporation in the VACES on Agglomerate Structure:

A significant fraction of the ultrafine particles in the Los Angeles atmosphere are agglomerate structures, mainly emitted from diesel and other high temperature

sources. Agglomerate structures have higher surface areas than spherical particles with the same equivalent diameter; agglomerate transport properties in both gas and liquid phases differ from spherical particles as well (Friedlander, 2000). These differences in surface area and transport properties may influence the biochemical effects of inhaled ultrafine particles. For these reasons, it is important to know whether condensation and evaporation that precedes aerosol concentration in the VACES is likely to affect the morphological properties of the ultrafine particles.

Atmospheric ultrafine particles and those concentrated by the VACES were sampled using a low-pressure impactor (LPI) on the UCLA campus, in west Los Angeles. Concentrated ultrafine aerosols generated by the VACES were sampled after they were dried by diffusion. The LPI is an eight-stage single jet impactor equipped with a critical orifice that maintains a flow rate of 1 LPM under the appropriate pressure drop (Hering *et al*., 1978 and Hering *et al*., 1979). The stages have 50% efficiency cutoffs in aerodynamic diameter of 4.0, 2.0, 1.0, 0.5, 0.26, 0.11, 0.075, and 0.05 μm for stages one to eight, respectively. The particles were collected on a nickel grid. To minimize the effects of particle bounce, only one stage at a time had a grid attached for sampling; the grid is secured at the center of a 25 mm diameter glass stage, while the other glass stages are coated with apiezon grease. Air is drawn through the impactor by a vacuum pump for 5 minutes per stage. Analysis was done for changes in structural characteristics of the agglomerate fraction. These agglomerates were collected on stages 7 and 8, which have particle aerodynamic diameter ranges of 0.075 - 0.11 μm and 0.05 – 0.075 μm, respectively. Transmission Electron Microscope (TEM) photomicrographs of the grids were taken using a JEOL 100CX and 2000FX TEM at a magnification of 10^5. The morphology of ultrafine particles (d_p< 0.10 μm) was characterized using the fractal concept applied to TEM micrographs. More details on fractal analysis can be found in Xiong (2000).

Experiments and simulations have shown that the fractal concept can be applied to aggregates of nanometer primary particles (Forrest and Witten, 1979: Witten and Sander, 1981). In applying the fractal concept, the fractal dimension and the prefactor for both ambient and concentrated particles were calculated. The fractal dimension (D_f) is a measure of the stringiness of the agglomerate and the prefactor (A) is a measure of denseness of the agglomerate. An agglomerate with the same fractal dimension as another may have a higher prefactor if it contains a higher primary particle overlap. Agglomerates produced by computer simulation algorithms help in the understanding of structure and fractal dimension (Friedlander, 2000). Figure 8 illustrates two examples for diffusion limited aggregation. An agglomerate with a chain-like structure has a lower fractal dimension than a more compact, spheroidal one. The structure of an agglomerate with a D_f value of 1 is a linear chain of primary particles. For a D_f value of 2 the agglomerate structure is a two-dimensional arrangement of closely packed primary particles with six nearest neighbors; and the structure for a D_f

value of 3 is a three-dimensional closely packed sphere. The agglomerate fractal dimension and prefactor arise from the following relationship (Weber et. al., 1995):

$$N_p = A\left(\frac{R_g}{R_o}\right)^{D_f} \qquad (1)$$

where D_f is the fractal dimension, N_p is the number of primary particles in the aggregate, A is the fractal pre-factor or structural coefficient, R_o is the average primary particle radius, R_g is the radius of gyration. The radius of gyration can be calculated using the relation: $[(1/M)\Sigma(m_i r_i^2)]^{1/2}$, where m_i is the mass of the i^{th} primary particle, M is the total mass given as Σm_i, and r_i is the distance of the i^{th} primary particle from the center of mass. The fractal dimension and prefactor of randomly sampled ambient and concentrated particles were obtained by plotting the number of primary particles positioned radially from the center of mass to the radius of the gyration of the agglomerate. The fractal dimension was determined from the slope and the prefactor was determined from the inverse log of the intercept of the least squares fit line.

Results and Discussion of the Field Study:

In each of the sampling lines of the VACES, coarse, fine and ultrafine particles were concentrated from an intake flow of 110 to a minor flow of 5 l-min^{-1}. Thus the ideal concentration enrichment factor for any chemical PM species is expected to be 22. Results from these field tests are summarized in Tables 1-3 and in Figures 9-14. In each figure, the concentrations determined by the VACES are compared to those determined by the MOUDI; the coordinates are fit by a linear regression and the tightness-of-fit by correlation coefficients. The slopes of the regression lines thus provide an average estimate of the overall concentration enrichment factor obtained by means of the VACES for a given PM fraction and species.

Table 2 presents the sulfate and Table 3 the nitrate content of the coarse fraction of ambient (MOUDI) and concentration-enriched ambient aerosol (VACES). The corresponding enrichment factor (defined as the ratio of the VACES coarse aerosol concentration to that of the MOUDI) is presented for each of 5 samples. Figure 9 presents paired measurements of ambient coarse aerosol mass concentrations obtained by the MOUDI versus those concentrated by the VACES.

As indicated by the slope of the regression line in Figure 9 the average concentration enrichment of the VACES is 22.5 (± 3.8), thus, very close to the ideal value of 22. The rather limited data obtained for coarse particle sulfate and nitrate (Tables 1a and 1b, respectively) also indicate a close agreement between

the VACES and MOUDI, with the concentration enrichment factors for sulfate and nitrate of 22.1 (± 4.9) and 19.9 (± 2.6), respectively. The limited data for these inorganic ion measurements are due to the very low nitrate and sulfate content within the coarse fraction of PM in the specific Los Angeles location. Thus, a greater level of uncertainty exists in the measurements made with the MOUDI (which samples at about one fourth of the flow rate of each sampling line of the VACES). Nevertheless, the overall agreement between the VACES and the MOUDI for coarse particle mass, sulfate, and nitrate is near "ideal".

Figure 10 shows the $PM_{2.5}$ mass concentrations measured by the MOUDI and VACES. The overall concentration enrichment obtained for the fine PM mode is slightly higher (25.6 ±3.7) than the ideal value of 22, as indicated by the slope of the regression line. As further discussed below, this may be due, in part, to losses of volatile species, such as ammonium nitrate, from the MOUDI substrates in the lower stages. Evidence of this phenomenon was not the case for the coarse PM collected in the upper stage of MOUDI, where the pressure drop is much lower than the smaller cutpoint stages. Moreover, coarse particulate nitrate in south and western Los Angeles (i.e., areas closer to the coast) is mostly associated with stable sodium nitrate (Noble and Prather, 1996).

These experiments were conducted during the months of May and June 2000, with temperatures averaging 32 (± 3) °C and low relative humidity values (i.e., about 35% or less). These conditions have been shown to favor loss of ammonium nitrate from impactor samplers (Chang *et al.,* 2000; Zhang and McMurry, 1987) due to the higher values of the dissociation constant of ammonium nitrate. For this temperature and humidity range, the study by Chang and colleagues (2000), which was conducted at the same site, presented that the total losses of nitrate from the MOUDI averaged between 40-60%. Furthermore, a previous study by Kim *et al.* (2000) showed that concentration enrichment through a smaller-scale portable fine PM concentrator, which has similar design parameters to that of the VACES (in terms of aerosol saturation and cooling temperatures), occurs without any measurable loss of particulate nitrate, despite heating and saturation of the aerosol to about 35 °C. In that earlier study, ambient nitrate concentrations were determined by means of the Harvard/EPA Annular Denuder System (HEADS;), used as the reference sampler. The HEADS measures total particulate nitrate without losses (Koutrakis et al., 1988). Thus, any bias in the enrichment factor of ammonium nitrate above the "ideal" value (i.e., inlet-flow divided by minor-flow), may be due to its losses in the MOUDI, and concerns of a negative bias, due to potential ammonium nitrate losses in the saturation-condensation segment of the VACES, which may be masked by this effect, is discussed below.

Ammonium nitrate dissociates to ammonia and nitric acid, with its dissociation constant increasing exponentially with temperature. However, the dissociation constant decreases sharply as the relative humidity (RH) exceeds 90-95%

(Stelson and Seinfeld, 1982). For example, at 50°C and at RH=95%, the dissociation constant of ammonium nitrate is approximately 7 ppb, which is also the value of the dissociation constant at 18°C, and RH = 50%. Therefore, despite the increase in the aerosol temperature (which would have increased, exponentially, the value of the dissociation constant), aerosol exposure to high water vapor conditions in the VACES seems to prevent nitrate losses due to volatilization.

These conclusions are further supported by the results shown in Figure 11, where the $PM_{2.5}$ nitrate concentrations measured by means of the MOUDI are compared to those measured by the VACES. The average concentration enrichment based on nitrate is 43.8 (± 20.3), roughly twice the value of the ideal concentration enrichment. Given that nitrate losses depend significantly on several parameters such as temperature, humidity and overall particle concentration, the MOUDI-to-VACES agreement should be highly variable, which is indicated by the somewhat lower correlation coefficient (R^2=0.66) of the VACES vs. MOUDI data.

By comparison, the concentration enrichment obtained for the non-volatile fine particulate sulfate (shown in Figure 12) was 19.8 (± 4.3) and thus in very good agreement to the ideal value of 22. The above results confirm that the disparity between the ideal and actual concentration enrichment factors based on nitrate is due to sampling artifacts of the MOUDI.

The results plotted in Figure 12 also show that there is no significant difference (p=0.38) in the sulfate-based concentration enrichment values obtained with the *in vivo* version of the VACES (in which concentrated particles are dried by diffusion and collected on filters) and the *in vitro* version (in which particles are collected by the BioSampler). The concentration enrichment obtained by means of the BioSampler was 21.2 (± 3.5), compared to 18.9 (±2.5) obtained using the diffusion-dried concentrated particles collected on Teflon filters. Given the high values and random nature (due to meteorological factors) of nitrate losses within the MOUDI during the sampling period, a similar comparison of the *in vivo* and *in vitro* versions of the VACES based on fine particulate nitrate would be difficult, if not meaningless.

The MOUDI fine PM mass concentrations were corrected for nitrate losses as follows:

$$PM_{corr} = PM_{MOUDI} + 1.29\,(\frac{NO_{3,VACES}}{22} - NO_{3,MOUDI}) \qquad (2)$$

where $NO_{3,VACES}$ and $NO_{3,MOUDI}$ are the nitrate concentrations measured by the VACES and MOUDI, respectively, and PM_{MOUDI} is the total MOUDI fine PM mass

concentration determined gravimetrically. The above equation assumes that all nitrate found in the fine particulate mode is associated with ammonium nitrate. The corrected values of the MOUDI mass concentrations are also shown in Figure 10, along with the adjusted concentration enrichment factor. The nitrate-adjusted concentration enrichment factor becomes 22.8 (±3.4), thus very close to the ideal enrichment value of 22. These results imply that the discrepancy between the PM2.5 mass concentrations between VACES and MOUDI can be entirely attributed to the difference in the nitrate concentrations measured by these two systems.

The results of Figure 11 also indicate that the overall impact of nitrate losses from the MOUDI substrates on the mass concentration determined by the MOUDI is rather small. This is because ammonium nitrate accounts on the average for 30 - 40% of the total $PM_{2.5}$ mass concentration at Downey, CA (Sioutas *et al*., 2000). Thus, even if nitrate losses are as high as 50%, the overall difference between the uncorrected and nitrate-adjusted mass concentrations is not substantial, as indicated by the data presented in Figure 10.

Results from the concentration enrichment obtained for selected trace elements and metals are shown in Figure 13. Due to the low ambient concentrations of trace elements and metals measured by the MOUDI, quantifiable concentration enrichment values were obtained only for the following metals: Mg (coarse PM only), Al, K, Ca, and two iron isotopes (i.e., Fe^{56} and Fe^{57}). Measurable amounts of Zn, Cu, Ni and Mn were also identified in the filters connected to the fine concentrator of the VACES, but not in the corresponding MOUDI stages. The average and the standard deviation values of concentration enrichment shown in Figure 13 correspond to seven (of ten) field experiments. In the remaining four field tests, the ambient concentrations of the aforementioned metals were either comparable to the blank content of the Teflon filters or lower than the ICP/MS limit of detection (defined as three times the standard deviation of the laboratory blank filters).

The data in Figure 13 indicate that the Al, K, Ca, Fe^{56} and Fe^{57} content of fine and ultrafine PM is enriched by a factor of 21.2 (±4), 19.4 (± 3.3), 22.1 (± 3.8), 24.3 (± 3.1) and 22.4 (± 3.4), respectively. Similarly, the Mg, Al, K, Ca, Fe^{56} and Fe^{57} content of coarse PM is enriched by a factor of 18.6 (± 4.2), 20.4 (± 3.3), 19.3 (± 3.8), 18.3 (± 4.2) 22.1 (± 3.4) and 21.6 (±3.5), respectively. These concentration enrichment values are also close to the ideal enrichment value of 22, thereby indicating that the concentration enrichment process preserves the concentrations of these elements and trace metals in both coarse and fine PM.

Table 4 shows the concentration enrichment achieved by the ultrafine concentrator of the VACES based on particle counts, using a condensation particle counter (3022 CPC; TSI Inc., St. Paul, MN). The first column of Table 4 shows the ambient concentration based on particle counts; the second column

shows that the number concentration, measured immediately downstream of the 0.18 μm cut-point impactor; and the third column corresponds to the particle number concentrations measured immediately downstream of the diffusion dryer of the ultrafine concentrator of the VACES. The fourth column shows the ratio of particle counts downstream to that upstream of the 0.18 μm impactor, indicating that about 84% of ambient particle counts are associated with particles smaller than that size. The final column of Table 4 shows the concentration enrichment obtained for ultrafine particles, defined as the ratio of the count-based concentration downstream of the VACES to that downstream of the 0.18 μm impactor. The overall concentration enrichment for ultrafine particles was 20.8 (± 1.4), thereby indicating that ultrafine particle are concentrated with very high efficiency by the VACES.

Earlier investigations of the size distribution of ambient elemental carbon (EC) in Los Angeles (Venkataraman and Friedlander, 1994), showed that EC displays a bimodal size distribution, with peaks within the 0.05 – 0.12 μm (mode I) and 0.5 – 1.0 μm (mode II) size ranges. Mode I was attributed to primary emissions from combustion sources while mode II was attributed to the accumulation of secondary reaction products on primary aerosol particles. Mode I contained 75 – 85 % of EC, by mass, in the Los Angeles air basin during the summer season. Therefore, the performance of the ultrafine particle concentrator of the VACES was characterized by further comparing EC concentrations obtained with the VACES to those measured in the afterfilter of the MOUDI (collecting 0- 0.18 ? m particles).

Results from these field comparisons are shown in Figure 14. Similar to the results based on particle count and mass concentrations, a high level of comparability resulted between the VACES and MOUDI EC concentrations, with the average concentration enrichment factor being 22.2 (±2.3). Ultrafine particle EC concentrations obtained by means of the MOUDI and VACES are also very highly correlated (R^2 = 0.94).

It should be noted that the ability of the VACES to enrich the concentrations of all particles in the fine mode (including its ultrafine component) is a particularly important feature of this technology, as it enables inhalation toxicologists to conduct exposures to any selected sub-range of $PM_{2.5}$. For example, previous studies in California presented the presence of two sub-modes within the accumulation mode of ambient PM (Hering et al., 1997; John et al., 1990). One mode peaks at around 0.2 μm, consisting mainly of gas-to-particle reaction products, such as carbonaceous PM and the other peaks at about 0.7 μm, mainly associated with hygroscopic PM species, such as ammonium sulfate and ammonium nitrate. These observations have been confirmed by our recent yearlong measurements at the facility of Rancho Los Amigos in Downey (Sioutas et al, 2000). By thus placing a conventional impactor upstream of the fine concentrator of the VACES, having for example a 0.35 μm cutpoint, inhalation

studies could be conducted to ultrafine PM plus the elemental and organic carbon content of the accumulation mode, however, without the majority of its sulfate and nitrate constituents.

Effect of Condensation and Evaporation in the VACES on Agglomerate Structure:

Changes in agglomerate structure were investigated by comparing the fractal dimension and the prefactor of concentrated ultrafine particles from the VACES to ambient particles. Our results, shown in Figures 15 and 16, indicate that the concentrated and ambient particles show very similar morphology. The fractal dimension and prefactor values were determined for a total of 38 ambient and 39 concentrated ultrafine particles. Figures 15 and 16 show the fractal dimension value distributions for concentrated and ambient aerosols, respectively. The count median fractal dimension is very similar (between 1.6 and 1.8) for both concentrated and ambient particles. Furthermore, the average prefactor for the particles collected from the VACES is 2.73 and for the atmospheric is 2.83. Higher prefactor values are typically associated with denser agglomerates, but similar to what was found with the fractal dimension, the difference between the concentrated and atmospheric aggregate prefactor is not significant.

Previous research suggests that chain agglomerates may become more compact when subjected to condensation and evaporation processes (Colbeck *et al*., 1990: Hallet *et al*., 1989: Wells *et al*., 1976). A study shows that for diesel chain-agglomerate particles the fractal dimension increased from 1.56 to 1.76 and 1.40 to 1.54 for mid and low sulfur fuel after condensation and evaporation processes (Huang *et al*., 1994). However, in our study the average fractal dimension showed practically no change in value following condensation and evaporation in the VACES. An explanation is that in the study by Huang et al., the particles underwent up to 3 cycles of condensation and evaporation while in our study the particles only went through 1 cycle. We can therefore conclude that the condensation and evaporation process used with the VACES is effective in concentrating the sampled ultrafine particles but causes little change in the compactness or denseness of the particles, as measured by the fractal dimension and prefactor. However, both the sources of the fractal-like structures and associated trace gases may affect this phenomenon. Since the measurements were made for one sampling site, more experiments will need to be made in different sites to make these conclusions generalizable

Finally, Figure 17 shows the concentration enrichment as a function of particle size obtained by measuring the size distributions of ambient aerosols upstream of the VACES and immediately downstream of the diffusion dryer of the VACES line sampling fine PM by means of the SMPS. These experiments were conducted at a minor flow rate of 20 LPM (thus the ideal concentration enrichment is by a factor of 11). Each experiment started by first measuring the

ambient particle number concentration by means of the TSI 3022 Condensation Particle Counter for 5 minutes. Subsequently, the concentration immediately downstream of the 0.18 μm impactor was measured for an additional 5 minutes, followed by a concentration measurement downstream of the ultrafine VACES concentrator for 5 minutes. The above cycle was repeated three times in each experiment.

It should be noted that the lowest particle size that could be detected with the specific SMPS configuration was 17 nm. Due to the very low concentration of ambient particles below that size, ambient readings for particle smaller than about 20 nm are somewhat unreliable. Overall, the results of Figure 17 show categorically that there is absolutely no distortion in the size distributions between ambient and concentrated aerosols, as the number median diameters (41 nm) and geometric standard deviation (1.7) of the concentrated and ambient aerosols are virtually identical. These results confirm that drying by diffusion returns the concentrated droplets to their original size with minimal distortion.

3.5 Conclusions for the Laboratory and Field Evaluations of the VACES.

The experimental characterization of the versatile coarse, fine and ultrafine concentrators demonstrated that concentration enrichment does not depend on particle size or chemical composition. Volatile species such as ammonium nitrate are preserved through the concentration enrichment process under the laboratory conditions used in this study. Furthermore, the concentration enrichment based on particle counts showed clearly that no particle coagulation occurs during the enrichment process, for any of the three minor-to-total flow configurations tested.

The ability of the VACES to enrich the concentrations of all particles in the fine mode including its ultrafine particle component enables inhalation toxicologists to conduct exposures to any selected sub-range of PM2.5. For example, previous studies in California showed the presence of two sub-modes within the accumulation mode of ambient PM (Hering et al., 1997; John et al., 1990); one mode peaks at around 0.2 μm consisting mainly of gas-to-particle reaction products, such as carbonaceous PM and the other peaks at about 0.7 μm mainly associated with hygroscopic PM such as ammonium sulfate and nitrate. These observations have been confirmed by our recent yearlong measurements at the facility of Rancho Los Amigos in Downey. By thus placing a conventional impactor upstream of the fine concentrator of the VACES, having a 0.4 μm cutpoint, inhalation studies could be conducted to ultrafine PM plus the elemental and organic carbon content of the accumulation mode, but without the majority of its sulfate and nitrate constituents.

4. *In vitro* Experiments at UCLA using the Portable Particle Concentrators

In addition to the animal and human exposures, we are currently using the newly developed versatile concentrators for direct PM collection for *in vitro* tests. Collection and chemical characterization of coarse, fine and ultrafine particles for *in vitro* tests using the combined concentrator/BioSampler method will be conducted in the primary SCPCS locations as well as in the sites where animal exposures to freeway originated aerosol. We have already initiated these studies, by collecting PM samples using the VACES at UCLA and at Rancho Los Amigos. In our current sampling scheme, outdoor particles are collected concurrently with human exposure studies for approximately 5-6 hours using autoclaved BioSamplers. The BioSamplers are connected immediately downstream of the ultrafine plus fine particle concentrator and the coarse concentrators of the VACES. Given that particle growth is based on mixing and saturation with warm water vapor, it is imperative that no bacterial growth occurs during the saturation process. Preliminary analysis of the BioSampler extracts has shown that no bacterial growth occurs during the saturation process. In addition to the biological content of ambient or indoor PM, we also monitor the following parameters over the 6 hours of the experiment: particle number concentration (continuously) and particle mass concentration (time-integrated).

5. Current *In Vivo* Experiments using the Portable Particle Concentrators

In addition to the *in vitro* tests described in the previous paragraph, we conducted our first series of animal exposures to ultrafine and fine particles. These studies were conducted jointly by investigators from UCLA, University of Southern California, UC Irvine and UC Davis. Healthy rats were exposed to fine and ultrafine PM, concentrated by a factor of 22, harvested at UCI (in June 2000) and UCLA (in July 2000) in west Los Angeles. Preliminary measurements in the later location have indicated an unusually high number concentration of both ambient as well as indoor PM on the order of 10,000-50,000 particles/cm^3, roughly 5-10 times higher than levels typically encountered in urban areas of the East Coast of the U.S., which makes these experiments of particular interest. The same particle sampling protocol, currently followed for the *in vitro* tests, was used to monitor the physico-chemical PM characteristics during the animal exposure studies, monitoring of particle mass, number concentration, elemental composition and selected PAH. Biological analyses from these exposure studies are currently under way.

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Table 1. Comparison of ultrafine mass concentration after the multi-slit impactor of the VACES and MOUDI

Ambient ultrafine mass concentration ($\mu g/m^3$)	Multi-Slit Impactor ultrafine mass concentration ($\mu g/m^3$)	Ratio of mass concentrations between Multi-slit impactor and MOUDI[b]
1.89	2.48	1.31
2.78	2.47	0.89
3.28	3.16	0.96
3.81	4.13	1.08
4.23	5.05	1.19
Average		1.07
Standard Deviation		0.15

a. Determined by reference MOUDI sampler.
b. MOUDI Collected particles in the size below 0.18 μm.

Table 2. Coarse Ambient Particle Sulfate Concentrations Determined with the MOUDI and the VACES

Ambient ($\mu g/m^3$)	VACES ($\mu g/m^3$)	Enrichment Factor
1.0	20.8	21.6
1.4	22.0	15.2
0.8	16.6	20.8
1.4	34.0	24.3
2.1	58.5	28.5
	average	22.1
	SD	4.9

Table 3. Coarse Ambient Particle Nitrate Concentrations Determined with the MOUDI and the VACES

Ambient ($\mu g/m^3$)	VACES ($\mu g/m^3$)	Enrichment Factor
4.68	83.85	17.91
6.43	135.87	21.13
3.71	57.80	15.58
6.78	155.77	22.98
5.41	118.28	21.86
	Average	19.90
	SD	2.6

Table 4. Ultrafine PM Number Concentrations Upstream and Downstream of the 0.18 μm Cutpoint Impactor and Downstream of the Ultrafine Concentrator of the VACES. All concentrations are averaged over 30 minutes sampling time.

VACES Particle Number Concentration ($particles/cm^3$)	Particle Number Concentration Downstream of the 0.18 μm Impactor ($particles/cm^3$)	Ambient Particle Number Concentration ($particles/cm^3$)	Ratio of Downstream-to-Upstream the 0.18 μm impactor Concentration ($particles/cm^3$)	Concentration Enrichment
551429	26714	32185	83%	20.7
801429	35000	43166	86%	22.9
420000	23000	31750	74%	18.3
600000	29857	33666	85%	20.1
648571	30428	35714	85%	21.3
795714	38285	45142	84%	20.8
574286	26880	31523	86%	21.4
		Average	0.83	20.8
		SD	0.042	1.41

FIGURE LIST

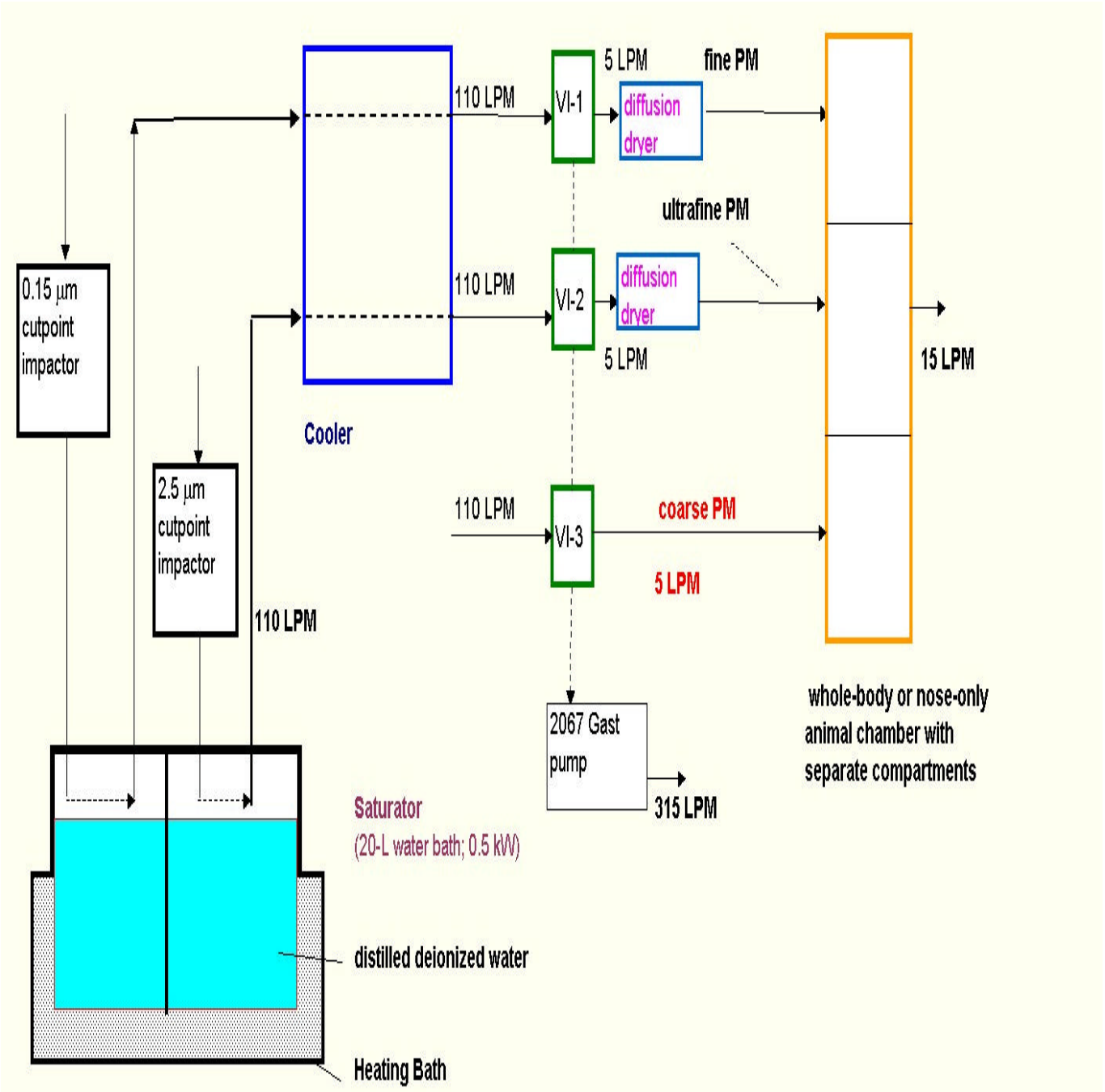


Figure 1a. Versatile Aerosol Concentration Enrichment System (VACES) for concurrent in vivo studies to coarse, fine and ultrafine PM

VI = virtual impactors

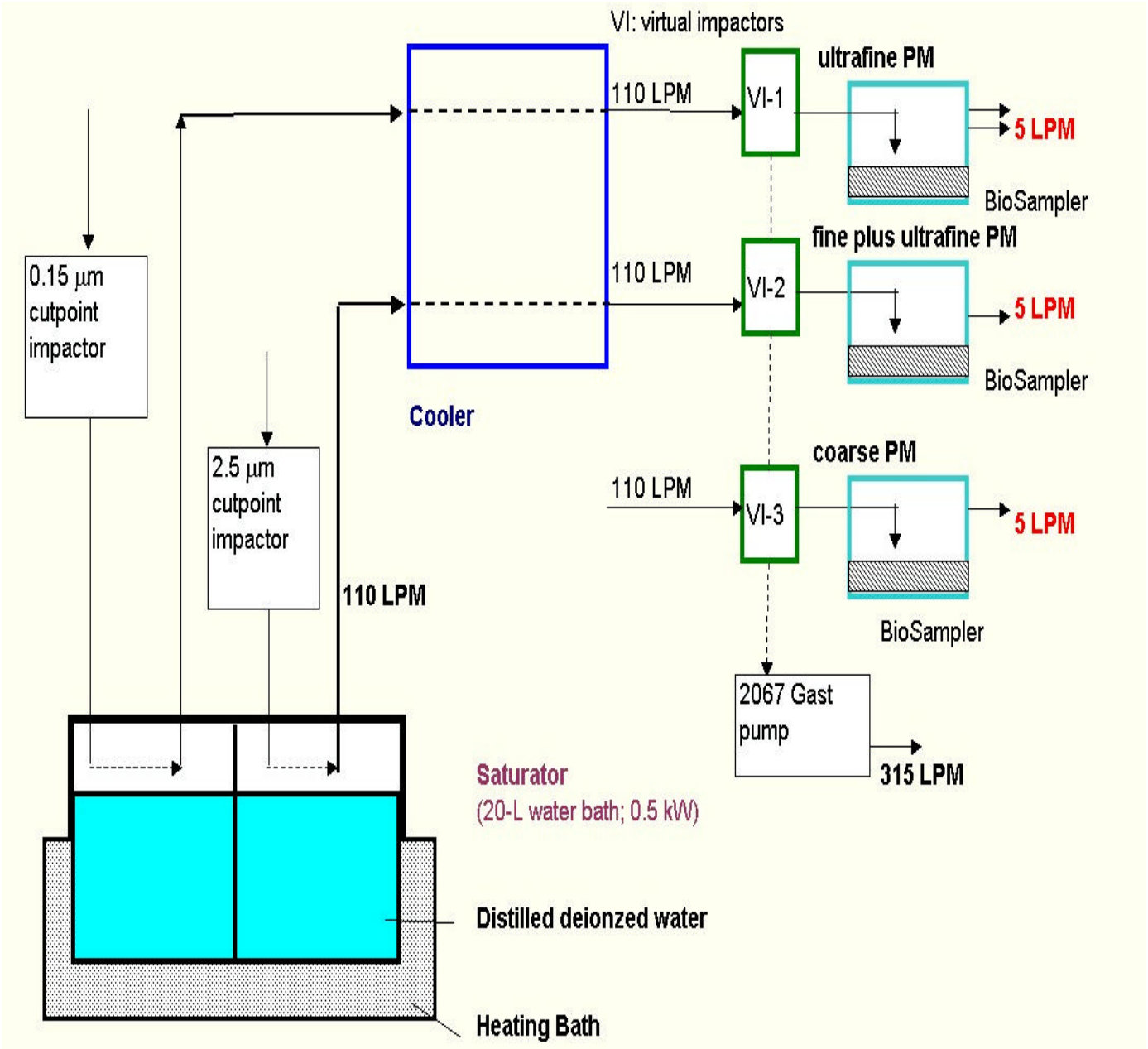


Figure 1b. Versatile Aerosol Concentration Enrichment System (VACES) for in vitro studies

Figure 2. Particle Collection Efficiency of the 2.5 μm Cutpoint Slit Nozzle Impactor. Flow Rate; 110 LPM

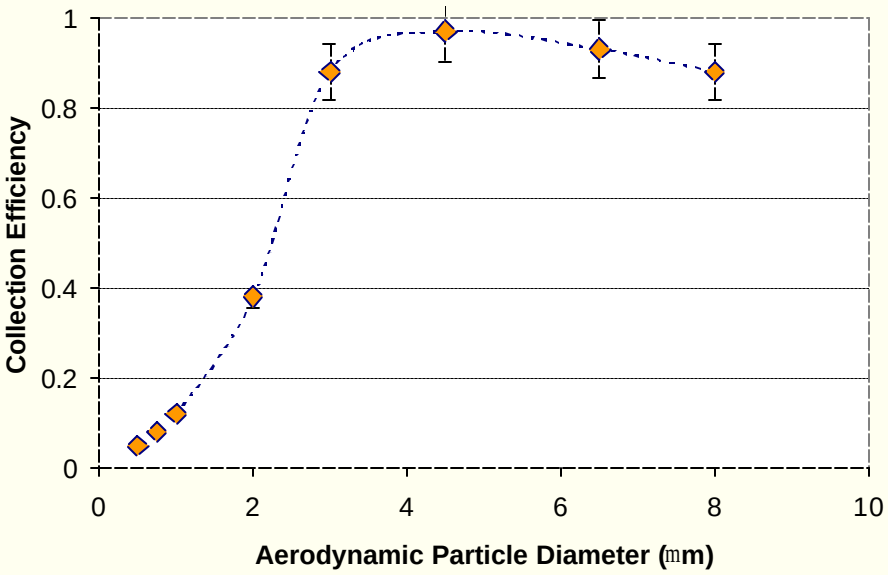


Figure 3. Pressure drop across the 0.18 μm cutpoint, multi-slit impactor as a function of flow rate

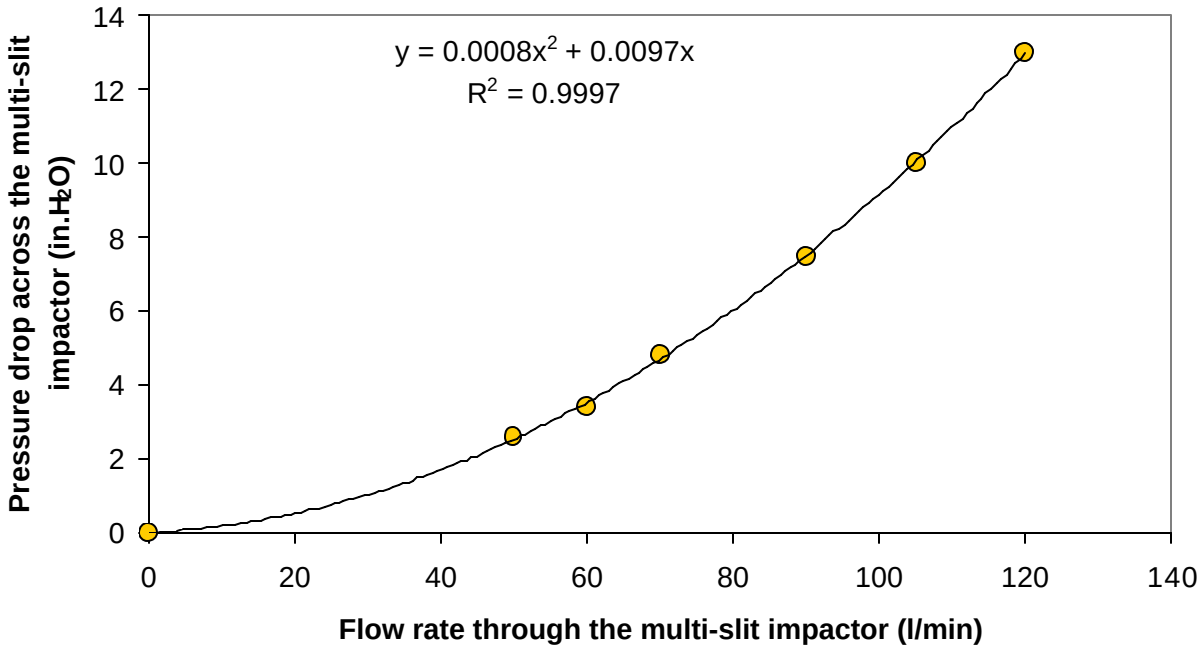


Figure 4. Removal efficiency of multi-slit low pressure drop impactor as function of particle diameter.

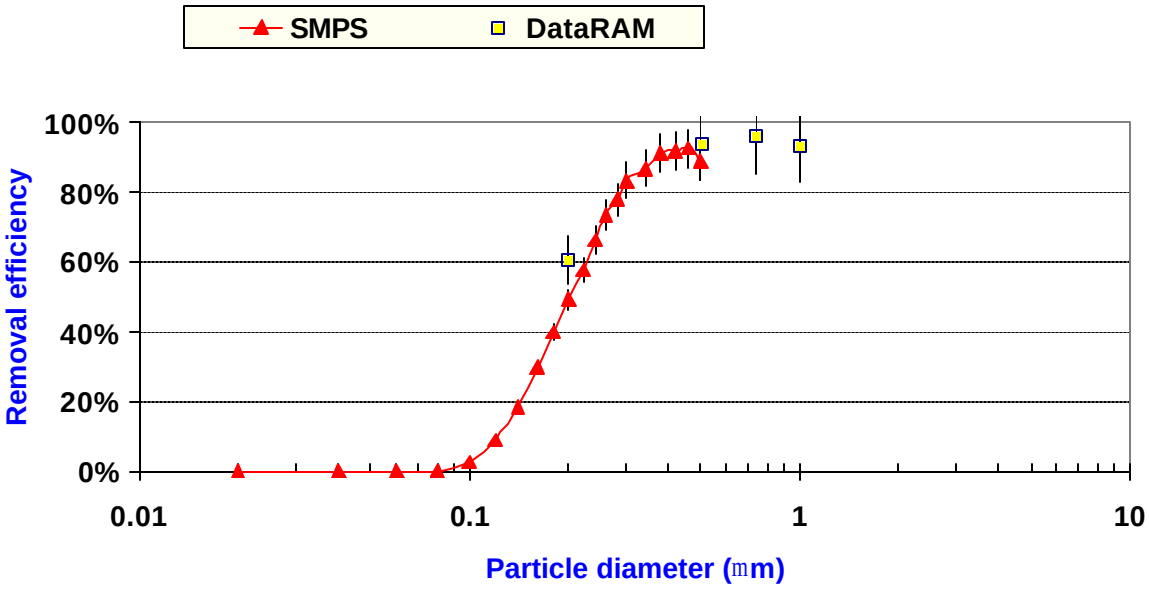


Figure 5. Pressure drop across the BioSampler nozzle as a function of flow rate

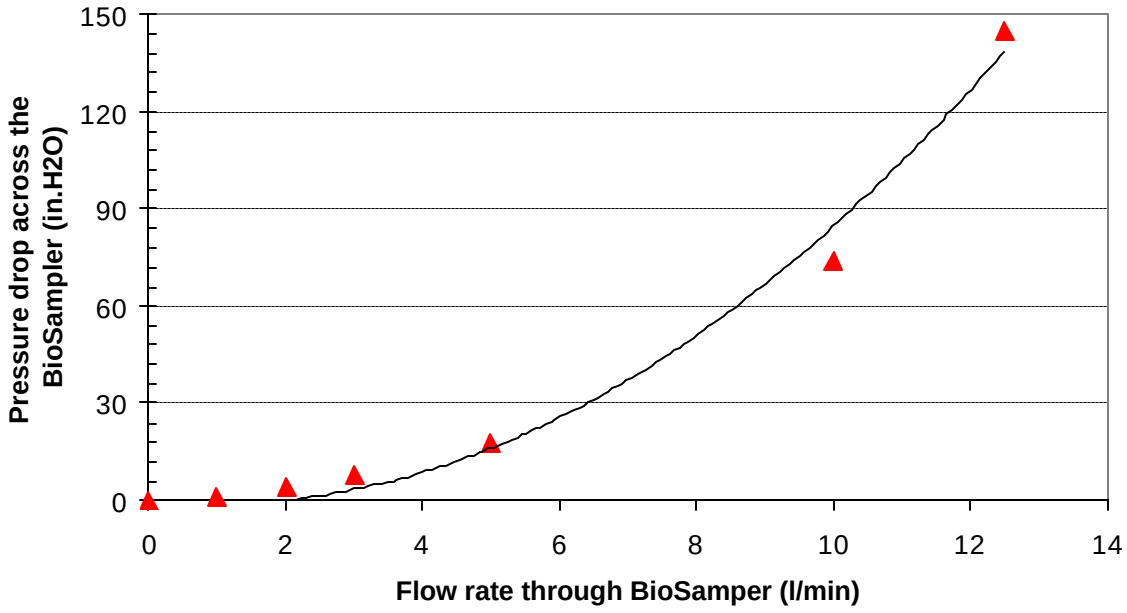


Figure 6. Particle collection efficiency of BioSampler as a function of particle aerodynamic diameter. Sampling flow rate: 5 LPM

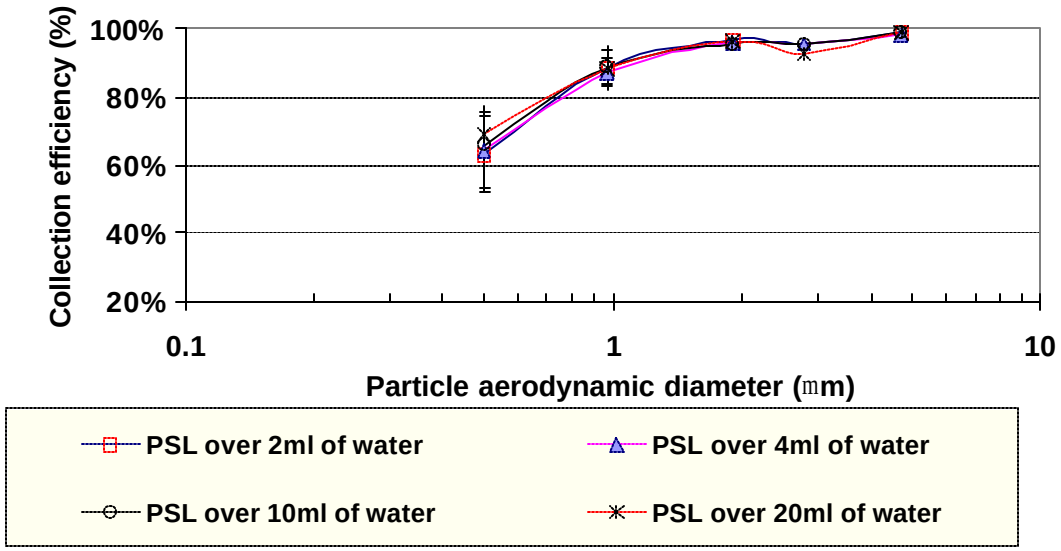


Figure 7. Chracterization of the Versatile Aerosol Concentration Enrichment System for three minor flows. Total intake flow: 220 lmin^{-1}

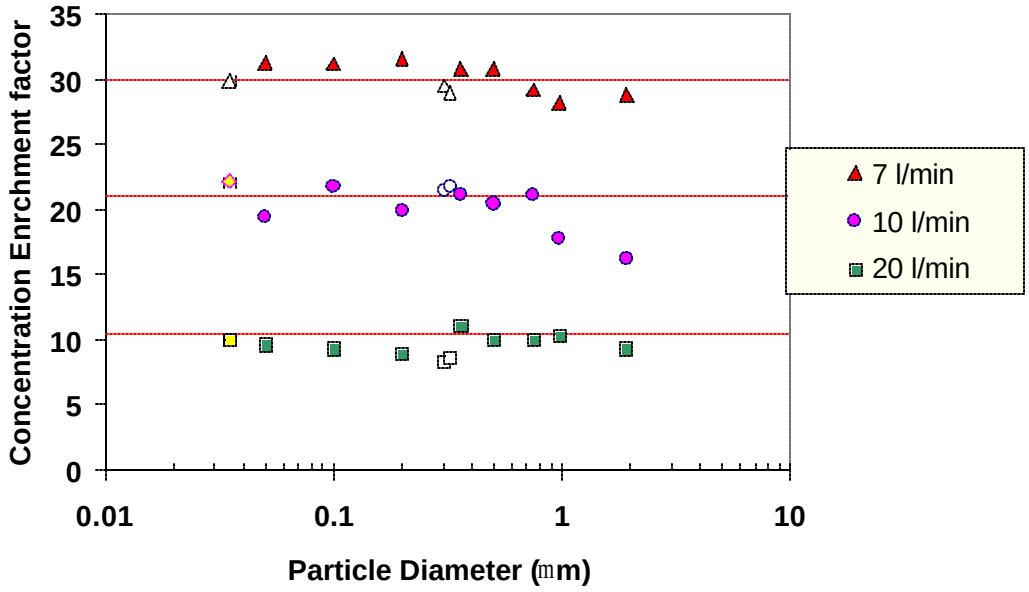
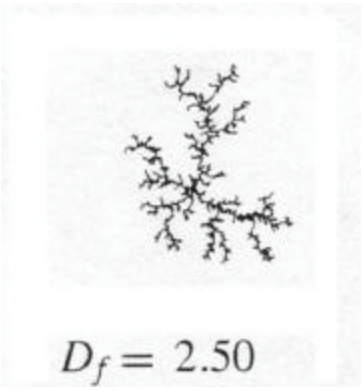
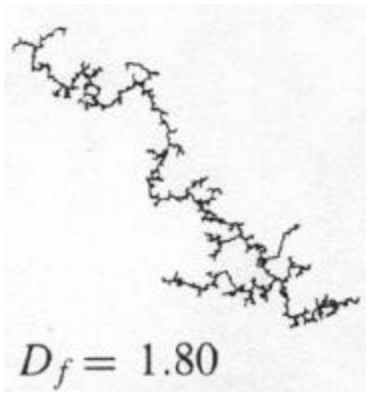


Figure 8. Structure and fractal dimension of agglomerates produced by two computer simulation algorithms (after Schaefer, 1988). Diffusion-limited aggregation was simulated for two subcases, (a) particle-cluster aggregation and (b) cluster-cluster aggregation. Particle-cluster aggregation refers to the release of single particles which attach to a growing cluster by Brownian diffusion. In cluster-cluster aggregation, agglomerates of primary particles are released and collide by Brownian motion.



(a) particle-cluster aggregation



b) cluster-cluster aggregation

Figure 9. Ambient (MOUDI) and VACES Coarse Particle Concentrations

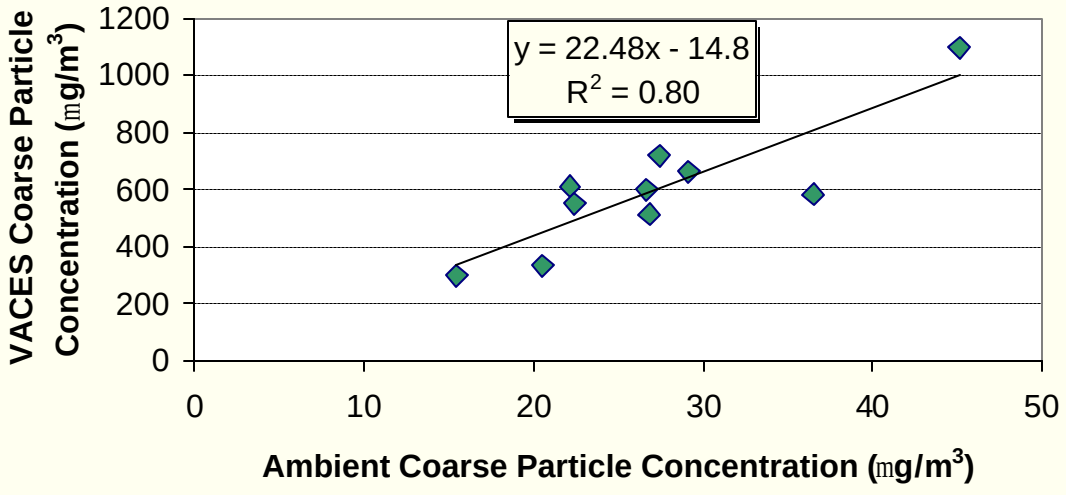


Figure 10. Ambient (MOUDI) and VACES $PM_{2.5}$ Mass Concentrations

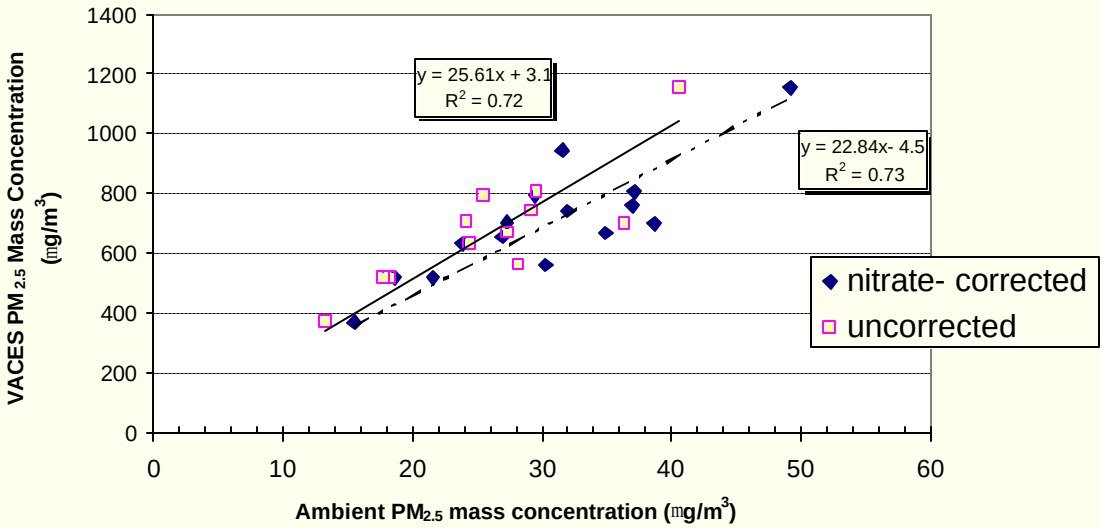


Figure 11. Ambient (MOUDI) and VACES PM2.5 Sulfate Concentrations

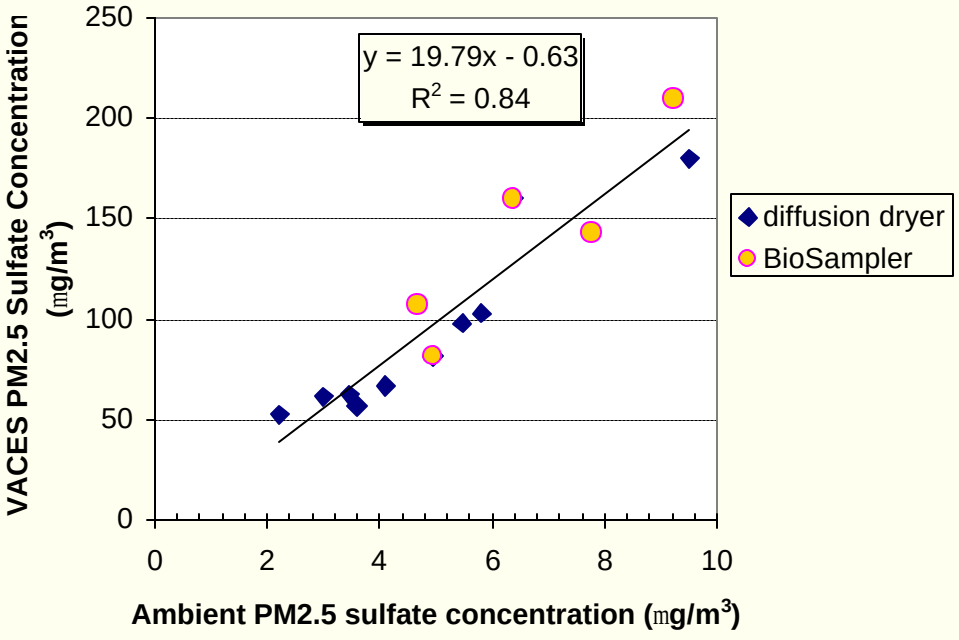


Figure 12. Ambient (MOUDI) and VACES PM2.5 Nitrate Concentrations

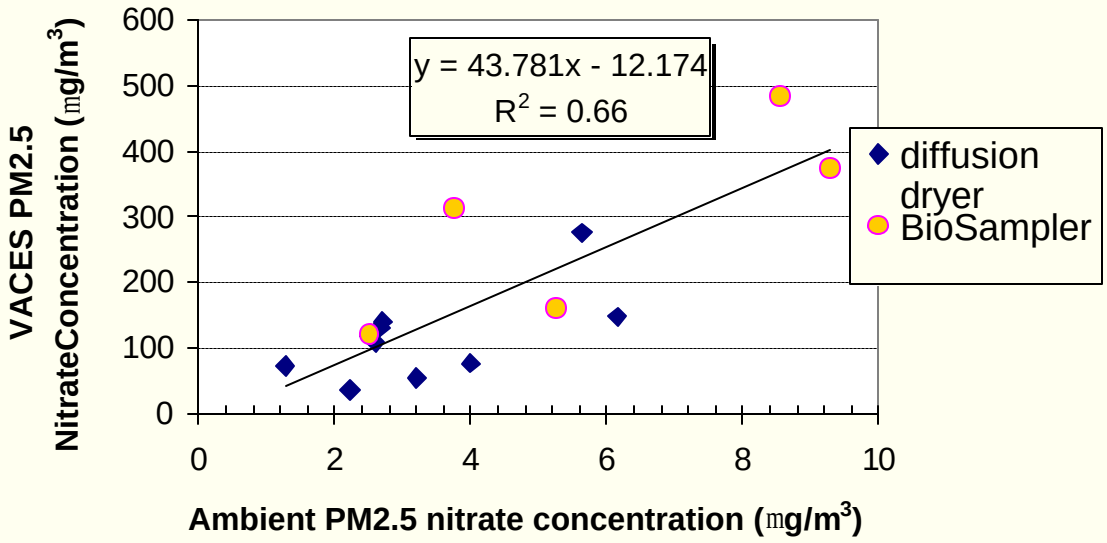


Figure 13

Concentration Enrichment of Selected Trace Elements and Metals by VACES

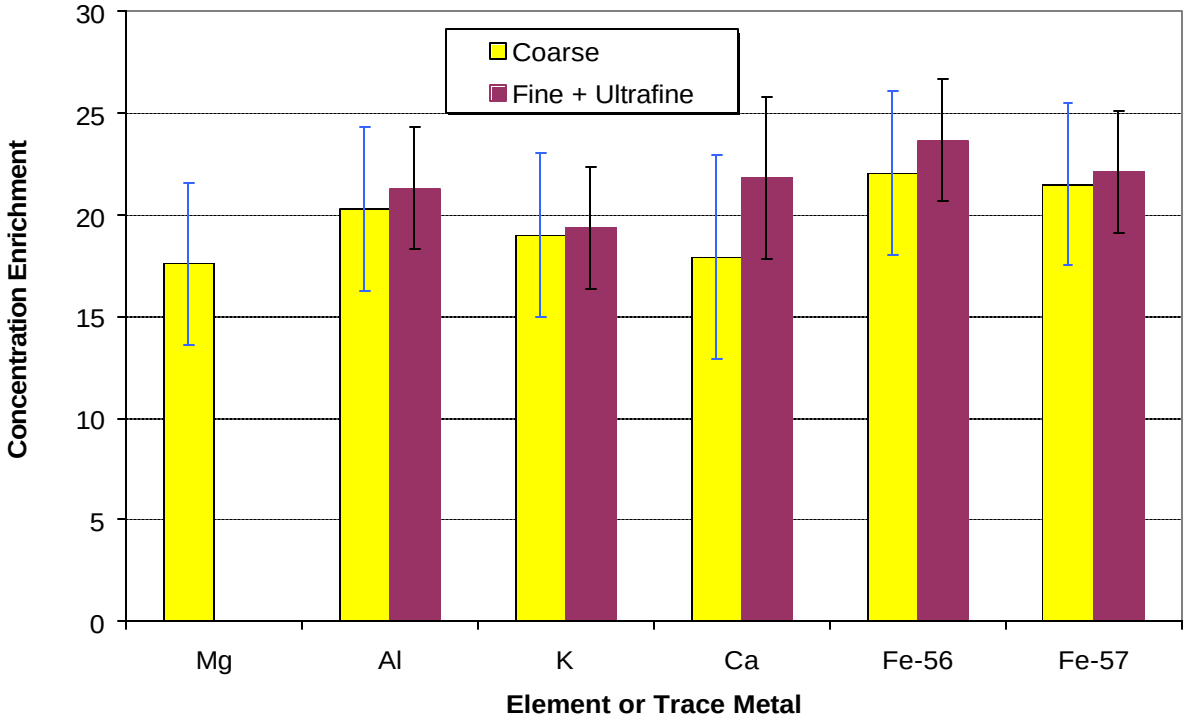


Figure 14. Concentration enrichment of ambient ultrafine particle Elemental Carbon (EC) by VACES

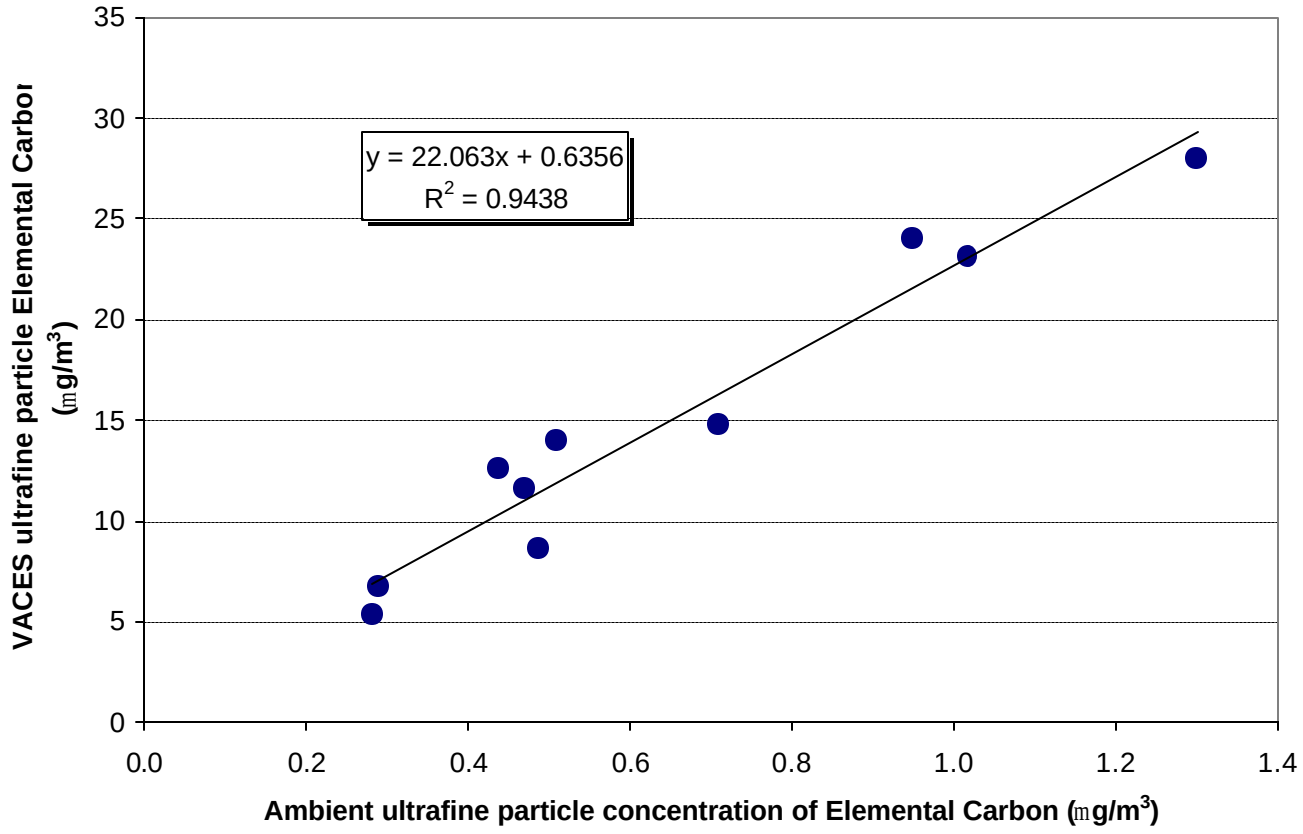


Figure 15. Fractal dimension distribution for agglomerates from the VACES. The count mean D_f value was found to be between 1.6 and 1.8. Samples were taken at the Center for Health Sciences at UCLA using a LPI.

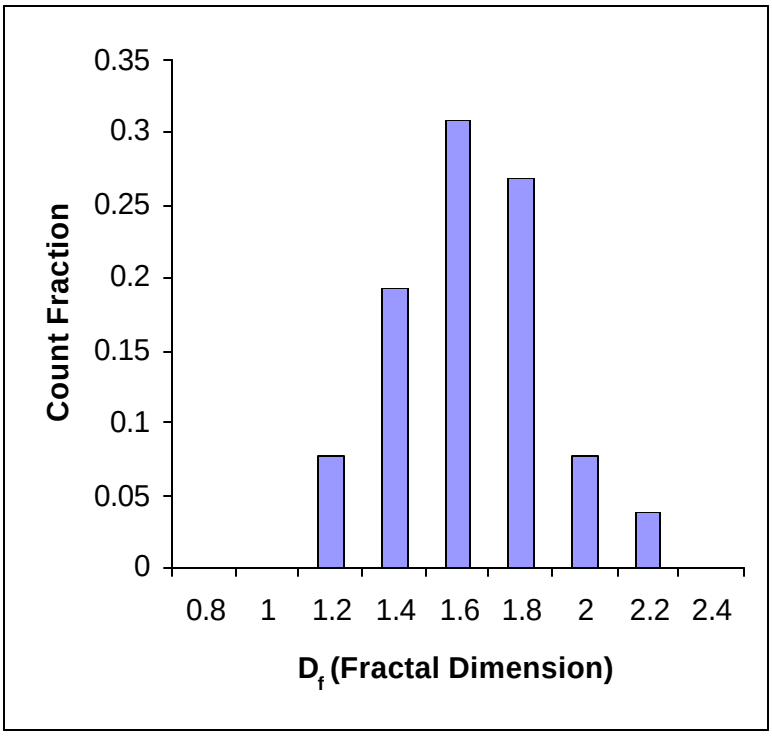


Figure 16. Fractal dimension distribution for agglomerates sampled from the ambient aerosol. The count mean D_f value was found to be between 1.6 and 1.8. Samples were taken at the Center for Health Sciences at UCLA on 8/22/00 using a LPI.

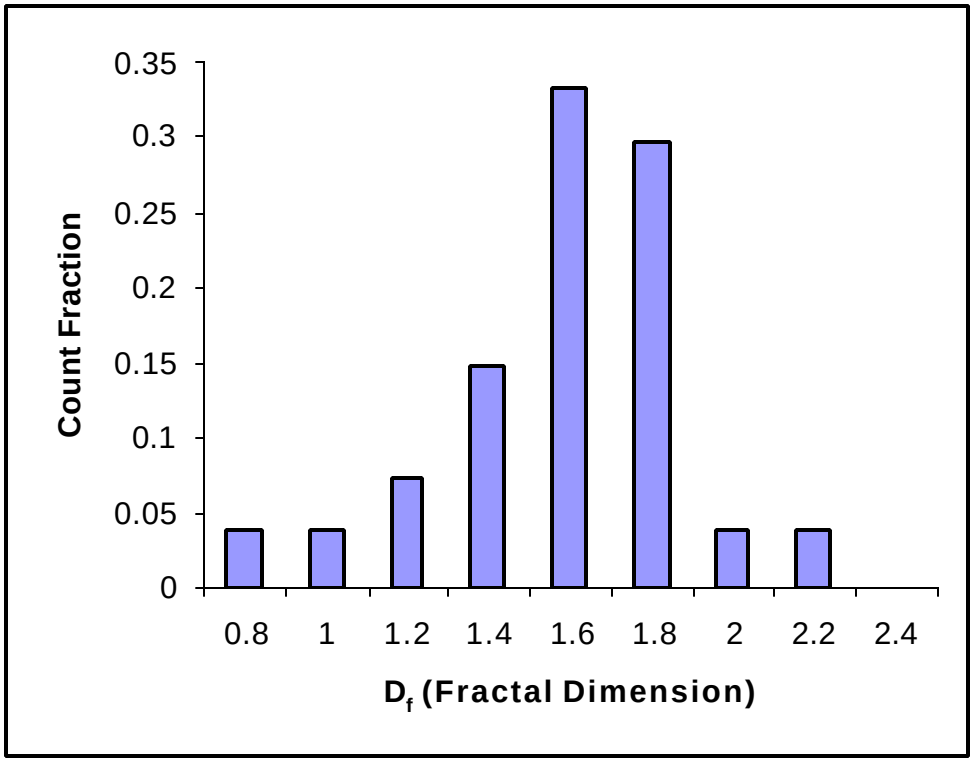
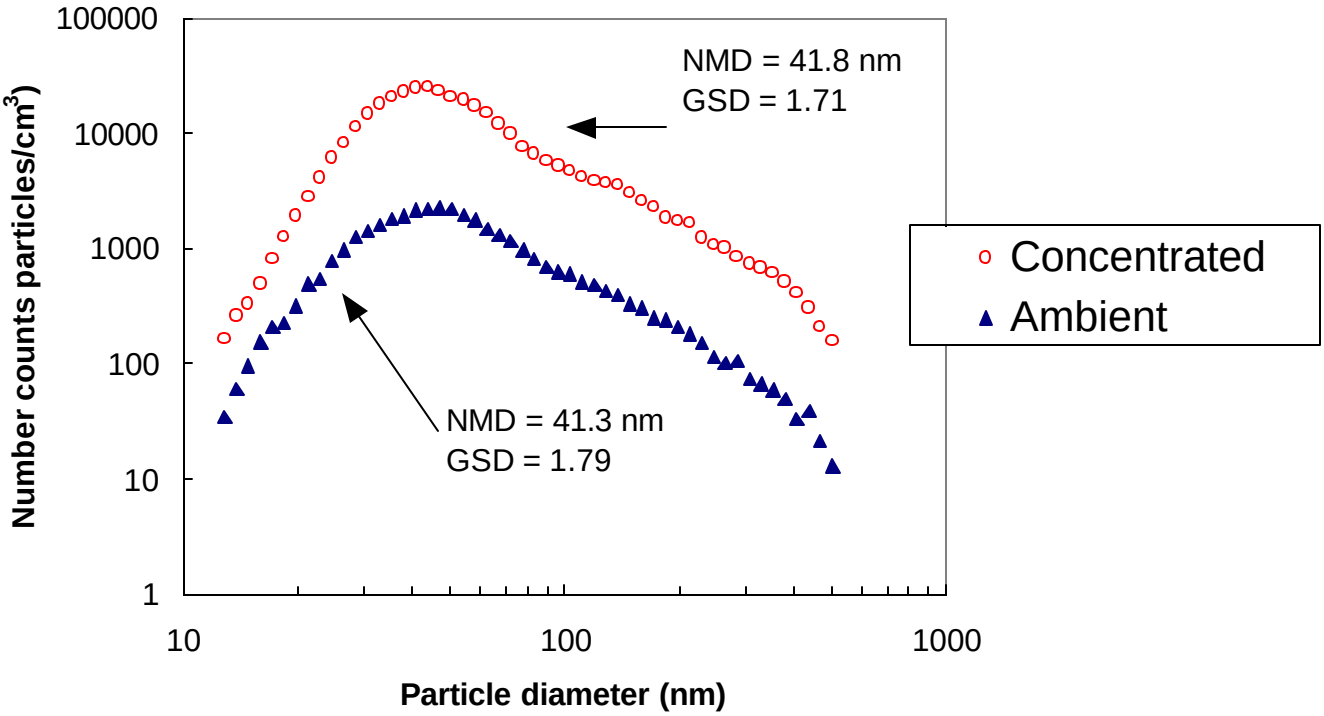


Figure 17. Size distribution of ambient aerosols before and after the VACES measured by SMPS



FINAL REPORT

Development and application of ambient aerosol concentrators to conduct health effects studies in the Los Angeles Basin

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Part 2

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Development and application of ambient aerosol concentrators to conduct health effects studies in the Los Angeles Basin

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ABSTRACT

A transportable inhalation exposure facility that provides concentrated coarse, fine and ultrafine ambient particulate matter for studies in experimental animal models has been developed, constructed, and validated during the course of this five year research program. The facility can be configured to collect large quantities of size-selected particulate matter for compositional analysis of particles and for in vitro toxicology experiments. A similar concentrator system suitable for human exposure to coarse, fine and ultrafine ambient particles was also developed and validated. The concentrator systems were applied to investigations of hypotheses concerning the public health impacts of exposure to particulate matter, including field studies of mice and rats exposed to size selected particulate matter in locations around the Los Angeles Basin, such as near-source sites at freeways. A series of clinical studies on human volunteers has been initiated using the transportable concentrator systems, and extensive characterization studies of the chemical, physical and toxicological properties of ambient particulate matter were carried out on samples of particles that were collected for in vitro studies.

EXECUTIVE SUMMARY

Background

The chemical composition and size distribution of particulate matter (PM) varies significantly by location and time because of a wide range of PM sources, meteorological and atmospheric chemistry differences and related factors. There are significant uncertainties associated with defining the consequent variation of health risks, yet this knowledge is critical for the design of a public health oriented management strategy. The development of particulate matter concentrators has fundamentally changed research designed to determine the physical and chemical characteristics of particles that are associated with adverse health effects, because they allow exposures to ambient particles at concentrations sufficient for sensitive experimental studies. The work that was accomplished under this contract created new particle concentrator technologies and applied them to investigate hypotheses concerning spatial and temporal variability in chemical and physical characteristics of particles and how these factors affect toxicological responses to inhalation exposure of ambient PM. Highly versatile mobile research facilities that provide proven capability for exposing experimental animals and human volunteers to highly concentrated, size selected ambient PM at locations across the Los Angeles Basin were designed and validated. The new concentrators were applied to studies of animal and in vitro toxicological testing and a series of human clinical studies. The combination of mobility, particle size selectivity, and extensive concentration capability create unique and very powerful research tools that can generate health effects and mechanistic data on which health-based management policies can be based. The work for this contract was coordinated and directly linked to extensive studies on particulate matter supported by the U.S. EPA through the Supersites program and the Southern California Particle Center. The linkage of these and other major funding sources has led to the production of an exceptionally rich body of research findings that would not have been possible without the initiative undertaken by the ARB to provide funding provided for concentrator technology that has been a critical component of our investigative program.

Materials and Methods

A concentrator system for coarse, fine and ultrafine PM was developed and validated. The concentrators were designed to operate at a very low pressure drop and at high flow rates. They are suitable for human exposures in clinical studies and can provide significant concentration of PM over ambient levels. A Versatile Aerosol Concentration Enrichment System (VACES) was applied to studies of mice and rats exposed near freeways and at locations across the LAB to test hypotheses concerning the chemical composition and size factors of PM that dictate the adverse health effects associated with exposure. Specific hypotheses explored include (1) PM from mobile emissions near a heavily trafficked roadway will exacerbate airway inflammation and/or induce allergic airway effects in a sensitized experimental model in a dose-dependent manner; (2) PM components associated with automotive emissions will associate more strongly with allergic inflammatory responses than will components more representative of background or regional PM; (3) chemical transformations during transport from source to receptor sites in the LA Basin modify the toxicity and allergic potency of the urban PM. After exposure, biological markers of responses associated with airway allergies were examined. In other studies, the VACES has been used to collect large amounts of size segregated PM for chemical and toxicological analysis back in the laboratory. These *in vitro* collections were the subject of extensive analyses of chemical composition and toxicological properties. Toxicological studies were focused on assays that measure the potential of the sample to produce oxidative stress. The concentrators for human exposure were installed in an exposure facility and applied to inhalation

studies of human volunteers.

Results

In vitro analysis of concentrated PM has shown that Los Angeles Basin ambient particles possess oxidative properties that can result in cellular oxidative stress and related pro-inflammatory processes. These properties are more prevalent on a mass basis in the ultrafine fraction as compared with larger size fractions and this association may be the result of combustion derived carbon compounds. Exposure to concentrated ambient particles (CAPs) very near a heavily trafficked roadway (50 meters) enhanced inflammatory and allergic responses in relation to unexposed mice. The inflammatory and allergic changes did not occur in mice exposed 150 m downwind. Exposure-response relationships were observed between airway allergy responses and the concentrations of elemental or organic carbon in the CAPs at the site 50 m downwind of the roadway. Studies in mice and rats of allergic airways effects at source and receptor sites did not confirm the hypothesis that PM from receptor locations possesses altered toxic properties in comparison to the source sites sampled. Limited findings were consistent with the hypothesis that PM collected during low photochemical season or from source sites may have increased activity toward the biological markers examined. This is generally consistent with findings from the *in vitro* and animal results of the freeway study that suggest very fresh ultrafine emissions may have increased oxidative potential. Further studies of location and source effects on particle composition and toxicity are underway. Markers of brain inflammation were increased in mice exposed to CAPs at the freeway location, indicating that more studies on nervous system endpoints are warranted.

Conclusions and Recommendations

We have designed and developed uniquely powerful research tools in the mobile concentrator facilities and put them to work in a wide array of investigations. The materials collected with the concentrators have led to numerous publications describing the measurement, sources, size distribution, chemical composition, physical state, and spatial and temporal variability of suspended particulate matter (PM) in the Los Angeles Basin (LAB). Sampling locations were chosen to provide wide geographical and seasonal coverage, including urban "source" sites and downwind "receptor" sites. Intensive PM measurements were conducted up and downwind of freeways of the LAB, to characterize near-roadway exposure environments, producing data that can help inform particulate matter management strategies. The concentrators have been and will continue to be central to *in vivo* and *in vitro* health effects research, including extensive toxicological studies in mice and rats and a series of studies of elderly human volunteers. *In vitro* study of the chemical and toxicological properties of ambient PM has demonstrated differences related to particle size and composition. Ultrafine particles and high organic and elemental carbon content have been associated with increased activity in our *in vitro* assays for oxidative properties to date. Further analysis of the chemical composition correlates of toxic properties are ongoing and will provide additional information on which particle constituents may be most important for specific toxicity parameters. Chemical composition data are also important for dose-response analyses of the experimental results of the animal studies that go beyond mass-based doses. Compositional parameters that are identified as useful explanatory variables can be compared to those with toxic properties identified in analysis of the in vitro data, thus expanding the overall understanding of key mechanisms in PM associated health effects. The findings of the animal work indicate the value of follow up experiments to clarify suggestive but inconclusive results of allergic airway endpoints in mice and rats in our source/receptor studies, using perhaps different exposure parameters and outcome measurements.

INTRODUCTION AND BACKGROUND

The overall goal of the five year research program was to develop, construct, and validate particle concentration systems and to apply them to investigate hypotheses concerning the health effects associated with exposure to airborne particulate matter (PM). The concentrator systems funded by the Air Resources Board (ARB) consists of mobile, ultrafine, fine, and coarse concentrators for animal exposure studies and in vitro sample collections, and a comparable concentration enrichment system suitable for human clinical studies.

In 1997, U.S. EPA developed new standards for $PM_{2.5}$ on the basis of epidemiological findings of consistent associations between PM exposures and various adverse health effects including premature mortality, exacerbation of asthma and other respiratory tract illnesses and decreased lung function. Scientific understanding of the underlying biological basis of these associations has progressed significantly since that time, but is still under investigation. Currently, uncertainty remains about the interpretation of findings on $PM_{2.5}$ and PM_{10} given the limited scientific information about the types of particles or particle composition that caused the identified adverse health effects in epidemiological studies, the toxicological mechanisms by which the particles cause effects, the relationship between particle source and personal exposures, and other related questions. There are significant differences in the range of chemical composition and size distribution of PM because of the wide range of sources, meteorological conditions, atmospheric chemistry, diurnal and seasonal factors across the Los Angeles Basin. Which types of PM are most critical for public health remains unclear. Because of these uncertainties there is a need for improved mechanistic and hypothesis-driven research on the health effects of PM. Identification of the critical particles will support cost-effective abatement strategies for particulate matter.

The development of particle concentrators has dramatically expanded the field of experimental research on PM. Epidemiological studies rely on exposure measurements of particles at ambient concentrations that are necessarily imperfect, especially with respect to individual exposures. Historically, controlled experimental exposure studies of human volunteers or animals had to rely on either ambient concentrations that were too low to provide sufficient sensitivity of outcome measures, or laboratory generated aerosols that were physically and chemically unlike the ambient aerosols that pose the public health threat of concern to policy makers and management agencies. With concentrator technology experiments can now be devised to test the toxicological properties of ambient aerosols with all their attendant complexities, but at exposure levels that can provide meaningful results. Using these experimental systems studies of the biological mechanisms underlying the adverse health effects associated with particle exposures as well as dose-response studies can be performed. This technological advance has and will continue to have long term significance in PM research.

An especially difficult problem has been the concentration of ultrafine particles. Although the mass fraction of the ultrafine mode is low this size range contains the highest number of ambient particles by counts as well as the surface area and there has been increasing toxicological and epidemiological evidence linking respiratory health effects and exposures to ultrafine particles. Epidemiological studies have not yet provided clear indication of whether it is particle mass, surface area, number concentrations, or chemical composition that may be responsible for observed health outcomes presumably attributable to particulate matter (PM), and experimental studies are needed. In order to assess the toxicity of just the ambient ultrafine fraction, ultrafine particles need to be separated from the much larger mass of the accumulation mode of $PM_{2.5}$. Ideally, this separation should also occur under near-atmospheric pressure conditions in order to make human or animal exposures to these particles feasible. The new impactors and concentration methods that were developed under this ARB

funding have made ambient ultrafine material available for a range of research approaches. Direct toxicological comparisons between fine and ultrafine PM can now be performed, a feature that will ultimately be of paramount importance in assessing the health effects of these PM modes.

The additional capability gained by installing concentrators in a mobile facility provides a means to conduct animal studies to assess the toxicity of PM emissions that arise from different sources, in that certain sources may dominate PM exposure at selected locations. For example, we are now able to pursue hypotheses concerning mobile source PM toxicity by positioning the concentrators at varying distances from roadways to characterize the toxicological properties of PM collected near the freeway in exposed animal models or in vitro tests. Mobile exposure systems allow exploration of other hypotheses related to spatial variability in exposure, such as the differences in aerosol toxicity resulting from photochemical and other changes as an air mass moves and is subjected to atmospheric chemical processes. The availability of both a fine and ultrafine concentrator which can be used in animal studies provides a basis to study the effects of ultrafine particles in comparison with ultrafine plus fines.

The research performed under this contract with ARB has been interlinked with major research initiatives developed as part of the Southern California Particle Center and Supersite and funded by U.S. EPA. The EPA funding for the SCPC followed the ARB award, and the research proposed for the Particle Center and for the Supersite was dependant upon the ARB-supported concentrator development. Thus this project has had a major impact beyond the specific research applications reported here. The availability of Supersite analytical capabilities has vastly enriched the possibilities for analysis of particles collected with the ARB-funded concentrators, which allows much more complex analysis of the data resulting from animal and in vitro studies of particle toxicity. In this final report to ARB, we have focused discussion on the concentrator development and animal toxicology studies, since these were fully funded by ARB. The ARB-funded concentrator was also used in studies of acute cardiopulmonary responses to concentrated particulate matter in healthy, asthmatic and COPD-diagnosed volunteers. The human studies were primarily funded by USEPA and are thus not discussed in detail here. The concentrator was used to collect particles for in vitro toxicology studies that were funded by USEPA; ARB funding was used to support the actual collection process. The major conclusions of the in vitro studies are described in this report.

TASK 1: Development and application of a transportable ambient aerosol concentrator and exposure facility suitable for human exposure to coarse, fine and ultrafine particles

Objectives

The main goal of the research was to develop transportable inhalation exposure facilities that provide concentrated coarse, fine and ultrafine real-life ambient particles for studies of inhalation toxicology animal models, human volunteers and in vitro toxicological experiments. Specific aims for concentrator development included:

1. Construct, install, and test a transportable exposure facility that includes fine, coarse and ultrafine particle concentrators for animal exposures and collection of particles for *in vitro* studies.
2. Develop a coarse concentrator suitable for human exposures.
3. Apply the coarse concentrator in a clinical study of human volunteers funded by USEPA.
4. Develop and evaluate a high-volume, low pressure drop impactor for separation of ultrafine from accumulation mode particles.
5. Develop and evaluate a compact facility for exposure of humans to concentrated ambient ultrafine particles only, that can achieve concentration of a factor of 8-10 and operate with a low-pressure drop suitable for human exposures.
6. Quality assurance of the concentrators and components that have to be or have been developed by USC for mobile concentrator facilities by using test aerosols and reference sampling devices.

As the research proceeded and concentrators were tested, two sub-aims emerged:

7. Design and implement a new cooling system for the condensation step of the ultrafine concentrator.
8. Implement a facemask in the trailer used for human inhalation studies, to improve ultrafine particle exposure parameters.

Over the course of the five year funding period, all of these objectives and aims have been achieved. Development of a compact, easily transportable concentrator system that includes coarse, fine and ultrafine concentrators for animal exposures and collection of particles for in vitro study was completed in the first year of the project. Subsequently, the construction and evaluation of new coarse and fine plus ultrafine concentrators was completed. All concentrators have been laboratory and field tested and installed in mobile trailers or other vehicles for use in exposure studies. For the new ultrafine concentrator, an improved cooling system was developed and tested and a facemask was successfully introduced for use in human exposure studies. As reported under Tasks 2-4, significant research efforts including animal exposure studies in a variety of locations and collection of ambient particulate matter for in vitro toxicology experiments made use of new concentrator technologies, expanding the possibilities for research on the health effects of ambient particulate matter exposure.

In sections following that report on Task 1 activities, the above aims will be addressed in turn. Most of the work is published and rather than duplicate completed reports and manuscripts, which would result in an unduly lengthy document, we have provided summaries of each with selected publications included as appendices to this report. Finally, an overall summary and conclusions

section addresses the scientific and regulatory impacts of concentrator development as a whole.

AIM 1: Construct, install, and test a transportable Exposure Facility that includes fine, coarse and ultrafine particle concentrators.

We completed the construction of the Versatile Aerosol Concentrator Exposure System (VACES) during the first year of this funding. A final report on the work covering the Project Period: May 31, 1999 – August 31, 2000 is available on the ARB website under "Completed Projects, Particulate Matter" at: ftp://ftp.arb.ca.gov/carbis/research/apr/past/98-316.pdf. See also Kim 2000, 2001a, and 2001b for the published work. The abstract of the year 1 final report to ARB follows:

Particles in the atmosphere are a complex and heterogeneous mixture that have been difficult to reproduce in the laboratory. As a result, scientists have not been able to conduct toxicological and clinical experiments that replicate realistic conditions in the environment. Investigators have typically generated synthetic atmospheres that differ in significant ways from the true environment. This has made it difficult to address unanswered questions about the true nature and mechanisms of action of atmospheric particles (PM) on human health.

To study the health effect of PM in a realistic setting we designed, built and evaluated a Versatile Aerosol Concentration System (VACES) for testing the toxicological significance of concentrated atmospheric aerosols in animals. The system has been designed for conducting animal exposure studies, but it can be readily scaled-up for human exposures. This report describes the development and bench-testing of a VACES capable of simultaneously concentrating ambient particles of the coarse, fine and ultrafine size fractions for conducting in vivo and in vitro exposure studies to "real" ambient aerosols over a wide dynamic range of concentrations. The VACES consists of three parallel sampling lines (concentrators), each operating at an intake flow rate of 110 LPM. Coarse particles are concentrated using a single round nozzle virtual impactor. Concentration enrichment of $PM_{2.5}$ and ultrafine particles is accomplished by first drawing air samples through two parallel lines, having 2.5 and 0.18 μm cutpoint pre-impactors, respectively, to remove particles larger than these sizes from the air sample. Both of the smaller PM fractions are drawn through a saturation/condensation system that grows particles to 2-3 mm droplets, which are subsequently concentrated by virtual impaction. A diffusion dryer is used in the fine and ultrafine concentrators to remove excess vapor and return the concentrated particles to their original size, prior to supplying them for in vivo exposures. The VACES can also provide highly concentrated liquid suspensions of particles of these three modes for in vitro toxicity studies. This is accomplished by connecting the concentrated output (minor) flows of each of the VACES parallel concentrators to a liquid impinger (BioSampler), used in a modified configuration, to collect particles under near-ambient pressure.

Detailed laboratory characterization of the individual components of the VACES are presented in this paper, including evaluation of its ability to preserve particle mass, number, and chemical species during the concentration enrichment process. The experimental characterization of the VACES demonstrated that concentration enrichment is accomplished with very high efficiency, minimal particle losses and without any dependence on particle size or chemical composition.

During the field evaluation of the VACES, the enrichment and preservation of ambient ultrafine, fine and coarse particles by size and chemical composition is determined by comparisons made between the VACES and a co-located multistage MOUDI impactor, used as a reference sampler. Furthermore, preservation of the ultrafine fraction is measured by the enrichment based on ultrafine

particle numbers, morphological characteristics as well as their elemental carbon (EC) content. The results suggest that the concentration enrichment process of the VACES does not differentially affect the particle size or chemical composition of ambient PM. The following fractions: 1) mass (coarse and fine PM); 2) number (ultrafine PM); 3) sulfate (fine PM); 4) nitrate (fine PM, after correcting for nitrate losses within the MOUDI); 5) EC (ultrafine PM); and 6) selected trace elements and metals (coarse and fine PM), are concentrated very close to the "ideal" enrichment value of 22 – thereby indicating a near 100% concentration efficiency for the VACES. The field results also suggest that volatile species, such as ammonium nitrate, are also preserved throughout the supersaturation and concentration-enrichment processes. Furthermore, ultrafine particles are concentrated without substantial changes in their compactness or denseness, as measured by fractal dimension analysis.

AIM 2: Develop a coarse concentrator suitable for human exposure studies

During the period covered by this report, we developed and evaluated a Coarse Particle Concentrator (CPC). This system was designed to concentrate coarse, real-life ambient particles and to be installed in a transportable inhalation exposure facility to evaluate cardiopulmonary responses of different groups of human volunteers exposed to coarse PM. The CPC is portable and compact. It can increase coarse particle concentrations up to 40-fold for use in exposure studies, with a flexible minor-to-total flow ratio that allows the exposure level to be adjusted. CPC operations have been validated in the laboratory and field through detailed comparisons of mass, sulfate, nitrate, and selected trace elements and metals in ambient and concentrated aerosols. A summary of this work follows. A manuscript describing the development and performance of the CPC was published in Aerosol Science and Technology (Chang et al., 2002). For a full treatment of Materials and Methods, Results and Discussion of this work, please refer to the publication, included as Appendix A.

Materials and Methods

The CPC consists of 10 single-nozzle virtual impactors developed by the department of Civil Engineering at the University of Southern California, arrayed in a 2 x 5 layout. Each of the ten impactors operates at an intake flow rate of 100 LPM, yielding a total intake flow of 1,000 LPM. Particles smaller than 10 μm aerodynamic diameter are drawn through the virtual impactor and accelerated through a circular nozzle with a 50% cutpoint of about 2 μm at a 100 LPM intake flow. Particles smaller than this cutpoint are diverted through the major flow while the coarse-mode particles (2.5-10 μm) cross the deflected airstream and are drawn through the collection nozzle in the minor flow. The 10 minor flows are joined and enter a 5cm tube that can be connected to an exposure chamber. The minor flows can be adjusted from 33 to 120 LPM in order to enrich ambient coarse PM concentrations by a factor of 8–30, depending on the desirable exposure level and flow rate needed. See Figures 1 and 2 in Appendix A for illustration.

The CPC was evaluated in the laboratory as well as validated in field experiments at the University of Southern California, in Los Angeles, CA, and in Bilthoven, the Netherlands. Monodisperse aerosols (1-10 μm) of fluorescent polystyrene latex particles were used for laboratory evaluation of the virtual impactors at 3 minor flow rates (3.3, 7, and 10 LPM). Concentration enrichment of particles from 1-5 μm was measured by comparing mass concentration by nephelometer, connected either at the intake or after the minor flow of the concentrator. Similarly, concentration enrichment of particles from 5-9 μm was determined by collection on glass fiber filters.

For field evaluation, particles from the CPC minor flow and a collocated modified micro-orifice uniform deposit impactor (MOUDI, MSP Corporation, Minn. MN) were collected on PTFE

filters. The CPC was operated at a total flow rate of 1,000 LPM and a minor flow of 33 LPM, corresponding to an idea enrichment factor of 30, and the MOUDI was sampled at 30 LPM. The filters were analyzed for particle mass, sulfate, nitrate, trace element and metal concentrations.

Results

The laboratory evaluation indicated that extremely efficient concentration enrichment was obtained for 2.5–10 μm particles. For this size range, the size distribution of the concentrated flow was preserved, and did not depend on particle size. See Figure 3, Appendix A for the concentration enrichment at three minor flow rates over particle diameter ranging from 1-9 μm.

In the field tests, concentration enrichment factors in the range of 26 to 30 were achieved based on particle mass, sulfate, and nitrate as well as selected trace element and metal concentrations (Al, Si, Ca, Fe, K, Mn, Cu, Zn, Ti). CPC and MOUDI concentrations were highly correlated for all species, with R^2 in the range of 0.74 to 0.89. See Tables 1 and 2, appendix A, for enrichment factors and correlations. Taking all ten analyzed metals and elements together and plotting MOUDI concentrations versus CPC concentrations a very high correlation was observed that was independent of the amount of chemical constituent in the aerosol (see Figure 8, Appendix A).

Discussion

The use of round (compared to rectangular geometry) nozzle virtual impactors in the CPC results in a high concentration efficiency, which reduces the CPC size as well as the power requirement that is required for its operation. The compact size of the CPC makes it readily transportable to desired locations for exposure to coarse mode particles derived from different sources and thus of varying chemical composition. Characterization of the CPC in the laboratory and field confirmed high enrichment factors without distortion of aerosol composition.

AIM 3: Apply the coarse concentrator in a clinical study of human volunteers

The newly developed capability for concentrating coarse particles provided an opportunity to clinically assess the effects of coarse particles in a potentially susceptible group of asthmatic volunteers. The study of human volunteers was primarily funded by the EPA grant that established the SCPC. On average, 40-50% of PM_{10} in the Los Angeles area is coarse, depending on location, so that the effects of coarse particle exposure are of significant scientific and regulatory interest. A manuscript entitled "Altered heart-rate variability in asthmatic and healthy volunteers exposed to concentrated ambient coarse particles" was published in Inhalation Toxicology (Gong et al, 2004a). The manuscript is included with this report as Appendix B. Some key components of the paper that pertain to the ARB-funded concentrator use and performance are presented briefly below:

Materials and Methods

Along with associated air-monitoring and medical equipment, the concentrator and exposure chamber were installed in a trailer dedicated to human exposure studies. The system used for exposure is slightly different than the configuration used by Chang et al. for field tests of the CPC (Chang et al., 2002). There were five parallel circuits, each incorporating three 2.5μm cutpoint virtual impactors, operated at 110 LPM inlet flow per impactor. The total minor flow in this configuration allowed for concentration enrichment between 10 and 50-fold. Exposures were carried out at minor flow rate near 85 LPM and diluted with and equal flow rate of HEPA-filtered room air; at this setting the maximum

concentration enrichment was a factor 9 over ambient. The concentrator and exposure chamber set up are shown diagrammatically in Figure 1 of the manuscript (Appendix B).

Actual exposures were assessed by collecting samples upstream of the chamber inlet, diverting a small part of the minor flow for continuous monitoring and filter sampling. Continuous monitoring by nephelometer and 10-min averages of PM_{10} tapered element oscillating microbalance averages provided exposure data. Analyses performed from filter-collected material included x-ray fluorescence of Teflon filters for metals and trace elements; organic and elemental carbon analysis of quartz filters; and particle-bound endotoxin from glass filters. In addition, a Teflon filter sampling cassette for $PM_{2.5}$ was placed inside the exposure chamber at breathing level.

Results and Discussion

This study was the first to report responses to controlled coarse particle exposures in humans with and without asthma. Most individual exposure levels were close to the target concentration of 200 $\mu g/m^3$ and variation was mostly attributable to variation in ambient levels. On average, 80% of the PM to which subjects were exposed was in the coarse range. There was some concentration of PM in the upper range of the accumulation mode, and these particles contributed 20%. While study results were more exploratory than conclusive in nature, reduced heart rate variability with exposure was observed. This suggests that health effects from this kind of exposure scenario could be mediated through systemic responses, rather than derived from limited effects on the airways. The exact mechanisms by which coarse particle exposure could alter cardiac autonomic function and result in heart rate variability and other effects is not known and is under further investigation.

AIM 4: Development and evaluation of a high-volume, low pressure drop impactor for separation of ultrafine from accumulation mode particles (50% cut off size at 0.15 µm mean aerodynamic diameter).

An improved multiple rectangular (slit) geometry jet conventional impactor that can be used to separate ultrafine from accumulation mode particles at high flow rates (550 l min^{-1}) and under a very low pressure drop (i.e., 8 inches of H_2O or 0.020 kPa) was developed and characterized. High flow rate and low pressure drop are two extremely important features in making human exposures to concentrated atmospheric ultrafine aerosols possible, as these exposures require that the concentrated aerosol is supplied to the exposure chamber at a much higher flow than the typical human breathing rate (12-30 l min^{-1}) and at near-atmospheric pressure. The key feature of our technique is the utilization of a multiple slit nozzle conventional impactor with a small 50% cutpoint (0.15 µm) to sample atmospheric particulate pollutants at high flow rates. Particles larger than 0.15 µm in diameter are collected on narrow strips (12.5 cm x 0.254 cm), which serve as the impaction substrate. The development of the ultrafine impactor has been published in the Journal of Aerosol Science (Misra et al., 2002) and can be found in this report as Appendix C.

Materials and Methods

A conventional impactor with a low cutpoint was designed to allow high-volume collection of size-fractionated particulate matter. It is made of aluminum, compact and lightweight. The impactor has relatively simple operation needs, making it convenient and versatile for field air sampling. The impactor consists of four parts: an inlet block, followed by ten parallel slit nozzles, collection block and outlet. Nozzle parameters and sampling flows were designed such that the predicted cutpoint of the impactor would be 0.15 µm. Aerosol enters the circular inlet, accelerates through the slit nozzles

and then particles larger than the 0.15 μm cutpoint impact on a collection substrate while the ultrafine particles pass through the impactor outlet. For photographs, please see Figs. 1a-c in Appendix C.

Three impaction substrates were tested using indoor air and polydisperse atomized ammonium sulfate and ammonium nitrate as the test aerosols for PM in the 0.25-0.50 μm size range. The ratio of the concentration measured downstream of the impactor to that measured upstream by a Scanning Mobility Particle Sizer (SMPS Model 3936, TSI Inc., St. Paul, MN) was determined. Similarly, for particles in the 0.3-2.5 μm range, a nephelometer was used to compare mass concentrations upstream and downstream of the separator. In these experiments the test aerosol was monodisperse polystyrene.

In field tests, the impactor was used together with a commercially available high-volume virtual impactor to remove coarse PM mode upstream of the USC ultrafine impactor inlet. A collocated MOUDI was used as a reference sampler. Quartz strips were used as the impaction substrate. For the MOUDI stage collecting particles between 0.16 and 2.5 μm, a prebaked aluminum foil disk was used; a prebaked quartz filter was used for the backup which collected particles smaller than 0.16 μm. Disks and filters were analyzed for sulfate, nitrate, elemental carbon and organic carbon.

Results

The quartz substrate was found to be the superior impaction surface, yielding higher collection efficiency than the other two substrates for any particle size (Fig. 3, Appendix C). Collection efficiency on the quartz substrate increased to more than 90% as particle size increases past about 0.2 μm. The 50% cutpoint of the impactor was shown to be approximately 0.15 μm. Particle collection efficiency for ammonium nitrate was nearly identical to the efficiency with which ammonium sulfate and indoor air were collected, indicating that physical properties such as hygroscopicity and volatility do not affect the function of the impactor (Fig. 4, Appendix C.)

Laboratory tests of particle loading demonstrated that collection efficiency of the impactor did not decrease for any size particle measured (50-700 nm) to loadings up to 60-70 mg (fig. 5, Appendix C). Above about 60-70 mg, the impactor's collection efficiency for particles in the 0.1-0.2 μm range and the pressure drop across the jets increased, indicating 60 mg as a practical maximum loading level.

Field evaluation results from the USC impactor as compared to the MOUDI reference sampler indicate excellent agreement on ultrafine sulfate, organic carbon and elemental carbon. Nitrate could not be evaluated due to below detect concentrations measured by the MOUDI, potentially due to volatilization losses. Data are shown in figures 6-9 of the manuscript in Appendix C.

Discussion

The USC ultrafine impactor, a high-volume low pressure drop slit impactor for separation of ultrafine from accumulation mode particles, has been evaluated in the laboratory and field. The data show an excellent agreement between both accumulation and ultrafine mode concentrations of test substances, and high correlation between the MOUDI and USC impactor concentrations. This impactor has been developed primarily as a separator of ultrafine particles for use in human and animal inhalation toxicology studies. High volumes of particles that penetrate the impactor can also be collected on quartz or Teflon filters for use in *in vitro* toxicology or other experiments.

AIM 5: Development and evaluation of a compact facility for exposure humans to concentrated ambient ultrafine particles.

In order to conduct the first ever studies of the health effects of real-world ultrafine particles on humans, we developed an ambient ultrafine PM concentrator (UFPC). The UFPC is capable of concentrating particles up to 40 times ambient concentrations while preserving particle size distributions, thereby enabling toxicological and human clinical studies which were heretofore impossible. Removal of particles above 0.15 μm (if desirable) is achieved by passing the air through the inertial impactor developed under Aim 2 above, which allows for exposures to only ultrafine particles. The concentration enrichment process is accomplished by first growing ultrafine PM to super-micron size (a size that can easily be concentrated using a virtual impactor) by means of supersaturation/condensation, followed by virtual impaction. The concentrated ultrafine particles are reduced to their original size distribution by passing through diffusion dryers containing silica gel, prior to entering the exposure chamber.

A paper reporting the development and validation of the UFPC was published in Aerosol Science and Technology in 2004 (Misra et al, 2004). The paper is included in this report as Appendix D and the work is summarized below.

Materials and Methods

The major components of the UFPC include, (i) an ultrafine impactor to separate ultrafine from the accumulation mode PM, (ii) 4 rectangular saturators (stainless steel containers), each 30 x 15 x 40 cm in size, (iii) 12 parallel condensers, (iv) 12 virtual impactors and (v) 12 diffusion driers. The UFPC operates at a total sampling flow rate of 1200 LPM. If concentrated accumulation mode particles are preferred, then the concentrator can be adapted for this purpose by substituting the ultrafine impactor with a 2.5 μm commercially available impactor. In the saturators, the aerosol passes over ultrapure deionized water kept at 30-32°C by quartz immersions heaters, each adjusted by means of a voltage regulator. The saturated aerosol is then drawn through 12 condensing tubes in which it is cooled to about 22°C; the ultrafine particles grow to supermicron size as they become supersaturated. The cooling system is described further below, in Aim 6. The minor-to-total flow ration of each of the 12 virtual impactors can be varied such that the enrichment concentration of the total ensemble can reach a factor of 20-50 fold. The concentrated aerosol passes through parallel diffusion dryers, to return the particles to their original size, humidity and temperature conditions. Prior to entering the exposure chamber, the concentration level can be adjusted by mixing with particle-free air. The entire UFPC system has been placed inside the mobile trailer described above in Aim 2, to allow for animal or human exposures in a variety of locations.

The performance of the UFPC was evaluated by comparing measurements made with a scanning mobility particle sizer (Model 3936, TSI Inc., St. Paul, MN) of the concentration-enriched ultrafine aerosol and comparing them to direct ambient measurements. Measurement of concentrated and ambient ultrafine PM was also performed using an aethalometer to test for enrichment of EC and PAHs. In addition to these continuous monitoring procedures, the UFPC was also evaluated in time-integrated experiments in which ultrafine PM was collected on Teflon filters placed before and after the UFPC. Sampling was done at 30 LPM for both filters to minimize adsorption and volatilization artifacts. Filters were weighed to determine mass concentration, and then extracted and analyzed for nitrate and sulfate by ion chromatography.

Results

Results from the SMPS studies of particle number enrichment indicated ideal enrichment in number concentrations could be obtained for all of the minor flow ratios and that the concentrator could enrich the particles by the desired factor while preserving the original particle size distributions (Figs 3-4, Appendix D). In the experiments with minor flow of 60 LPM, corresponding to a minor-to-total flow ratio of 5%, particles as small as 15 nm were concentrated at near-ideal factors. At the lower minor flows, there was some decrease in concentration enrichment for particles below 20 nm.

Aethalometer results show that the average EC enrichment observed was 19.55 (±2.5), 28.9 (±0.67) and 42.5 (±4.1) corresponding to the minor-to-total flow ratios of 5, 3.3 and 2.5% respectively while the PAHs were enriched by factors of 16.7 (± 0.22), 25.1 (± 1.86) and 38.21 (± 3.52) (Figs 5-7, Appendix D).

Time-integrated tests in which the concentration enrichment was determined based on ultrafine PM mass as well as nitrate and sulfate indicated a near ideal enrichment with the average ratio of enrichment factor being 18.3, 20.6 and 17.8 for ultrafine PM mass, sulfate and nitrate, respectively (Figs 8-9, Appendix D).

Discussion

The SMPS results show clearly that the UFPC works very well to concentrate ultrafine particles up to 40-fold over ambient concentrations. High enrichment factors were found for all of the minor flow ratios tested. The supersaturation process captures ultrafines down to very small aerodynamic diameter particles, without loss to coagulation. The drying process returns ultrafine particles to their original size. The Aethalometer findings demonstrate that the UFPC can concentrate hygroscopic (EC) and some semi-volatile constituents of ambient aerosol such as PAHs by near-ideal enrichment factors. Thus, field experiments indicated that the concentration enrichment process is not affected by particle size and chemical composition, at least for particles larger than 0.02 μm in diameter.

The UFPC opens new avenues of research into the health effects of particulate matter, allowing real-life ambient aerosols to be used at the high exposure levels required in toxicological studies of inhalation health effects. Human and clinical studies and experimental animal work that was previously impossible can now be designed and carried out, leading to a vastly improved understanding of the range of health effects associated with exposure to particulate matter, and the mechanistic bases of those effects.

AIM 6: Validation and quality assurance studies

Validation and quality assurance studies have been performed on each component of the concentrators and mobile exposure facility as part of development processes. The VACES system, the coarse concentrator, and the ultrafine human exposure system have all been subjected to laboratory and field evaluation studies, which are described in the sections above and in more detail in the references cited (e.g. Kim et al, 2000, 2001a, 2001b and Geller et al., 2002). Two additional papers that specifically address the preservation of size distribution and chemical composition characteristics of concentrated aerosol are included as appendices E and F.

Zhao et al. (2005, and Appendix E) describes a field study of the VACES performed by coupling to a rapid single-particle mass spectrometer (RSMS-3, Phares et al., 2002) to investigate the hit rate increase effected by the VACES and importantly, potential chemical composition changes. The study was conducted as part of the U.S. Environmental Protection Agency Supersite program in Pittsburgh during March 2002 with the primary goal of increasing applicability of RSMS-3 to

conditions with low ambient particle concentrations. The RSMS-3 sampled alternately before and after concentration by the VACES. To sample concentrated PM, the minor flow from the VACES was connected directly to the RSMS-3 sample port. Spectra were classified by the Adaptive resonance Theory-2a algorithm. The results showed that there was an excellent conservation of particle composition during the concentration process. Depending upon sampling day, there was an 8-10% shift in spectral nitrate classification to carbon nitrate from other nitrate classes such as ammonium nitrate. Upon careful analysis of the spectral data, there was no evidence to suggest that the VACES had caused the shift. The shifts could have been introduced by changes in the composition of ambient air, since the RSMS-3 sampling is done alternately for ambient and concentrated particles. It was also apparent that the algorithm presents limitations for classification of carbonaceaous particles with a wide range of ammonium nitrate contents, so that subtle changes due to initial conditions, instrument operation or atmospheric changes could cause the class shift. Overall, the study provided validation for preservation of chemical composition during concentration.

In another study, supported in part by this ARB funding, we describe field observations from the VACES concentrator coupled to an Aerodyne Aerosol Mass Spectrometer (AMS) (Khylstov et al., 2005, appended to this report as Appendix F). The measurements were conducted during the Pittsburgh Air Quality Study at the central monitoring site in Pittsburgh, PA, during September 2002. The main aim of this study was to determine the applicability of the VACES-AMS combination to measurements of aerosol chemical composition during nucleation events and assess the effects of water condensation on semi-volatile aerosol species during concentration. Because of the very low aerosol concentrations during these events attention was given to small changes in aerosol composition (of the order of 5% of the concentrated mass) that were not relevant to the previous characterization studies of the VACES (Geller et al., 2002; Kim et al., 2001b; Misra et al., 2004). The AMS study confirmed minimal artifact during the water-based concentration process. The size distribution of sulfate was preserved during all study conditions, and sulfate concentration factors were very close to those determined by CPC. When sampling was carried out during relatively light pollution, size distributions of ammonium, organics and nitrate were preserved. Under more polluted conditions (e.g. sulfate at 20 $\mu g/m^3$), these species show a small increase at lower particle sizes. The increase was especially prounced for nitrate, although the absolute amount of artifact nitrate was small relative to the total aerosol mass. The artifact is likely due to the redistribution of gas phase nitrate to aerosol during the condensation process and will be most pronounced under ammonia limited conditions, while remaining negligible under ammonia rich conditions. When the VACES is used for exposure studies, concentration factors for individual components could be used if comparison of outcome measures with individual components is of interest.

AIM 7: Development of a cooling system for the UFPC

In our original ultrafine concentrator prototypes, used in the VACES system for animal exposures and *in vitro* particle collections (Kim et al. 2001a, b), the cooling media in the condensers was a mixture of ice, salt and water, cooling the aluminum sampling lines in a commercially available Coleman cooler. For the development of the larger scale UFPC Human Exposure Facility, it became imperative to devise a new cooling method, which would be self-sustaining, require minimum maintenance and very importantly, would provide much more uniform cooling over the entire length of the condensing tubes. A somewhat more detailed summary of the chiller development and testing may be found in Misra et al., 2004 (Appendix D).

Materials and Methods

Two refrigerated circulating chillers rated at 900 Watts maximum cooling capacity each (Programmable Refrigerated Circulator, Model 9710, Polyscience, Niles, IL) were employed, each to provide sufficient cooling load to accommodate 600 LPM of air (thus sufficient for 6 of the 12 condenser tubes of the UFPC). The 12 condensing tubes each consist of two concentric stainless steel tubes and polyethylene insulation on the outer shell. The flow of coolant circulated by the chillers can be controlled using a valve.

The performance of the Model 9710 refrigerated circulator was evaluated in order to determine whether the circulator was able to provide an optimum and, very importantly, a stable temperature for the growth of particles over a prolonged period of time, thus achieving a steady state coolant temperature circulating through the condensers. The evaluation used one circulator to cool six condensers (corresponding to a flow of 600 LPM) in the UFPC. Each of the 6 condensers consisted of a virtual impactor at its outlet, where the grown ultrafine particles were concentrated. The minor flow of each virtual impactor was connected to a diffusion dryer and the flow from all six diffusion dryers was then merged into one minor flow (of 30 LPM). The performance of the circulator was then evaluated by periodically checking the enrichment in terms of particle number concentration measured before and after the concentrator by means of a Condensation Particle Counter (CPC, Model 3022A, TSI Inc., St. Paul, MN). The circulator reservoir temperature was also recorded periodically. The saturator temperature in these experiments was maintained at 28^{o}-30^{o}C. The coolant flow rate in these experiments was set at 2 LPM per condenser in order to provide adequate residence time in the circulator reservoir for cooling.

Results and Discussion

The results demonstrated that each of the two Model 9710 circulators have sufficient cooling power to cool 6 condensers (corresponding to a total aerosol flow of 600 LPM) over long periods of time (well in excess of the 2 hours needed in the current scheme). Thus, the applicability and robustness of the new cooling system was confirmed and two circulators are employed to provide adequate cooling to operate the UFPC at 1200 LPM. For more detail, please refer to the publication in Appendix D.

AIM 8: Development of a facemask exposure system for the UFPC

Preliminary human exposure experiments with the new ultrafine particle exposure system indicated that the enrichment factor of the ultrafine concentrator was found to be lower than the earlier validation studies had led us to expect, i.e., ~4-6 instead of ~9. Measurements of number counts immediately downstream of the ultrafine impactor (concentrated ultrafine aerosol) and at the breathing zone (diluted ultrafine aerosol) inside the exposure chamber lead to the conclusion that there was an unaccounted dilution effect that could not be explained by the dilution flow only. Leak checks were performed with the gas meter and all the chamber-sampling instruments were operated as in a regular experiment, with the exhaust pump adjusted to draw 120 L/min out of the chamber, and HEPA-filtered dilution air was supplied at a flow rate of 76 L/min. The chamber negative pressure during this operation was found to be 2.5 in. H_2O. Total flow out of the box was 136 L/min, and theoretical flow through the concentrator was expected to be 60 L/min. Repeated gas-meter readings showed a flow rate of 25-26 L/min, i.e., a 34-35 L/min shortfall. Therefore, the drop in the enrichment factor inside the exposure chamber was explained by the lower flow from the concentrated ultrafine stream and more dilution effect from the ambient air through the leaks.

Several options were considered to solve the leaking problems and the resulting lower than ideal enrichment, with better sealing material and different flow rates, and the performance of the UFPC improved somewhat, but could not reach the ideal enrichment factor of ~9 times the ambient. Consequently, a facemask for human exposures has been introduced. The research and findings presented for this aim below have not yet been published, although the technology is currently in use in human exposure studies.

The New Configuration of the UPFC

A facemask has been introduced for the subject inside the exposure chamber. The subject breathes the concentrated ultrafine aerosol stream of 70 LPM by means of the facemask. A valve-controlled leak is introduced at the back of the chamber. This leak allows ambient air circulation inside the chamber. With this configuration, we ensured a total flow rate of 120 LPM inside the chamber, of which 70 LPM is supplied from the concentrator. Human exposures are being carried out under the new configuration and with very satisfactory results (enrichment factor ~10-12). The ultrafine PM mass concentrations ranged from 60 $\mu g/m^3$ to 150 $\mu g/m^3$ depending upon the ambient concentrations. During the exposure, the ambient and concentrated ultrafine PM counts are recorded and results from the improved exposures to-date are tabulated in Table 1.

Table 1: Ambient and Concentrated Ultrafine PM Counts during Subject Exposures

Ambient UFPM (#/c.c.)	Concentrated UFPM (#/c.c.)	Enrichment Factor
22169	203250	9.2
23535	219883	9.4
19638	223567	11.4
24033	224650	9.4
8057	150150	18.6
13103	126067	9.6
12435	139300	11.2
13415	112372	8.4
13778	141417	10.3

A representative time series of a subject exposure on 04/20/2004 describes the performance of the human ultrafine particulate concentrator facility.

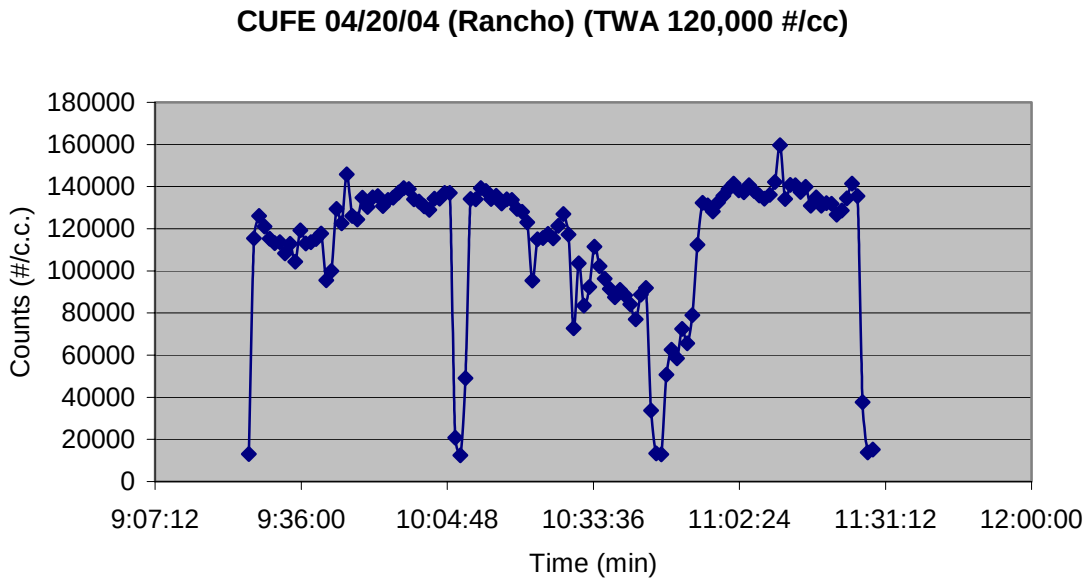


Figure 1: UFPC Time series for CUFE (Concentrated Ultrafine Exposure) on 04/20/2004.

Summary Discussion for Task 1

Several advancements in technology for concentrating ambient particulate matter for use in scientific research have been made during the course of this contract funding. A Versatile Aerosol Concentrator Exposure System (VACES) capable of simultaneously concentrating ambient particles of the coarse, fine and ultrafine size fractions for conducting in vivo and in vitro exposure studies to "real" ambient aerosols over a wide dynamic range of concentrations has been completed and applied. This equipment allows us to carry out sophisticated animal exposure studies in a variety of locations without substantial changes in the physical and chemical characteristics of the particles as they traverse the system from the ambient air to exposure chamber. Animal exposure studies are described in this report under Tasks 2 and 3. The ability to carry out the animal exposures is also an important part of the research we proposed in our successful application for competitive renewal of funding for the Southern California Particle Center. The VACES can provide highly concentrated liquid suspensions of particles of the three size modes for in vitro toxicity studies, again preserving particle characteristics that are potentially very important factors in their toxicity. These new concentrators provide the technology to collect large amounts of particulate matter for toxicological investigations in the laboratory, such as the *in vitro* studies detailed under Task 4 of this report. The collection of a wide range of material supported by this contract has been indispensable to the analytical work performed over the past six years as part of the activities of the Southern California Particle Center and Supersite (SCPCS), funded by U.S. EPA.

The VACES in both animal exposure and liquid BioSampler configuration has been tested extensively in the laboratory and field using a variety of diagnostic tools. The system performs very well in terms of its ability to achieve high concentration enrichment factors with minimal pressure drop and excellent preservation of size distribution and chemical characteristics of concentrated aerosols.

A new conventional impactor for ultrafine particles was developed, validated, and applied as part of a new ultrafine particle exposure system suitable for human exposure studies. This system, for concentrating size selected ambient particles at high flow rates and with low pressure drop, has been installed in a trailer configured for exposures associated with human clinical studies. An expanding set of studies of human volunteers, elderly, with or without asthma or chronic obstructive pulmonary disease has now been carried out (Gong et al., 2003, 2004a, 2004b, 2005) using the coarse, fine and ultrafine concentrator capabilities we have developed for human exposure applications. Further studies of asthmatics and other potentially sensitive subgroups will continue to provide valuable data to better characterize the risks associated with PM exposure.

TASK 2. Conduct near-source studies of concentrated fine and ultrafine ambient particles (Freeway Study - Animal).

Objectives and Introduction

The overall objective of the freeway study was to assess the role of fine (F) and ultrafine (UF) particles in producing toxicity associated with mobile sources, and to assess toxicity as a function of distance from a heavily used freeway. The study hypotheses included: (1) exposure to mobile emissions from mobile sources close to a heavily trafficked roadway will exacerbate inflammation and allergic airway responses; (2) the magnitude of allergic airway disease responses will decrease with increasing distance from the roadway; and (3) allergic responses will be greater at sites with higher concentrations of ultrafine particles.

To pursue these hypotheses, ovalbumin (OVA)-sensitized BALB/c mice were exposed to freeway-derived, concentrated fine and ultrafine PM at progressively increasing distances downwind from a freeway in the Boyle Heights area of Los Angeles, California. The effect of background aerosol was assessed by including an upwind site that is not influenced by the freeway. The versatile aerosol concentration enrichment system (VACES) was used to concentrate ultrafine (UF; particle diameter [d_p] $\leq$ 150 nm) and fine (F; $d_p \leq 2500$ nm) PM which were delivered at a constant target concentration to a mobile exposure system to expose mice under relatively controlled conditions in a real world on-site setting. Note that the fine particle fraction includes the ultrafines. After exposure, biomarkers of allergic responses in blood and pulmonary fluid and proinflammatory biomarkers in brain were measured. In addition, samples were collected and analyzed to characterize the chemical composition of the concentrated particles. The resulting biomarker data could then be analyzed with respect to a number of different exposure variables.

The freeway exposure studies have produced two published papers to date: Campbell et al., 2005 and Kleinman et al., 2005. Both are appended to this report (Appendices G and H, respectively) and the presentation of the published work is limited to brief discussions below. We also published a paper describing the mobile facility we have developed for animal inhalation exposures to concentrated aerosol (Oldham et al. 2004; Appendix I). The focus of this report is a more detailed analysis of the allergic response biomarkers with respect to chemical composition of the particles than has been published to date. The results and discussion of the recent analysis work are presented in greater detail in this report than the earlier findings, since they are as yet unpublished and have not been the subject of progress reports to ARB.

Methods

The methods for the freeway study are presented in detail in Appendix J as well as in the publications that resulted from this study (Appendices G and H). Methods are presented briefly below. Much of the methods here are also relevant to Task 3, the Source Receptor study.

Ovalbumin sensitized Balb/c mice were selected as the model for this study because they display some key characteristics of asthma or allergic airways diseases including pulmonary inflammation and pulmonary eosinophilia. Mice were sensitized by nasal instillation of OVA before each PM exposure, and then challenged with OVA after exposure, before sacrifice. Exposure to PM was four hours per day, five days per week, for two consecutive weeks. The target concentration for fine PM was 400 $\mu g/m^3$; the VACES was operated at maximum efficiency for UF, due to lower ambient concentration levels for this size fraction. Actual exposure concentrations varied and are

reported below in the results section. The VACES was connected to a mobile exposure facility that also provided purified air for control animals and for use during transport of animals to the study location. The actual performance of the exposure system was determined prior to the study, and subsequently published (Oldham et al, 2004; Appendix I).

After exposure, mice were challenged with OVA, euthanized and tissues were prepared for assays. The biomarkers we selected for the study are associated with responses of Type 2 T-helper lymphocytes (T_H2 cells) that mediate allergic responses. Cells present in bronchoalveolar lavages were analyzed to determine eosinophil (EOS) influx and infiltration of polymorphonuclear leucocytes (PMN). The levels of two cytokines, interleukin-5 (IL-5) and interleukin-13 (IL-13) were measured in lavage fluid. IL-5 supports the proliferation and differentiation of mouse B cells, and enhances secretion of antigen-specific immunoglobulins IgG1 and IgE. IL-5 is also a chemoattractant for eosinophils and is strongly implicated in the pathogenesis of asthma. IL-13 is a cytokine that plays a pivotal role in the induction of T_H2 cells; inhibition of IL-13 blocks the development of allergic responses. To determine if PM exposure could have adjuvant effects in the development of airway allergies, levels of two OVA-specific immunoglobulins E (IgE) and G1 (IgG1) were compared in blood from mice exposed to concentrated PM and control mice that were ovalbumin sensitized but exposed to filtered air.

Data were analyzed primarily by ANOVA and linear regression methods. Further description of the statistical methods can be found in Appendix J and in Kleinman et al, 2005.

Results and Discussion

1. Animal exposure system

Performance of the portable whole-body mouse exposure system was determined for 0.5 to 2.0 μm diameter aerosols by measuring (1) uniformity of aerosol distribution and (2) particle deposition in the tracheobronchial and pulmonary regions of mice exposed in the system. The average data from three runs showed no statistically significant difference among individual compartments. Particle deposition efficiency in adult male BALB/c mice was measured after exposure (30 min) in the system using monodisperse fluorescent polystyrene latex particles (0.5, 1, and 2 μm aerodynamic diameter). The measured deposition efficiency in this mobile exposure system for the combined tracheobronchial and pulmonary regions of the adult male BALBc mice was 21% for 0.5 μm, 11% for 1.0 μm, and 6.5% for 2.0 μm particles. These deposition efficiencies are similar to those reported for mice exposed in a nose-only exposure system, which indicates that particle losses to animal fur and exposure system surfaces were acceptable (Oldham et al., 2004; Appendix I).

2. Preliminary studies to determine exposure parameters

Initial exposures were carried out to test responses to the OVA sensitization protocol and to optimize the exposure protocol. Sensitized mice showed eosinophils in bronchoalveolar lavage; controls did not. Significant effects of the OVA sensitization protocol on the biomarkers was desirable with respect to producing an animal model with high sensitivity, but elevated levels of biomarkers in some sensitized controls also increases the variability in the background upon which PM exerts toxic effects. Groups of OVA sensitized mice were exposed to target concentrations of 400 and 800 $\mu g/m^3$ F + UF PM four hours per day for five consecutive days and at 400 $\mu g/m^3$ for ten days. The actual concentrations were were 415 and 609 $\mu g/m^3$ in the five day exposures and 361 $\mu g/m^3$ for the ten day

exposure. Two weeks after the last exposure mice were challenged with inhaled OVA, euthanized and bioassays were performed. Both the five and ten day exposures to the lower concentration resulted in increased IL-5, IgE and eosinophils with the ten day exposure producing greater responses. Interestingly, the 5 day exposure to the higher concentration resulted in suppression of these three allergic biomarkers, in comparison to sensitized controls that were exposed to filtered air. A target concentration of 400 $\mu g/m^3$ for a ten day duration was selected as the most sensitive exposure scenario to capture enhancement of allergic responses in this experimental system. It is of note, however, that the animal model showed suppression at high exposure levels. Suppressive effects of PM exposure are consistent with observations in our rat experiments, performed as part of the source-receptor study (Task 3, Appendix K). The difference in fine PM mass that enhanced versus suppressed allergic responses was quite narrow, which complicates biological interpretation of biomarker measurements. It may be that overt toxicity associated with the high exposure concentration limits the ability of the immune system to mount a response to the allergic stimuli. But since mass may not be the best measure of PM toxicity and allergic effects, the dose dependence is difficult to interpret.

3. Brain Inflammatory Markers in Mice Exposed to PM near the Freeway

There has been a great deal of recent interest in systemic effects of PM in organs other than the lung and heart. To further examine potential roles of PM exposure in systemic inflammatory effects, brains from mice that were exposed to F and UF PM at a location 150 m from the freeway were analyzed for biomarkers of oxidative stress and inflammatory responses. The selected biomarkers were NFκB, a transcription factor that elicits the expression of proinflammatory cytokines, and IL-1α and TNFα, two of the proinflammatory cytokines regulated by NFκB.

We demonstrated that exposure (4 h, 5 days per week for 2 weeks) to concentrated airborne particulate matter increases inflammatory indices in brain of ovalbumin sensitized BALB/c mice. Animals were divided into three exposure groups: filtered air (control), ultrafine particles, or fine and ultrafine particles. The levels of pro-inflammatory cytokines interleukin-1 alpha (IL-1α) and tumor necrosis factor alpha (TNF-α) were increased in brain tissue of mice exposed to particulate matter compared to that of control animals. Levels of the immune-related transcription factor NF-κB were also found to be substantially elevated in the brain of exposed groups compared with the control group. These data indicate that components of inhaled particulate matter may trigger a pro-inflammatory response in nervous tissue that could contribute to the pathophysiology of neurodegenerative diseases. This study has been published in Campbell et al. (2005); the publication is appended to this report (Appendix G).

4. Biomarkers of allergic airways response and distance from the freeway

Exposure Concentrations

The particle mass, particle count and chemical composition of the F and UF CAPs atmospheres at the 50 m and 150 m sites are summarized in Table 2. These experiments were conducted over the 4-year period 2001 to 2004 and during different seasons of the year. This provided for a fairly wide range of values for PM mass, number and constituent concentrations over which to examine exposure-response relationships.

Table 2. Mass, number and composition of F and UF CAPs 50 m and 150 m downwind of a heavily trafficked roadway (Mean ± SD).

Component	Location			
	50 m		150 m	
	F	UF	F	UF
Mass	394 ± 94	297 ± 189	387 ± 68	213 ± 95
Number (x 10^{-3})	210[a]	490 ± 141	160[a]	440 ± 212
EC	8.1 ± 2.9	9.9 ± 11.4	8.8 ± 6.3	42 ± 37
OC	104 ± 74	61 ± 11	151 ± 147	159 ± 27
Total Metal	28 ± 32	12 ± 7	34 ± 41	19 ± 15

[a] Note: Only one measurement of F particle number was made at each site

The mass concentration ranged from 163 μg/m^3 to 500 μg/m^3. The F concentration masses were greater than the UF fraction masses. This was to be expected since the UF fraction of PM represents a much smaller fraction of the total mass than does the F PM fraction and even with the VACES performing at maximum efficiency the target concentration of 400 μg/m^3 was not attainable for UF. The F particle mass at 50 m downwind of the roadway was not significantly different from the F particle mass measured 150 m downwind. However, the UF particle mass was decreased 30% 150 m downwind of the roadway as compared with the UF particle mass measured 50 m downwind. F particle number concentrations seem to be decreased by about 30% going from 50 m to 150 m downwind. However, only one set of measurements were made for this parameter and we therefore do not know if the decrease is significant. Averaged UF number concentrations are not significantly different between the two sites. The average EC concentrations showed high variability; they ranged from about 2 to about 68 μg/m^3. The OC concentrations tended to be higher in the F than in the UF fractions and OC concentrations were greater in the samples collected 150 m downwind than in those collected 50 m downwind. This was biased by the samples collected during the last year of the study, perhaps suggesting a change in source emissions during that year. Metal concentrations were greater in the F particle fraction than in the UF fraction, and the average concentrations 150 m downwind were slightly but not significantly greater than those measured 50 m downwind.

Biological Responses

Initially, we performed an analysis that focused on the effects of exposure (plus/minus CAPs) and location (50 vs. 100 m) on the five biomarkers of allergic airways responses. The results of the initial analyses have been reported in past progress reports to ARB, and were published in Kleinman et al. (2005). The published paper is included in this report as Appendix H. Briefly, our initial analysis of the data showed that exposure to CAPs 50 m from the freeway elicited significant increases in biomarkers of airway allergies and effects were not observed in mice exposed 150 m downwind of the freeway. There were not enough data at that time the publication was written to examine the hypothesis that the toxicities of F and UF particles were different. As described below, we have continued the freeway study and have now analyzed a more complete data set to determine exposure-

response relationships between allergic responses and important PM components. We have also addressed the issue of the role of UF PM, as a component of $PM_{2.5}$, in responses to airborne particles.

The number of mice in each group and the average concentrations of the T_H2 cytokines, IL-5 and IL-13, PMN and EOS in BAL, and OVA-specific immunoglobulins, IgE and IgG1, in blood plasma from OVA-sensitized mice exposed 50 m and 150 m downwind of the roadway are summarized in Table 3. The data were analyzed using a 1-way ANOVA to test the null hypotheses: 1) that responses in Air-exposed animals were = to responses in CAPs-exposed animals (i.e. a main effect of EXPOSURE). Tukey Multiple Comparison Tests were performed to test the null hypotheses: 2) that responses in animals exposed 50 m downwind were = to responses in animals exposed 150 m downwind (i.e., an effect of SITE); 3) there was a statistically significant interaction between the factors LOCATION and EXPOSURE. The results of the ANOVA for each of the parameters are summarized in Table 3.

Table 3. Biological responses in mice exposed to F or UF CAPS 50 or 150 m downwind of a heavily trafficked roadway

		Location			
		50 m		150 m	
Biological Response	Exposure	n	Mean ± SE	n	Mean ± SE
IL-5	Air	33	4.4 ± 1.0	27	2.7± 2.2
	F	27	11.8 ± 1.5[a,b]	26	2.1 ± 1.2
	UF	18	8.5 ± 1.3[c]	18	≤1.3
IL-13	Air	31	5.0 ± 0.5	27	8.1 ± 1.1
	F	27	5.9 ± 0.8	26	10.4 ± 1.6
	UF	17	4.5 ± 0.9	18	6.5± 0.9
OVA-specific IgE	Air	34	5.9 ± 2.0	27	1.3 ± 0.5
	F	27	5.7 ± 2.2	26	1.1 ± 0.3
	UF	18	8.2 ± 3.4	18	2.2 ± 1.1
OVA-specific IgG1	Air	32	1800 ± 300	27	3900 ± 1000
	F	26	6600 ± 1500[a]	26	4300 ± 900
	UF	16	5000 ± 1000	18	4400 ± 1200
PMN	Air	34	1700 ± 340	27	1400 ± 300
	F	27	4500 ± 2500	26	1400 ± 400
	UF	18	1400 ± 500	18	1100 ± 200
EOS (GM±GSD)	Air	33	8 ± 5.1	27	4 ± 2
	F	27	13 ± 12	25	2 ± 1
	UF	18	3 ± 3	18	2 ± 1

Notes: [a]Tukey Air ≠ F_{50}, $p \leq 0.05$; [b]t-test (unequal var.) $F_{50} \neq F_{150}$, $p \leq 0.001$; [c]t-test (unequal var.) $UF_{50} \neq UF_{150}$, $p \leq 0.01$.

Table 4. ANOVA Probability that the null hypotheses should be rejected

	50 m							
	One-Way ANOVA		Tukey					
			50 m			150 m		
Biological Response	p_{50m}	P_{150m}	Air vs. F	Air vs. UF	F vs. UF	Air vs. F	Air vs. UF	F vs. UF
IL-5*	0.0002	0.48	≤0.05	N.S.	N.S.	N.S.	N.S.	N.S.
IL-13	0.37	0.14	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVA-specific IgE	0.77	0.44	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
OVA-specific IgG1*	0.003	0.95	≤0.05	N.S.	N.S.	N.S.	N.S.	N.S.
PMN	0.29	0.79	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
EOS	0.31	0.77	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Significant Effect of Exposure in One-way ANOVA; N.S. = non-significant in Tukey Multicomparison Test

There were statistically significant effects of EXPOSURE for IL-5 and IgG1 in BAL or plasma from mice exposed 50 m downwind from the road. The Tukey multiple comparison test was used to test for significant differences between group means for these two parameters. As shown in Table 3, except for IL-13, there was an overall pattern for parameters associated with airway allergies to be numerically elevated after CAPs exposures at the site 50 m downwind of the roadway compared to those measured in mice exposed at the 150 m site. Only the findings for IL-5 and IgG1 were significant at the $p \leq 0.05$ level. None of the measured biomarkers were significantly elevated in mice exposed 150 m downwind of the roadway. As shown in Table 4, the significant One-way ANOVA results for IL-5 and IgG1 at the 50 m site were driven by the differences in group mean value comparisons between Air and fine CAPs exposure.

Cytokine Responses: The mice exposed to F CAPs 50m downwind of the freeway had significantly greater IL-5 concentrations in BAL than did mice exposed to F CAPs 150m from the freeway ($p \leq 0.05$). The mice exposed to UF CAPs 50m downwind of the freeway also had significantly greater IL-5 concentrations in BAL than did mice exposed to UF CAPs 150m from the freeway ($p \leq 0.05$). However, although IL-5 concentrations in mice exposed to F CAPs tended to be greater than that of IL-5 in mice exposed to UF CAPs, these differences were not statistically significant. The IL-13 concentrations followed the same general patterns as those observed for IL-5, but the effects of EXPOSURE were not significant. Concentrations measured at the 150 m site tended

to be higher than those measured at the 50 m site for all three exposure atmospheres (Air, F and UF) but the differences were not statistically significant. The analyses were performed in different batches and the observed differences could possibly represent some unidentified measurement bias.

Immunoglobulin Responses: IgE concentrations were generally higher in plasma from mice exposed to CAPs vs. mice exposed to purified air. The group mean differences (control vs. exposed) were not significant. The IgE concentrations in mice exposed to CAPs 50m downwind of the freeway were slightly increased over the IgE concentrations in plasma from mice exposed 150m from the freeway, but the differences were not statistically significant. IgG1 concentrations were higher in the BAL from mice exposed to CAPs vs. mice exposed to purified air at the 50 m site ($p = 0.003$).

Cellular Responses: EOS and PMN numbers in BAL from mice exposed to F CAPs 50 m downwind were higher than those in mice exposed to purified air but neither increase was statistically significant. It should be noted that the distribution of the EOS data was non-normal, that is, many mice had no detectable EOS cells in their BAL. We therefore used a log transform to evaluate the data. The EOS values in Table 3 are the geometric means and the upper tail of the geometric standard deviation.

Bivariate regressions were performed on the IL-5 and IgG1 data as measures of PM composition to test the hypothesis that allergy responses were elicited by components of the PM. These biomarkers were identified in the ANOVA as having significant group mean differences. Linear regressions were performed as functions of EC, OC, metal ions, particle mass and particle number. These are shown in Figure 2.

Figure 2 shows that for both biomarkers the slopes of the regression lines as functions of EC and OC are greater for mice exposed 50 m downwind than for mice exposed 150 m downwind. These differences in slopes for the two sites are statistically significant ($p \leq 0.05$). The figure also demonstrates graphically that the distributions of component concentrations are different between the two sites. It is interesting to note that there was a statistically significant association between IL-5 responses and metal ion content at both sites. This suggests that metal ions may play some role in eliciting allergy responses. However, it can be seen that the slopes of the regression lines are greater for the carbon constituents than for the metal ion constituents. Additional data on the specific organic chemical composition should provide more specific ideas of components and pollutant sources that affect airway allergies near heavily trafficked roads.

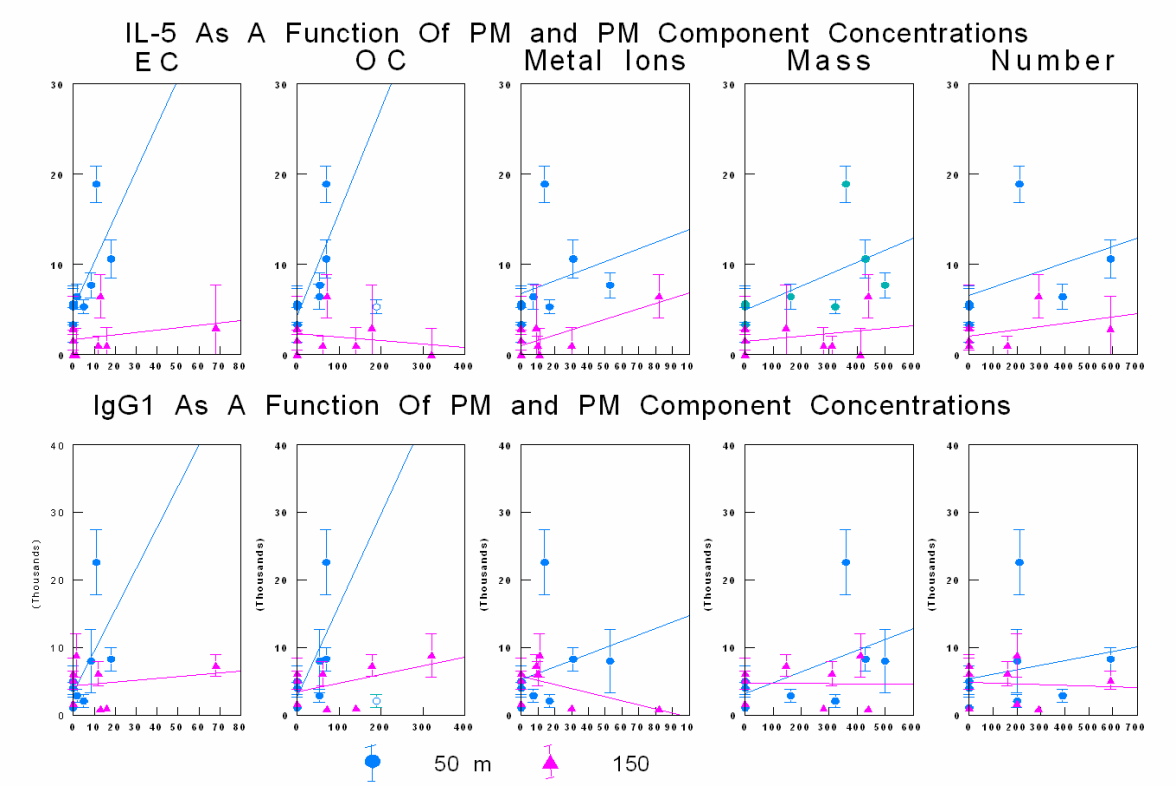


Figure 2. Exposure-response relationships between PM components and biomarkers of allergic response have significantly different ($p \leq 0.05$) slopes for animals exposed 50 m vs. 150 m downwind of a heavily trafficked roadway.

Discussion

The objectives of this study were to test the hypotheses that 1) CAPs exposure would enhance inflammatory and allergic responses in OVA-sensitized BALB/c mice compared to sensitized, clean air controls; 2) there would be differences in response at two distances downwind of heavily traveled freeways suggestive of greater toxicity of PM closest to the freeway; and 3) that UF PM resulted in more significant responses than F PM. In general, the data provide some support for the first two hypotheses. First, the results indicate that CAPs enhanced some inflammatory and allergic responses in relation to controls (Table 3) in mice exposed 50 m downwind from the freeway as compared to mice exposed 150 m downwind for IL-5 ($p \leq 0.05$) and anti-OVA IgG1 ($p \leq 0.05$), and these were supported by a similar, but non-significant trend for anti-OVA IgE. Second, for those biomarkers that had significant CAPs exposure-related responses, bivariate regression analyses showed that there were significant differences between the slopes of the relationships with specific parameters of PM composition in mice exposed 50 m downwind compared with mice exposed 150 m downwind from the roadway. Overall the differences between responses to UF CAPs at the 50 m site were not significantly different from responses to F CAPs at the 50 m site. It is important to note that UF materials made up a significant portion of the material that we classified as F PM, since the fine fraction contains all PM below 2.5 µm in diameter. This is apparent from the data in Table 2 and is especially notable when EC and OC levels are compared. It is therefore not surprising that F PM and UF PM have similar effects. These results suggest that allergic response could be modeled as a joint function of F and UF particles. Since metal ions were more important in the F PM, and OC was more

important in the UF PM, we tested a multivariate regression that IL-5 response was a function of both OC and metal ion PM content. The multiple regression provided a larger R^2 than did regression on the single components; however, in the multiple regression, neither of the slope parameters were statistically significant, most likely because the degrees of freedom were reduced.

The effects appeared to indicate that PM could act as an adjuvant of the immune response. Taken together, this field study demonstrates that freshly-generated CAPs within 50 m of a roadway can have adjuvant effects which are consistent with the modest effects seen in other laboratory studies (Takano et al. 1997, Tao et al. 2003, Walters et al. 2001, Whitekus et al. 2002, Diaz-Sanchez 2000, Nordenhall et al. 2001). However, these effects are not seen in mice exposed 150 m downwind from the roadway.

We have tried, in several ways, to put these results into context with the atmospheric characterization data that were obtained during the exposures. Zhu et al. (2002) have described the decay in particle count with distance from two freeways as a monoexponential function, with different asymptotes. The decay function is greater for the diesel rich 710 freeway (>25% diesel traffic) than for the gasoline rich 405 freeway (<5% diesel traffic); and as the freeway used in this study had 30-40% diesel traffic, its PM characteristics were assumed to be similar to those of the 710 freeway. Accordingly, the decay constant (0.053 m^{-1}) for the 710 freeway was used to estimate values for the asymptote and the coefficient for the exponent. At the distances used in this study, the predicted decline in particle number in going from 50 m to 150 m is rather limited, about 15% to 37% by their estimates and, between 20 and 25% which is consistent with the values shown in Table 2.

The greater biological effects observed closer to the freeway seems to be related to the differences in particle compositions between aerosols in varying distances from the roadway and UF particles are likely to be important contributors to the overall responses. As pointed out by Zhu et al. (2002), the concentrations of sub-25 nm particles decrease very rapidly with distance from the freeway compared to the concentrations of larger size ranges. Zhang et al. (2003) suggest that this rapid decrease may be related to the volatility of these smaller particles, which makes them evaporate with distance from freeways, as the atmospheric dilution reduces the gas phase concentrations of organic vapors associated with the formation of these particles. There is also evidence that the chemical composition of the particles closer to the roadway may be different than that of the larger range of the ultrafine mode, which may consist of less volatile, more refractory material (Sakurai et al. 2003). Our results support the hypothesis that the carbonaceous fraction of PM may be the most toxicologically important fraction. A more detailed chemical analysis of the PM samples may, with respect to the carbonaceous components, prove useful in identifying specific source characteristics associated with PM-induced or PM-exacerbated airway allergies.

The logistics of the exposure, that is, a van with limited numbers of exposure chambers, restricted us to a single control group that consisted of OVA-sensitized mice exposed to filtered air. Thus the background level of response in the control animals was greater than that in unsensitized mice for some endpoints, and introduced inter-individual variability within exposure groups. This was certainly the case for IL-13, which had high background values in all of the controls. These high backgrounds made it difficult to detect group mean differences with statistical certainty. Despite this, we found that the levels of biomarkers of inflammation and allergic responses were greater in relation to controls and that responses supported greater effects in mice exposed 50 m downwind from the freeway compared to mice exposed 150 m downwind from the freeway.

The overall pattern of responses in these markers resembled those obtained by Takano et al. (1997) who exposed OVA-sensitized mice to diesel exhaust particles using intratracheal installation. Takano et al. (1997) observed substantial increases in eosinophil count, IL5 and IgG1, among other

markers, when OVA-sensitized mice were exposed by inhalation to diesel exhaust particles (DEP) at levels of 3500 $\mu g/m^3$ for six weeks, and changes that approached significance when exposures to DEP were 350 $\mu g/m^3$. These findings contrast somewhat with our observations. The DEP doses used were substantially greater than the CAPs concentrations to which mice were exposed in this study. Since our biomarker changes were significant at these much lower doses, the results would suggest that exposure to ambient PM under the conditions used here was much more effective or that ambient PM derived from mobile sources in close proximity to a freeway with heavy diesel traffic are much more active in stimulating inflammatory and/or allergic responses. Thus, our studies of concentrated ambient particles at levels ranging from about 200 to 500 $\mu g/m^3$ for 5 days per week for two weeks produced significant responses that were consistent with T_H2 effects. Our earlier exploratory studies had found that higher exposure levels, on a mass basis, were associated with suppression of responses. It is possible that compositional differences in the DEP compared to ambient CAPs can explain why Takano et al did not observe similar suppression at their high exposure level.

The data in this study indicate that CAPs exposure can elicit a significant T_H2 response relative to controls. *In vitro* studies (Li et al., 2003) conducted in the Los Angeles Basin which separated PM into three size ranges (coarse, F, and UF) have demonstrated effects on macrophages and epithelial cells. UF particles were most potent in inducing oxidative stress as measured by heme oxygenase induction, GSH/GSSG ratio and the generation of reactive oxygen species as measured by dithiothreitol (DTT). UF PM produced significantly greater oxidative stress, redox activity, and mitochondrial damage than F and coarse on a per-microgram basis. Therefore, the study reported here represents one of the first investigations that links PM-related effects found with *in vitro* assays with findings from *in vivo* investigations in relation to the impact of differing PM size distributions on toxicity. The findings of the two studies demonstrate a consistent impact of PM on toxicity and our study is the first to link these effects specifically to ambient carbonaceous PM components. The availability of the mobile PM concentrators has made possible the conduct of both *in vitro* and *in vivo* assays in the field to evaluate the physical/chemical and the spatial/temporal characteristics of PM related toxicity.

TASK 3. Conduct animal inhalation studies at Source and two Receptor sites (Source/Receptor Study).

Objectives

The objective of the source-receptor study was to apply the VACES to examine the hypothesis that chemical transformations occur during transport of aerosols from source to receptor sites in the LA Basin that modify the toxicity and allergic potency of urban PM in rodent models. The VACES and mobile animal exposure facility were used to perform exposures at sites representative of sources and receptors along a trajectory of airflow across the basin. Intermediate receptor sites and sites at longer distance along the trajectory were selected. An additional objective of the study was to explore the potential for season, as a surrogate for photochemical activity, to modify effects of location on the study endpoints. At each location, exposures were carried out during periods of high photochemical activity and low photochemical activity. Exposure studies were carried out in both rat and mouse models.

The animal work and sample collection for the mouse study was fully funded under this ARB contract and is reported below. U.S. EPA funding was used for support of personnel and supplies required for operating the particle analysis equipment and for performing the physical, inorganic and organic chemical characterization of the aerosol at each site. ARB funding was used to support the operation of the concentrators, the animal exposures and the biological assays performed. A study in rats was funded by U.S. EPA, with the concentrator operations necessary to carry it out supported by ARB. For methods, results, and discussion of the rat source/receptor study please see the progress report from Drs. Jack Harkema and Costas Sioutas submitted to EPA December 22, 2004 under EPA Agreement Number R-82921601, attached as Appendix K.

Methods

The exposure protocol was the same as that used for the freeway study (all methods are described in detail in Appendices H and J). Mice were randomized into 3 exposure groups. Group 1 was exposed to purified air, group 2 was exposed to UF particles and group 3 was exposed to F + UF particles for 5 days per week for 2 weeks. OVA was administered via nasal instillation to each mouse prior to each day of exposure. Animals were challenged with OVA aerosol (30 mg/m^3) 2 weeks after the last exposure and euthanized 24 hours after the challenge. The lungs were lavaged, and prepared for biomarker assays. The biomarkers included interleukin-5 (IL-5), interleukin-13 (IL-13), OVA-specific immunoglobulin E (IgE), OVA-specific immunoglobulin G1 (IgG1) and eosinophil (EOS) influx.

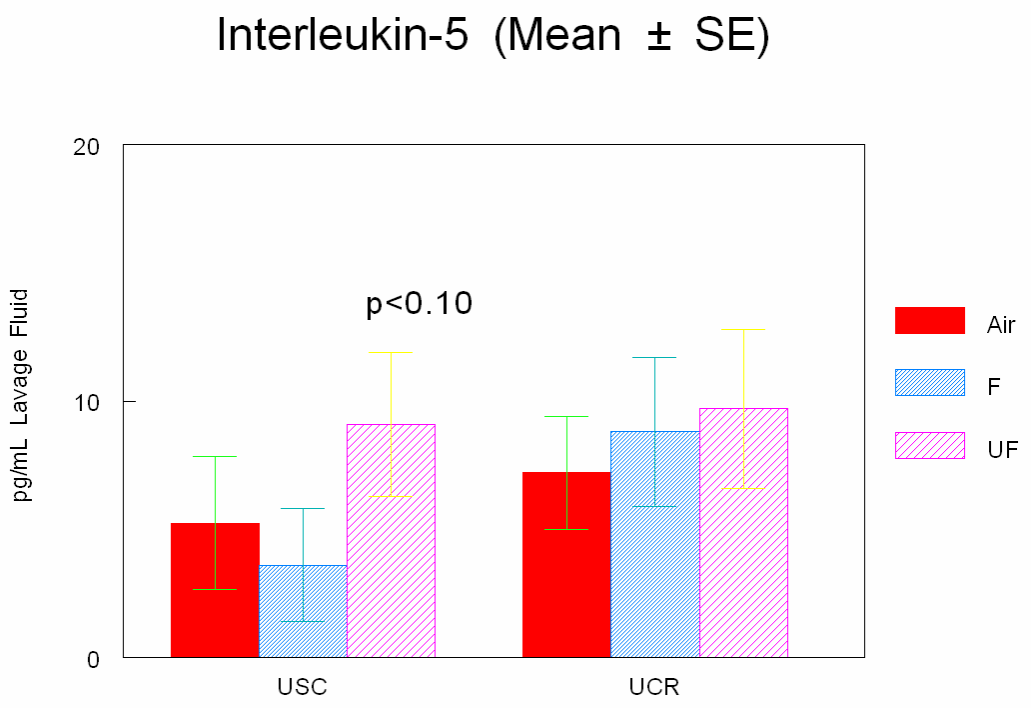
Experimental Design

The original power calculations suggested that data from about 25 mice would be needed to have adequate power to see significant differences between group mean values as a function of particle size or exposure location. An important part of our analysis was the recognition that we were using a minimally-sensitized model so as to enable us to differentiate subtle effects of PM exposure. This resulted in the model being more variable in response than the more robust responses obtained by i.p. injection of OVA used by other investigators. However, other investigators using the more 'robust' model required exposure concentrations that were orders of magnitude greater than those we

successfully used for the Freeway study. Thus we have a useful model, but one that requires substantial sample size.

In the original design we had a source site, a receptor site, and an intermediate site. We initially used a site in Claremont as our intermediate site, however that site was managed by the Department of Water and Power and for several reasons, one of which was intensified efforts at maintaining security of water supplies, we could not complete our studies there. The AQMD graciously allowed us to use their Diamond Bar facility as an intermediate site and we completed a field exposure there, to bring us to two total intermediate site exposure studies. In January 2005 we reported that it was not possible for us to obtain a sufficient sample size (n ~ 25) as per our original power calculations for all three sites to test the hypothesis that there were site-related differences in the responses of the allergy model. A preliminary analysis (Figure 3) for exposures with group sizes of 16 to18 mice suggested that exposure to UF particles at USC resulted in increased IL-5 compared to air exposure, and this difference approached statistical significance ($p = 0.10$). The IL-5 levels at UCR showed only a small difference between samples from air and UF-exposed mice. The data showed a trend ($p = 0.15$) for a greater UF effect compared to air or F particle exposures. It is possible that if the group size were enlarged, there might be significant differences between the source and receptor sites. Accordingly, during the last year of this project we focused on increasing the sample size for the USC (source) and Riverside (downwind receptor) sites, and did not continue evaluations at the intermediate sites. This decision was supported by ARB staff.

Figure 3: Comparison of IL-5 at the Source and Receptor Sites



Results

Exposures were completed in Summer 2005. The exposures and bioassays performed are summarized in Table 5. By focusing our efforts on the USC and Riverside sites we achieved $n \geq 25$ in each particle size/location group, although each group included mice from three different exposure dates. If the data are pooled across sites we have completed four exposures during periods of high photochemical activity and four exposures during periods of low photochemical activity, providing $n \sim 36$ for each period in each size fraction.

The bioassay and atmospheric chemistry data are presented in Tables 6, 7 and 8, for the USC, Intermediate and Riverside sites, respectively. The values shown represent the number of animals exposed for which valid data were obtained; the group mean value and pooled standard error for each location were analyzed. There is substantial animal to animal variation. Some data values were identified as possible outliers. Outliers were provisionally identified as values that when removed from a group mean significantly ($p<0.05$) reduced the variance. For the evaluation below we only eliminated data from mice that had two or more 'outliers', and in those cases, none of the data for that mouse was used. Data from 4 mice were dropped from the analysis for this reason.

Analyses of variance did not show any statistically significant effects associated with exposure to PM at either the source, intermediate, or receptor sites. However, as noted for the Freeway study data, ANOVA may not be the best way to analyze this type of data because compositional relationships of the PM at the various sites may be different. We therefore tested whether IgG1 was increased as a function of EC or OC, and whether the slope of the relationships were different between data from the exposures at USC (source) and Riverside (receptor) sites. We chose OVA-specific IgG1 for this analysis because it is a sensitive biomarker for T_H2 responses in the OVA-sensitized mouse. We chose EC and OC because the Freeway study demonstrated significant relationships of these PM components to changes in IgG1 at the site 50 m downwind from the roadway but not at the site 150 m downwind from the roadway. As seen in Tables 6 and 8, the average IgG1 values varied between sets of analyses, possibly due to slight differences in the reagents used for this assay. We therefore plotted the results in terms of percent of control in Figure 4. The figure shows that there is a greater slope for both EC and OC at the USC (source) site than at the Riverside (receptor) site. However, as can be deduced from the low correlation coefficients (r values shown on the plots), the differences in slopes are not statistically significant at the $p = 0.05$ level. However, the data are consistent with the findings of the Freeway study which are discussed in Task 2, in that exposures to PM near the source may be more toxic than exposures downwind of the source, at least with respect to development of airway allergic responses. It can also be seen by examination of Figure 4 that responses to F PM exposure are not different from those of UF exposure at either the source or receptor site. As was mentioned for the Freeway study, the most likely reason for this is that UF particles make up a substantial fraction of the total F particles and therefore measured effects may be due to the UF component.

Table 5: Exposures performed at Source and Receptor Sites and Completed Bioassays

Year	Season	Date	Site	Exposure	Photochemical Activity	EOS	IL-5	IL-13	IgE	IgG1
2002	Winter/Spring	2/18-3/01	Claremont	UF, F, FA	Low	x	X	X	x	x
	Fall/Winter	10/23-11/01	USC	UF, F, FA	Low	x	X	X	x	x
2003	Summer	8/4-8/15	Riverside	UF, F, FA	High	x	X	X	x	x
2004	Winter/Spring	3/1-3/12	Riverside	UF, F, FA	Low	x	X	X	x	x
	Summer	6/21-7/2	USC	UF, F, FA	High	x	x	X	x	x
	Summer	8/2-8/13	Diamond Bar	UF, F, FA	High	x	x	X	x	x
2005	Winter	March-April	USC	UF, F, FA	Low	x	X	X	x	x
	Summer	July - August	Riverside	UF, F, FA	High	x	X	X	x	x

Table 6: Summary Source-Receptor Data – Source Site

Analyte	Exposure	USC (Source) November 2002 Low Photochemical Activity			USC (Source) June/July 2004 High Photochemical Activity			USC (Source) March/April 2005 Low Photochemical Activity		
		n	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.
IL-5 (pg/mL)	Control	9	8.1	3.3	9	6.8	1.6	9	6.2	1.8
	F+UF	9	5.6	2.0	9	1.0	1.9	9	3.2	1.3
	UF	9	12.3	3.2	9	ND*	3.1	9	1.8**	2.3
IL-13 (pg/mL)	Control	9	5.5	1.7	9	5.8	1.5	9	4.1	2.0
	F+UF	9	5.2	1.6	9	2.8	0.6	9	4.1	1.3
	UF	9	3.0	0.6	9	2.2	1.1	9	3.2	1.0
IgE (Units/mL)	Control	5	6.1	4.0	9	0.8	0.4	9	0.03**	0.02
	F+UF	9	2.3	1.1	8	0.8	0.6	9	0.3**	0.2
	UF	7	5.9	2.0	8	1.3	0.5	9	0.2**	0.2
IgG1 (Units/mL)	Control	9	4000	1900	9	5400	1900	8	1100	300
	F+UF	9	2700	1000	9	5500	2100	9	3100	800
	UF	9	6400	2000	9	4500	1100	9	1600	500
EOS (# Cells in BAL)	Control	9	260	160	9	0	0	9	0	0
	F+UF	9	30	30	9	0	0	9	0	0
	UF	9	470	300	9	0	0	9	0	0
Total PMNs (# Cells in BAL)	Control	9	2000	900	8	100	50	9	1100	600
	F+UF	9	600	200	8	500	160	9	800	200
	UF	9	1400	400	9	200	150	9	1000	400
EC ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	9.1	-	-	3.6	-	-	9.8	-
	UF	-	7.4	-	-	4.2	-	-	2.1	-
OC ($\mu g/m^3$)	Control	-	-		-	-	-	-	-	-
	F+UF	-	97	-	-	119	-	-	117	-
	UF	-	48	-	-	70	-	-	59	-
Total Metals ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	22	-	-	71	-	-	16	-
	UF	-	17	-	-	9.2	-	-	12	-
Nitrate ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	75.5	-	-	183.5	-	-	117.9	-
	UF	-	55.6	-	-	45.2	-	-	15.1	-
Sulfate ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	161.2	-	-	151.8	-	-	110.2	-
	UF	-	42.8	-	-	54.4	-	-	8.1	-
Particle Count (#/cc)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	-	-	-	-	-	-	-	-
	UF	-	210000	-	-	-	-	-	-	-
Particle Mass ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	310	-	-	356	-	-	443	-
	UF	-	170	-	-	151	-	-	103	-

* Below the detection limit; ** Large number of values below the detection limit

Table 7: Summary Source-Receptor Data - Downwind Remote Receptor Site

Analyte	Exposure	Riverside (Distant Receptor) August 2003 High Photochemical Activity			Riverside (Distant Receptor) March 2004 Low Photochemical Activity			Riverside (Distant Receptor) July/August 2005 High Photochemical Activity		
		n	Mean	S.E.	n	Mean	S.E.	n	Mean	S.E.
IL-5 (pg/mL)	Control	9	10.3	3.6	8	8.4	2.1	9	8.2	2.4
	F+UF	8	9.4	3.7	9	8.7	2.8	9	4.8	2.1
	UF	9	9.0	2.6	9	12.0	3.8	8	7.7	3.0
IL-13 (pg/mL)	Control	9	0.9	1.0	9	5.9	1.5	9	1.1	0.6
	F+UF	8	5.1	1.2	9	3.9	0.7	9	0.6	0.8
	UF	9	0.6	0.7	9	4.7	0.9	8	2.0	0.5
IgE (Units/mL)	Control	8	2.2	1.2	9	1.3	0.9	8	0.3	0.1
	F+UF	8	0.2	0.1	9	2.7	2.1	9	0.4	0.2
	UF	9	2.9	0.8	8	2.2	1.1	9	0.6	0.3
IgG1 (Units/mL)	Control	9	4200	1100	9	9100	4800	9	3400	1400
	F+UF	8	4400	2100	9	8600	5300	9	4100	1400
	UF	9	7200	1600	9	8700	3800	9	3600	1500
EOS (# Cells in BAL)	Control	9	30	30	9	0	0	9	0	0
	F+UF	8	60	40	9	0	0	9	0	0
	UF	9	270	150	9	110	80	9	0	0
Total PMNs (# Cells in BAL)	Control	9	1700	700	9	1200	600	9	2000	500
	F+UF	8	2000	1100	9	6000	5600	9	1100	100
	UF	9	900	300	9	1200	700	9	1100	200
EC ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	7.2	-	-	5.4	-	-	5.8	-
	UF	-	4.7	-	-	2.0	-	-	2.2	-
OC ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	34	-	-	158	-	-	108	-
	UF	-	30	-	-	79	-	-	164	-
Total Metals ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	113	-	-	50	-	-	135	-
	UF	-	9.1	-	-	21	-	-	9.5	-
Nitrate ($\mu g/m^3$)	Control	-	-	-	-	-	-	-		
	F+UF	-	85.6	-	-	128.2	-	-		
	UF	-	13.3	-	-	15.2	-	-		
Sulfate ($\mu g/m^3$)	Control	-	-	-	-	-	-	-		
	F+UF	-	33.6	-	-	49.5	-	-		
	UF	-	7.8	-	-	9.3	-	-		
Particle Count (#/cc)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	-	-	-	-	-	-	-	-
	UF	-	-	-	-	130000	-	-	-	-
Particle Mass ($\mu g/m^3$)	Control	-	-	-	-	-	-	-	-	-
	F+UF	-	341	-	-	391	-	-	407	-
	UF	-	86	-	-	155	-	-	152	-

Table 8: Summary Source-Receptor Data – Intermediate Site

Analyte	Exposure	Claremont (Intermediate Receptor) February 2002 Low Photochemical Activity			Diamond Bar (Intermediate Receptor) August 2004 High Photochemical Activity		
		n	Mean	S.E.	n	Mean	S.E.
IL-5 (pg/mL)	Control	8	0.3	2.6	9	6.8	1.6
	F+UF	9	2.4	3.0	9	1.0	1.9
	UF	9	3.0	2.4	9	ND*	3.1
IL-13 (pg/mL)	Control	9	5.0	1.5	9	5.8	1.5
	F+UF	9	5.6	1.2	9	2.8	0.6
	UF	7	6.2	0.9	9	2.2	1.1
IgE (Units/mL)	Control	9	1.0	0.1	9	0.8	0.4
	F+UF	9	1.8	0.6	8	0.8	0.6
	UF	9	2.0	0.6	8	1.3	0.5
IgG1 (Units/mL)	Control	9	1000	200	9	5400	1900
	F+UF	9	900	60	9	5500	2100
	UF	9	900	50	9	4500	1100
EOS (# Cells in BAL)	Control	9	0	0	9	0	0
	F+UF	9	300	210	9	0	0
	UF	9	0	0	9	0	0
Total PMNs (# Cells in BAL)	Control	8	1900	1000	8	100	50
	F+UF	9	800	300	8	500	160
	UF	9	2200	800	9	200	150
EC ($\mu g/m^3$)	Control	-	-	-	-	-	-
	F+UF	-	6.8	-	-	3.6	-
	UF	-	3.3	-	-	4.2	-
OC ($\mu g/m^3$)	Control	-	-	-	-	-	-
	F+UF	-	110	-	-	119	-
	UF	-	31	-	-	70	-
Total Metals ($\mu g/m^3$)	Control	-	-	-	-	-	-
	F+UF	-	18	-	-	71	-
	UF	-	4.4	-	-	9.2	-
Nitrate ($\mu g/m^3$)	Control	-	-	-	-	-	-
	F+UF	-	166.3	-	-	183.5	-
	UF	-	0.7	-	-	45.2	-
Sulfate ($\mu g/m^3$)	Control	-	-	-	--	-	-
	F+UF	-	26.9	-	-	151.8	-
	UF	-	1.2	-		54.4	-
Particle Count (#/cc)	Control	-	-	-	-	-	-
	F+UF	-	176000	-	-	-	-
	UF	-	130000	-	-	-	-
Particle Mass ($\mu g/m^3$)	Control	-	-	-	-	-	-
	F+UF	-	370	-	-	356	-
	UF	-	36	-	-	151	-

* Below the detection limit

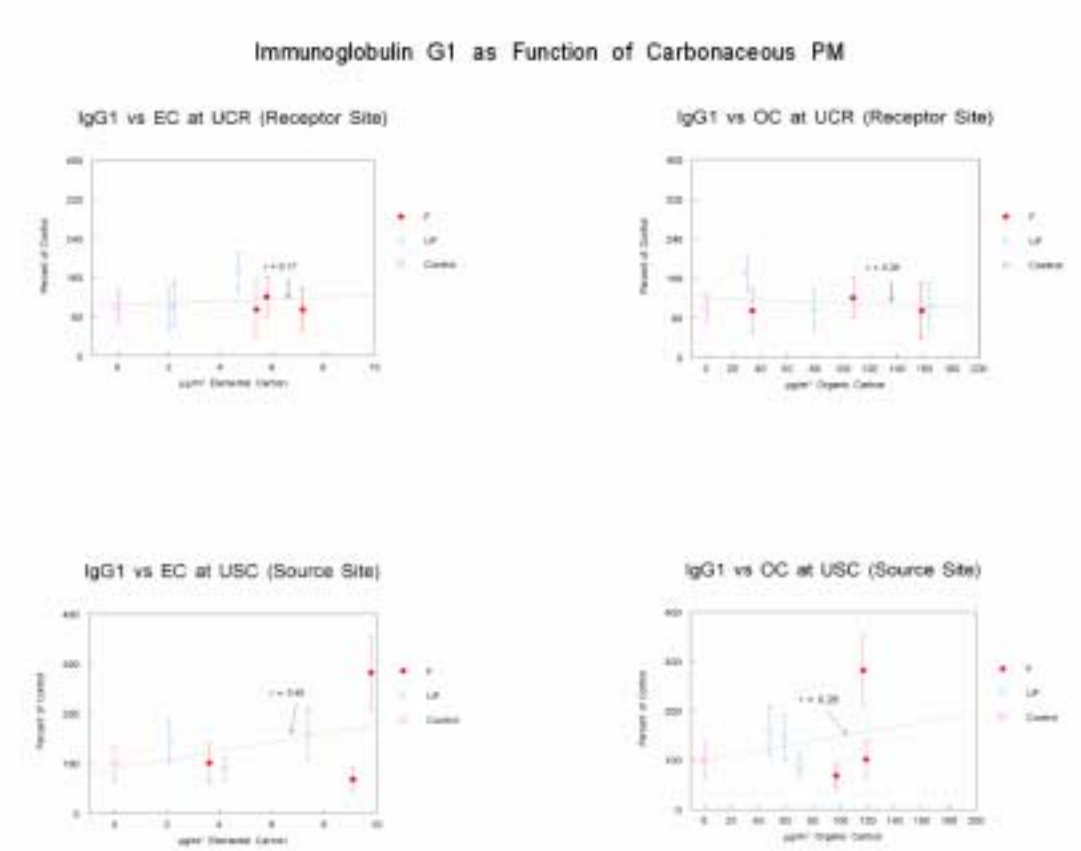


Figure 4: IgG1 as a Function of Carbon-Containing PM Shows Differentiation Between Source and Receptor Site Data

Discussion and Conclusions

We initially hypothesized that receptor sites and high photochemical periods would result in more substantial allergic airways responses. However, our data do not support these hypotheses. In order to test for differences between source and receptor sites, we needed to achieve an n ~ 25. We modified our original experimental design and used our resources to focus on exposures at the USC and Riverside sites to provide sufficient numbers of animals so that we had the power to test whether or not there are differences between source and receptor sites. The results do not support the hypothesis of a significant difference between treatment locations. Since we did not observe an effect of PM treatment when we take all locations together, the lack of clear difference in treatment effect by location is not surprising. When anti-ovalbumin IgG levels relative to controls were analyzed by the level of elemental or organic carbon in the PM samples, there was a stronger, albeit still quite limited, association with carbon levels at the source site. This suggests that fresh emissions may cause greater response in our experimental system.

There was a slight excess in IL-5 levels in mice exposed to UF PM during low photochemical periods (USC November 02, Riverside March 04 and Claremont February 02). IL-5 levels were also enhanced in the Freeway study (Task 2) in mice exposed at 50 m but not at 150 m from the freeway. At the outset of the source/receptor study, we hypothesized that high photochemical periods might produce greater responses. While the IL-5 data are not statistically significant, they contribute to the suggestion that fresh emissions may promote allergic responses in this experimental model to a greater extent than aged, atmospherically processed emissions.

The number of animals in the groups was also sufficient to evaluate the relative potency of the two PM size fractions (F vs. UF), if all locations are pooled. No clear differences were noted in any of the outcomes measured when comparing F vs. UF exposures. When evaluated in an exposure-response manner for EC and OC components of the F and UF CAPs, the data for the different size fractions appear to fall along the same lines. It is important to note that the F PM to which our mice were exposed was concentrated in such a way as to include the UF components. As for the freeway study discussed in task 2, there is a great deal of overlap between the effects of the F and UF CAPs, and we might not expect to see strong differences given the limitations of the study power. Since we saw a limited effect of EC and OC from source sites on IgG, and both carbon types are primarily found in the submicron fraction of the PM, our findings suggest that the UF fraction of PM could be the most important contributor to the effects of PM on biomarkers of airway allergies.

Further animal studies and support from our *in vitro* study findings may help to clarify the preliminary findings presented here. Sensitivity of the animal model could be improved by using longer-term exposure conditions. An asthma aggravation model rather than an asthma development model might deliver different results in future studies designed to test the possible effects of photochemical oxidation of particulate matter and other physicochemical changes associated with particle aging. A study of Brown-Norway rats exposed at source and receptor sites, partially supported by this contract, measured a different set of biomarkers than we have reported here for the mouse study. Still, the findings of the rat study also suggest that PM exposure was not associated with the assessed indicators of allergic response exacerbation (see Appendix K). In fact, PM exposure appeared to produce a suppression of pulmonary responses associated with allergic response. The observed particle-induced attenuation of allergic airway

disease was the greatest with exposures to transported (“aged”) F CAPs and UF CAPS at the receptor site (Claremont, CA).

TASK 4: Collect coarse, fine and ultrafine particles for in vitro toxicity studies from source and receptor sites as well as downwind locations from gasoline freeway (in conjunction with animal studies in Tasks 2/3)

Objectives

The objective of task 4 was to use the newly developed and tested concentrators to collect coarse, fine and ultrafine ambient particles from around the Los Angeles basin, to pursue hypotheses concerning climatological, spatial, temporal, and other correlates of the physical, chemical, and toxicological properties of ambient particulate matter.

Particle Collection Methods

Concentrated ambient air particulates were collected with the mobile particle concentrators developed through the ARB program, and with a liquid impinger, at numerous locations of the LA Basin and provided to investigators of the Southern California Particle Center and Supersite for further study. Investigations focused on how the physical and chemical characteristics and relative toxicity of particles varied with collection location, size fraction, time of day or season, and atmospheric chemistry factors. For example, the VACES system and associated sampling equipment was deployed to collect coarse, fine, and ultrafine particles at locations typically classified as source-dominated or as primarily receptor sites with attendant products of atmospheric chemistry reactions, to assess differences in particle size distribution and chemical composition at these types of locations. Extensive particle collection studies were carried out at freeway and tunnel locations, including roadways that carry predominantly gasoline or diesel engine traffic. Differences in physical and chemical particle characteristics as distance from the freeway increased were explored in detail. We have used the concentrators to collect particles indoors and outdoors to study penetration factors and disposition of particles with different size or chemical characteristics. Samples have been collected on filters to compare analytical findings to impinger samples. We have explored the ability of our concentrators to collect semi-volatile materials. ARB also funded concentrator collections of special samples taken during a major wildfire episode in Southern California.

Results

Based on analyses of concentrated particle collections supported by ARB during the course of this funding, the Southern California Particle Center and Supersite has published numerous studies reporting a wide range of properties of ambient particle samples. While the analytical work performed on the collected material has been funded from other sources, selected citations are included here because the analyses would not have been possible without the support from ARB to develop and operate the concentrators. The results of all the studies performed on PM samples collected with the concentrators during the funding years are too lengthy to summarize. Selected results can be found in the references cited below as well as in progress reports from the SCPCS to the U.S. EPA. SCPCS progress reports may be accessed at: http://www.ph.ucla.edu/scpcs/publications.html, and contain a comprehensive range of study summaries. Below, we present a list of selected studies that report findings on the physical and chemical characteristics from samples at various locations around the LAB,

collected in pursuit of a wide variety of study hypotheses. A discussion of toxicology findings from *in vitro* experiments that we have performed on collected particulate material follows.

The VACES system and associated sampling technology has been deployed in a wide range of projects funded by the U.S. EPA PM Center and Supersites Program. Size selected samples have been collected on filters and in impingers at source and receptor locations in the Los Angeles area, including USC, Downey, and Riverside, at freeway locations, and during a wildfire episode. Analyses of these samples led to the following publications, among others (short summaries of all papers listed below were provided in previous progress reports and are not duplicated here):

Fine PM, Chakrabarti B, Krudysz, Schauer J, Sioutas C. (2004). "Diurnal Variations of Individual Organic Compound Constituents of Ultrafine and Accumulation Mode Particulate Matter in the Los Angeles Basin." Environmental Science and Technology **38**: 1296-1304.

Geller MD, Sardar SB, Phuleria H, Fine PM, Sioutas C. (2005). "Measurements of Particle Number and Mass Concentrations and Size Distributions in a Tunnel Environment." Environ. Sci. Technol. **39**(22): 8653-8663.

Hasson AS, Paulson SE (2003). "An Investigation of the Relationship between Gas-phase and Aerosol-borne Hydroperoxides in Urban Air." Journal of Aerosol Science **34**: 459-468

Khlystov A, Zhang Q, Jimenez JL, Stanier CO, Pandis S, Wornsop DR, Misra C, Fine PM, Sioutas C. (2005). "In-situ concentration of semi-volatile aerosol using water-condensation technology." Journal of Aerosol Science **36**: 866-880

Kuhn T, Biswas S, Fine PM, Geller M, Sioutas C. (2005). "Physical and Chemical Characteristics and Volatility of PM in the Proximity of a Light-Duty Vehicle Freeway." Journal of Aerosol Science **39**(4): 347-357.

Kuhn T, Krudysz M, Zhu Y, Fine PM, Hinds WC, Froines JF, Sioutas C (2005). "Volatility of Indoor and Outdoor Ultrafine Particulate Matter Near a Freeway." Journal of Aerosol Science **36**(3): 291-302.

Phuleria HC, Fine P, Zhu Y, Sioutas C (2005). "Air Quality Impacts of the October 2003 Southern California Wildfires." Journal of Geophysical Research - Atmospheres **110**(D7, D07S20): doi:10.1.1029/2004JD004626

Sardar SB, Fine PM, Jaques PA, Sioutas C. (2005). "Seasonal and Spatial Variability of the Size-Resolved Chemical Composition of PM10 in the Los Angeles Basin." Journal of Geophysical Research **110**: D07S08.

Sardar SB, Fine PM, Mayo PR, Sioutas C. (2005). "Size Fractionated Chemical Speciation Measurements of Ultrafine Particles in Los Angeles Using the NanoMOUDI." Environmental Science and Technology **39**: 932-944.

Sardar SB, Fine PM, Yoon H, Sioutas C. (2004). "Associations between particle number and gaseous co-pollutant concentrations in the Los Angeles Basin." Journal of Air and Waste Management Association **54**(8): 992-1005.

Zhang KM, Wexler A, Zhu Y, Hinds W, Sioutas C. (2004). "Evolution of Particle Number Distribution near Roadways: Part II: The 'Road-to-Ambient' Process." Atmospheric Environment **38**: 6655-6665.

Zhao Y, Bein KJ, Wexler AS, Misra C, Fine PM, Sioutas C. (2005). "Field Evaluation of the VACES Particle Concentrator Coupled to the RSMS-3 Single Particle Mass Spectrometer." Journal of Geophysical Research **110**(D07S02): doi:10.1029/2004JD004644

Zhu Y, Hinds WC, Krudysz M, Kuhn T, Froines J, Sioutas C. (2005). "Penetration of Freeway Ultrafine Particles into Indoor Environments." Journal of Aerosol Science **36**(3): 303-322.

The collective results of analyses of concentrated ambient particles has been a tremendous increase in diverse knowledge of ambient particulate matter in the Los Angeles Basin. Please see the complete publications list from the Southern California Particle Center and Supersite, attached as Appendix L.

In Vitro Toxicology Methods and Results

The hypothesis that motivated this project is that the toxicity associated with airborne PM is due to reactive organic compounds present in the PM, which exert their toxicological actions through generation of reactive oxygen species and formation of covalent bonds with tissue nucleophiles. The ability of ambient coarse, fine, and ultrafine PM to generate reactive oxygen species (ROS), which provide pro-inflammatory stimuli to bronchial epithelial cells and macrophages, was investigated using particles collected in some of the field studies referred to above. Previous studies on the biological effects of diesel exhaust particles (DEP) have highlighted the role of ROS, catalyzed by organic compounds. We set out to establish whether this constitutes an oxidative stress model that can be used to study the biological effects of ambient coarse and fine PM. Towards this end, ambient concentrated airborne particulates, collected with the mobile particle concentrators developed through the ARB program and with a liquid impinger, to investigate their properties in assays that measure the potential to produce cellular oxidative stress.

Traditionally, particle collection for *in vitro* tests has been conducted by using collection substrates such as filters or impactors. Particles collected on filters are subsequently extracted before use in further experimental procedures. Incomplete removal from filters, loss of semivolatile PM constituents, and incomplete extraction of some compounds can be problems. Our studies took advantage of a special liquid impinger (BioSampler^{TM}, SKC West Inc., Fullerton, CA) in which particles are injected into a swirling flow and collected by a combination of inertial and centrifugal forces onto the surface over which the air flow swirls. Particles collected in the BioSampler were subjected to several assays related to the potential to induce oxidative stress in target cells. The assays included cell-free assays of chemical properties as well as tissue culture experiments on macrophage and epithelial cell types. Some of the assays we have applied and brief summaries of our findings from the assays follow. Please refer to the cited publications for complete and detailed presentations of methods, results and discussions.

DTT assay

We applied a method that assesses redox activity of a test substance by measuring the ability to catalyze electron transfer from dithiothreitol to oxygen. This assay has now been applied to a range of concentrator samples. Results indicated that activity in this assay was greater on a per-microgram basis in the ultrafine fractions as compared with fine and coarse fractions. DTT activity correlated well with the PM content of organic carbon, elemental carbon and to a lesser degree, polycyclic aromatic hydrocarbon content (Li et al., 2003; Cho et

al., 2005). Correlation of potency in the DTT assay with the concentration of benzo(g)perylene in the particulate sample was especially good in some tests; BgP is a tracer of vehicular combustion emissions and is emitted at relatively high levels from gasoline engines.

While ultrafine PM fractions were on average found to be more potent than fine and coarse PM, human exposure is a function of both potency and concentration in air. By summing the potency x concentration of coarse, fine and ultrafine for each location studied, a metric for the total redox activity of PM_{10} per unit of air sampled is generated that can be used to compare sampling times/locations.

Ratio of total to oxidized glutathione in cultured cells

The intracellular ratio of reduced (GSH) to oxidized (GSSG) glutathione is a tightly regulated homeostatic process that serves to maintain intracellular redox balance. A drop in the intracellular GSH/GSSG ratio signals the cell that it has become oxidatively stressed. This may initiate protective responses or, if the level of oxidative stress is more acute, could induce injurious effects. Glutathione ratios were significantly decreased after exposure to fine or ultrafine particles collected in winter 2002 at Claremont; coarse particles collected simultaneously did not have this effect (Li et al., 2003). The ultrafines were more potent than fines on a per-microgram basis.

Mitochondrial effects

We have applied a variety of assays to investigate possible impacts of particle and particle extract exposure on the mitochondria of cultured cells, including: Ultrastructural observation by electron microscopy; mitochondrial swelling related to opening of the permeability transition pore; mitochondrial membrane potential changes; mitochondrial calcium ion retention; changes in mitochondrial respiration. After exposure to concentrated particles collected at USC, we observed that ultrafine particles became located in the damaged mitochondria of macrophage and endothelial cell lines (Li et al., 2003). In cells exposed to fine particles, some particles were also observed within mitochondria but without the indicators of damage to the organelle. Coarse particles did not appear to reach the mitochondria. In subsequent studies, ultrafine particles were found to affect mitochondrial functioning, by inducing the permeability transition pore to open and effecting mitochondrial swelling (Xia et al., 2004). Calcium retention capability of mitochondria was reduced by ultrafines as well. These effects were also observed with extracts of diesel exhaust particles while polystyrene nanoparticles were inactive, indicating that the effects of ultrafine ambient particles were likely associated with the chemical constituents of the particles.

Activation of oxidant stress-related genes and gene products

We have developed and applied assays to measure cellular responses to particulate matter on a molecular level, as part of our interest in determining the molecular mechanisms by which particulate matter induces a variety of adverse health effects. In earlier publications, we showed that concentrated ambient particles induce expression of a gene product called heme oxygenase-1 (HO-1) (Li et al., 2002, 2003). HO-1 is one of a set of phase 2 detoxification and antioxidant enzymes thought to serve in protective functions, and the expression of which is upregulated in cells exposed to oxidants. Our data demonstrate that fine particulates induce HO-1 expression more readily than coarse particles. The ambient PM effect on HO-1 was concordant with a relatively high organic carbon (OC) and PAH content in fine versus coarse particles. While fine particulates consistently induced HO-1 expression during the entire

survey period, coarse particulates were more effective at inducing the same effect during the fall and winter months. Moreover, this biological effect was positively correlated to the higher OC and PAH content of fine versus coarse particles, as well as the rise in PAH content that occurs in coarse particles during the winter months. In a subsequent study, we developed an assay for a gene product (Nrf2) that regulates the expression of many of the phase 2 and antioxidant enzymes, including HO-1. A comparison of the effects of ambient ultrafine and coarse particles collected via the VACES demonstrated that the ultrafine fraction was more potent than the coarse fraction in assays that measure Nrf2 protein levels and Nrf2 activation of a target gene promoter, indicating that ultrafine particles are effective at inducing an antioxidant response in the cell lines studied (Li et al., 2004).

Discussion

Extensive discussion of our findings from toxicity assays on samples collected under the ARB concentrator program may be found in the published papers cited above. The assays have shown that particles from the Los Angeles Basin have the capacity to generate reactive oxygen species and promote intracellular oxidative stress. Ultrafine particles are more potent in comparison to fine and coarse particles, and this appears to be dependent on the greater organic carbon content per unit mass in ultrafines. Organic carbon in ultrafines is primarily of anthropogenic origin and we have investigated the hypothesis that vehicular combustion products are especially potent in promoting oxidative stress. Redox active quinones and other species are thought to be involved in the redox activity of ambient particulate matter, but other species that are metabolized to redox active products, such as polycyclic aromatic hydrocarbons, may also be of importance. Further studies on the role of metabolism and the disposition of the chemical constituents of ambient particles are needed.

We also conducted studies of mitochondrial toxicity. The smallest and potentially most toxic ambient particles, ultrafine particles, were identified by electron microscopy inside damaged mitochondria (Li et al. 2003). It is not clear whether UFPs target the mitochondrion directly or enter the organelle secondary to oxidative damage (Li et al. 2003), but PM-induced mitochondrial perturbation has important biologic effects, which include the initiation of apoptosis and decreased ATP production (Hiura et al. 2000). That ultrafine particles were able to induce mitochondrial swelling and alter calcium ion retention supports their ability to be involved in toxicity to this organelle, promoting apoptosis at high exposure levels.

Consistent with what was noted in Task 2 of this report regarding greater responses of mice to particulate matter when they were exposed at the site nearest to the freeway, we observed modestly higher oxidative potential of the ultrafine particles collected 50 m downwind of a freeway with heavy diesel traffic as compared with the ultrafines collected 150 m downwind (Cho et al. 2005). As we discovered with the studies of mice exposed at source and receptor sites (Task 3 of this contract), we were not able to determine major differences in assay results when we compared particle samples collected at source vs. receptor locations. However, it is important to note that it has not been possible yet to collect source and receptor samples simultaneously, so that the samples we have from these sites to date may not be directly comparable.

In vitro toxicology results from the ARB-supported particle collections have been reported in several publications from the Particle Center (Cho et al., 2005; Li et al., 2002; Li et al., 2003; Li et al., 2004; Xia et al., 2004).

SUMMARY AND CONCLUSIONS

The development of particle concentrator technologies has fundamentally changed research into the causal factors of health effects associated with exposure to PM. Historically investigators did not have the capability to concentrate ambient particles to conduct inhalation studies and the use of ambient exposure levels did not result in sufficiently sensitive animal toxicology experiments. For higher exposure levels, investigators were limited to laboratory generated aerosols, which cannot capture the chemical and physical complexity of ambient particulate matter. Now, with support from this contract, new particle concentrator technologies have now been developed and validated that can be used to provide elevated levels of ambient particulate matter for experimental exposures of animal or human subjects. Research and development under this contract has produced compact, highly versatile concentrators that can be applied to a variety of animal and human exposures in the ultrafine, fine, and coarse particle size modes. In addition to use in their exposure study capabilities, the new concentrators can be configured to collect high volumes of particulate material for further study in the laboratory using *in vitro* experimental techniques. The concentrators have been laboratory and field tested, and may be installed in mobile platforms to make transportable exposure facilities. We have used the concentrators in such a mobile exposure facility equipped to house rodents for extensive inhalation studies, examples of which are detailed under Tasks 2 and 3 of this report. Similarly, we have installed concentrators in a trailer configured for exposures associated with human clinical studies, and have put them to use in a series of such studies.

With the concentrator technologies developed or refined under this contract, it is now possible to identify and quantify dose-response relationships, and to design detailed mechanistic studies in laboratory animals. Because the concentrators are more compact than earlier technology, the system is easily mobile, which allows investigators to differentiate the effects of ambient aerosols originating from different sources, or to tailor studies to a particular community of interest.

We have applied the concentrators to sub-acute exposure studies of mice and rats that were also exposed to a known allergen (ovalbumin). In Task 2, a novel particle concentrator and animal exposure system were used to determine whether real-world ambient particulate matter near a heavily trafficked road might contribute to enhancement of allergic responses. We measured interleukin-5 and interleukin-13, key signaling molecules that act in helper T cell allergic responses (T_H2 cells). We also looked for whether co-exposure to particulate matter acted as an adjuvant to the sensitizing ovalbumin exposure, by measuring anti-ovalbumin antibodies (IgE and IgG1) in plasma. Mice exposed at a site 50 meters from the freeway exhibited a response consistent with a T_H2-type response whereas those exposed 150 m downwind had a considerably smaller response, suggesting that distance from the freeway was an important factor. Significant differences were observed between ovalbumin sensitized mice exposed to PM or to clean air. That is, even with the limitations of sample size and the variability in sensitization responses in this animal model, effects of both PM exposure and distance from the freeway could be identified. The findings also suggested an important role of the carbon-containing fraction of PM. Our findings are consistent with epidemiological observations of more wheezing and asthma-like symptoms in children that live near heavily trafficked roads, especially those that are traversed by large numbers of diesel-powered vehicles.

Brain tissues from some mice exposed to PM at the freeway sites were examined to determine the activity level of a nuclear transcription factor and two proinflammatory cytokines regulated by this factor. In exposed mice, the activity of the transcription factor was enhanced over control levels. Levels of both the proinflammatory cytokines were increased in the brains of treated mice. The extent to which induction of inflammatory parameters in the brain of PM-exposed animals may lead to potentially adverse consequences is at present unknown, but our exploratory study suggests that components of inhaled particulate matter may trigger a proinflammatory response in nervous tissue that could contribute to the pathophysiology of neurodegenerative diseases.

In Task 3, we carried out a mouse study with a design related to the freeway study performed in Task 2. Rather than freeway locations, however, we selected locations traditionally characterized as "source" or "receptor" sites, so that we could compare the allergic responses of mice exposed to PM closer to emissions sources to the responses of mice with comparable exposure to PM that had undergone atmospheric processing and aging. The source-receptor study examined the hypothesis that chemical transformations during transport from source to receptor sites in the LA Basin will modify the toxicity and allergic potency of the urban particulate. Conclusions from this project were limited. We did not observe a clear effect of exposure to PM on the allergic airways endpoints measured. As such, we were unable to distinguish differential effects of PM exposure that would be attributable to location. When data on a selected response variable (anti-ovalbumin immunoglobulin G) was analyzed using concentrations of elemental or organic carbon as the explanatory variable, we found that both EC and OC were more strongly associated with immunoglobulin levels at the source site than the receptor site. There was a limited effect of season observed on interleukin-5 levels, in that mice exposed to ultrafine particles during low photochemical periods showed a slight excess of IL-5 over mice without PM exposure. More analysis is needed, but we cautiously interpret these findings as support for the hypothesis that fresh emissions could be more toxic than aged, insofar as enhancement of the allergic responses measured is concerned.

Task 3 also funded the operation of concentrators for a source/receptor study carried out in rats exposed to ovalbumin. The results of this study suggested that the PM exposures did not enhance the allergic airways responses measured, but rather may have suppressed pulmonary inflammatory and epithelial responses to the inhaled allergen. We observed related suppressive effects of high PM exposure levels on some of the allergic biomarkers that we examined in mice during initial experiments in the freeway study. One interpretation is relatively simple: high exposure concentrations are overtly toxic and could result in inhibited allergic responses. The biology is like more complicated though. Modulation of mammalian immune responses is extremely complex and relies upon coordinated actions of numerous cell types and inter- and intra-cellular signaling pathways. Environmental exposures may not have simple, easily measured effects on something as intricate as allergic response, so that some elements could be enhanced by a particular exposure while others are suppressed, resulting in changes in measured biomarkers of effect that are not straightforward to predict or interpret. In the rat study, particle exposure may have stimulated an immune system pathway that enhanced certain responses while attenuating the downstream markers of allergic response. It appears that the development and severity of experimentally induced allergic airway disease may be highly dependent on specific exposure parameters, including the time at which the laboratory rodents are exposed to both the allergen and the airborne particles.

The work performed under Task 4 involved collection of particles from various locations around the Los Angeles Basin in support of studies on the chemical, physical, and

toxicological properties of ambient particulate matter carried out for the Southern California Particle Center and Supersite. Extensive findings on particle characteristics included particle number concentrations, size distributions, and detailed PM chemical composition as a function of particle size. Sampling locations were chosen to provide wide geographical and seasonal coverage, including urban "source" sites and downwind "receptor" sites, and special studies of mobile source particles at freeway locations. These findings are summarized in numerous publications some of which are cited in Task 4, and many of our conclusions relevant to this work are also summarized in a final report submitted to the U.S. EPA Supersites program. The Final Report of the Southern California Supersite is available for public download at http://www.epa.gov/ttn/amtic/finrep.html.

A series of toxicological studies were also carried out using the particles collected under Task 4. The assays indicate that ambient particles collected in the Los Angeles Basin have the capacity to generate reactive oxygen species and promote intracellular oxidative stress. In turn, oxidative stress in target cells may underlie inflammatory processes in airways and cardiovascular system, providing a biological mechanism for the cardiorespiratory effects associated with exposure to particulate matter. Ultrafine particles are more potent in comparison to fine and coarse particles in the assays of oxidative stress potential that were conducted, and this appears to be associated with greater organic carbon content per unit mass in ultrafines. Organic carbon in ultrafines is primarily of anthropogenic origin and our findings support the hypothesis that vehicular combustion products may be especially potent in promoting oxidative stress and attendant damage at the tissue and cellular level.

RECOMMENDATIONS FOR FUTURE RESEARCH

The concentrators we have developed will continue to support a wide range of research efforts, including important studies carried out in association with the Southern California Particle Center (SCPC), recently re-funded by U.S. EPA for a project period from 2006-2010. Extensive studies of particles using the concentrator technology will examine the relationships between particulate matter sources, exposure, and toxicity using a variety of experimental models and research approaches. The experiments performed on collected ambient particles that explored the association of chemical composition and physical characteristics of PM with activity in *in vitro* assays formed the basis for a new set of in vitro studies that will be performed in the SCPC. Further investigations of which particle properties best predict particle behavior in redox and electrophilic reactions in exposed cells will be performed and applied to future particle collections. We plan to conduct studies that will assess in vitro toxicity of particles collected at airports, seaports, and at locations influenced by residential wood combustion and road and brake dusts. To continue our work on motor vehicles, we are involved in studies of engine technology on particle composition and toxicity, and plan to continue our work with roadway particle samples.

The allergy model used in the mouse studies reported here was designed to be minimally sensitized so that the mice would be sensitive to lower exposure levels, more relevant to actual ambient exposures. We were able to observe small but significant effects in mice exposed 50 m downwind of a freeway but not 150 m downwind. However sample size was a limiting factor given the variability in response. Our preliminary work suggested that exposure duration was also an important variable and further studies should be carried out to explore exposure parameters that influence the association between particle exposure and allergic responses. In the source/receptor studies we exposed animals 4 hr per day, 5 days per week for

2 weeks. This duration was adequate to demonstrate effects near a freeway, however longer exposures might be needed to show effects at sites that are more remote from sources. In addition, more detailed exposure-response assessments that utilize the chemical and physical data we have collected on PM are likely to be more informative than analysis based on exposure defined simply by PM treatment and location. The Children's Health Study has demonstrated significant changes in pulmonary function in children living in both source and receptor areas. The chronic exposures experienced by the children in the study were associated with decreased pulmonary function. Studies of lung function and lung morphometry could be made with sensitive animal models to test hypotheses that specific compounds in ambient PM could have been causal factors in the reduced lung function in PM-exposed children.

The SCPC has proposed a study of the role of oxidative stress in PM-induced exacerbation of asthma that will use an OVA sensitized Balb/c mouse model, related to the model used for the freeway study. This study will build upon and expand the findings of the freeway study. In addition to the endpoints studied in the freeway work, lung histology, immunohistochemistry, and airway morphometry experiments are planned. Groups of mice will be exposed to three target PM exposure levels, in addition to purified air controls, to produce a comprehensive set of dose-response relationships. A new rodent model that is deficient in a gene thought to play an important role in anti-oxidant defenses will be used to investigate effects of susceptibility on PM-induced airway inflammation.

We reported above that PM present in air pollution may contribute to the progression of pre-existing changes relating to the pathology of age-related neurological diseases. The nervous system has only recently been identified as a target tissue for PM-induced toxicity, but there is great interest in this and other non-respiratory targets. The neuroanatomical loci of the inflammatory changes we measured remain to be elucidated. More epidemiological and mechanistic studies on the effects of inhaled particulate matter on the brain are warranted before concluding that exposure to PM increases inflammatory processes in the brain and that this may be relevant to the progression of neurodegenerative diseases. The use of transgenic animal models of age-related neurological disorders will be important in evaluating whether PM exposure can exacerbate formation of pathological lesions characteristic of neurodegenerative disease processes.

To build upon our interest in new target tissues, the SCPC has proposed a set of studies in an atherosclerotic animal model and in elderly human subjects with coronary heart disease that will investigate the effect of PM exposure on endpoints related to cardiovascular health. Findings from *in vitro* studies supported by the ARB-funded concentrator work were used to develop hypotheses for the atherosclerosis and cardiovascular health studies. The latter studies are built upon our findings that ambient PM possesses the capacity to generate reactive oxygen species and promote intracellular oxidative stress. Oxidative damage may play a role in the pathophysiological mechanisms of cardiovascular morbidity and mortality, and our future work intends to investigate specific mechanistic hypotheses.

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APPENDICES

Note: Because appendices A through H are copyrighted, they are not included in the publicly-available version of this report.

Appendix A: Chang MC, Geller MD, Sioutas C, Fokkens PHB, Cassee FR (2002). "Development and Evaluation of a Compact Highly Efficient Coarse Particle Concentration for Toxicological Studies." Aerosol Science and Technology **36**: 492-501.

Appendix B: Gong H, Linn W, Terrell S, Anderson K, Clark K, Sioutas C, Cascio W, Alexis N, Devlin R. (2004). "Altered Heart Rate Variability in Asthmatic and Healthy Volunteers Exposed to Concentrated Ambient Coarse Particles." Inhalation Toxicology **16**(6): 335-343.

Appendix C: Misra C, Kim S, Shen S, Sioutas C (2002). "A High Flow Rate, Very Low Pressure Drop Impactor for Inertial Separation of Ultrafine from Accumulation Mode Particles." Journal of Aerosol Science **33**(5): 735-752.

Appendix D: Misra C, Fine P, Singh M, Sioutas C (2004). "Development and Evaluation of a Compact Facility for Exposing Humans to Concentrated Ambient Ultrafine Particles." Aerosol Science & Technology **38**(1): 27-35.

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Appendix F: Khlystov A, Zhang Q, Jimenez JL, Stanier CO, Pandis S, Wornsop DR, Misra C, Fine PM, Sioutas C. (2005). "In-situ concentration of semi-volatile aerosol using water condensation technology." Journal of Aerosol Science **36**: 866-880.

Appendix G: Campbell A, Becaria A, Bondy SC, Meacher D, Oldham M, Sioutas C, Misra C, Kleinman M. (2005). "Exposure to Particulate Matter in Air Pollution Increases Inflammatory Paramenters in Mouse Brain." NeuroToxicology **26**(1): 133-140.

Appendix H: Kleinman M, Sioutas C, Stram D, Froines J, Cho A, Chakrabarti B, Hamade A, Meacher D, Oldham M (2005). "Inhalation of concentrated ambient particulate matter near aheavily trafficked road stimulates antigen-induced airway responses in mice." Journal of Air & Waste Management Association **55**: 1277-1288.

Appendix I: Oldham M J, Phalen RF, Robinson RJ, Kleinman MT (2004). "Performance of a Portable Whole Body Mouse Exposure System." Inhalation Toxicology **16**(9): 657-662.

Appendix J: Materials and Methods for Mouse studies performed under Tasks 2 and 3

Appendix K: Harkema JR and Sioutas CS. Effects of Airborne Particles on Allergic Airway Disease. NCER Assistance Agreement Annual Report for EPA Agreement Number: R-82921601. Submitted 12/22/04.

Appendix L: SCPCS Publications List 2000-2005.

APPENDIX J

Materials and methods for the animal studies reported in Tasks 2 AND 3.

Atmosphere Generation and Characterization

Fine (F; $d_p \leq 2.5$ μm) and ultrafine (UF; dp ≤ 180 nm) ambient particles were concentrated using a Versatile Aerosol Concentration Enrichment System (VACES), which has been characterized and described in detail by Kim et al. (Kim et al., 2001a; Kim et al., 2001b). The US EPA uses the term "$PM_{2.5}$" to define particles with aerodynamic diameters less than or equal to 2.5 μm. Thus the F particles used could be considered concentrated $PM_{2.5}$. Analogously, the UF could be considered concentrated $PM_{0.18}$. The VACES system is mobile and capable of enriching the concentration of particles ranging in size from 0.01 to 10 μm by up to 40 times, depending on the output flow rate. The VACES can incorporate size-selective inlets to provide concentrated ambient particles (CAPs) in a defined size range.

For this study, the VACES was installed inside a one-ton van (Dodge RAM 350 Custom), which had been internally modified for the safe handling of animals. Electric power for the VACES and all other sampling instruments was supplied by a 1.2 kW gasoline-powered portable power generator (Model EU 1000i, Honda Motor Co., LTD., Tokyo, Japan). The generator was placed approximately 18 m downwind of the sampling location of the van and PM emissions from the generator did not affect the input aerosol to the VACES (Kleinman et al., 2005). Ambient air samples for the exposures were drawn into the VACES via a 2 m long and 7.5 cm in diameter duct made of aluminum in order to avoid particle losses due to electrostatic deposition. The concentrated aerosols were delivered to whole-body animal exposure chambers (Oldham et al., 2004; Kleinman et al., 2005) as shown in Figure 2. Each exposure chamber was a sealed unit, sectioned for housing 9 mice per chamber. Temperature and airflow were controlled during the exposures to ensure adequate ventilation, minimize buildup of animal-generated contaminants (dander, ammonia, and CO_2) and to avoid thermal stresses. The animals were transported to and from the exposure site in an air conditioned vehicle and provided with purified air during transport. Between exposures the animals were housed at UCI in and AAALAC-accredited vivarium and provided with food and water, ad lib.

Figure 1. Schematic Diagram of VACES and Exposure System

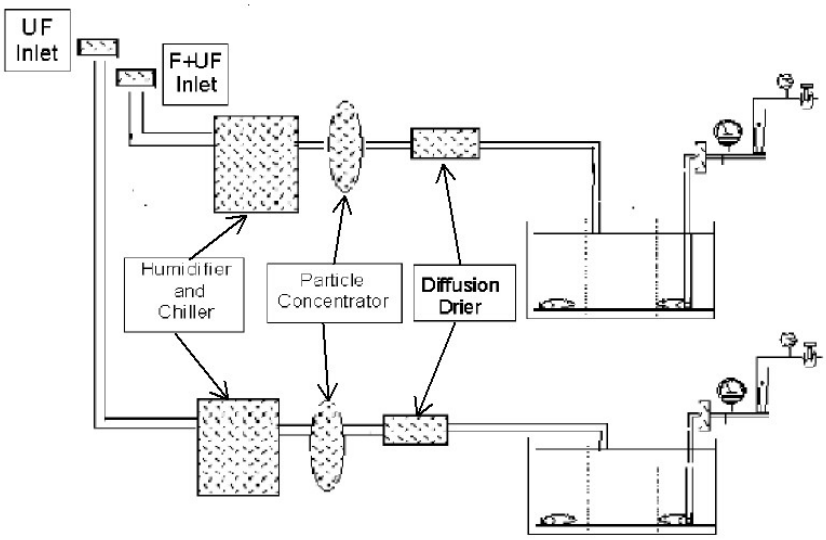


Figure 1. Schematic diagram of the VACES particle concentrator for simultaneous exposures of mice to fine + ultrafine (F+UF; $d_p < 2.5$ μm diameter) and ultrafine (UF; dp < 0.15 μm diameter) in a mobile exposure facility.

Physico-Chemical Characteristics of Concentrated PM

Samples of F and UF CAPs were collected from a port immediately upstream of the exposure chambers to determine physical and chemical characteristics of the exposure aerosols. Samples for analysis of inorganic constituents were collected on 37 mm Teflon filters (PTFE 2 μm pore, Gelman Science, Ann Arbor, MI) at a flow rate of 1 LPM and analyzed for particle masses and chemical composition. The filters were equilibrated at constant humidity and temperature overnight and weighed before and after each exposure session using a Mettler 5 Microbalance (MT 5, Mettler-Toledo Inc., Hightstown, NJ), under controlled relative humidity (40-45%) and temperature (22-24°C) conditions. The filters were analyzed by X-Ray fluorescence (XRF) to determine the concentrations of particle-bound trace elements and metals. Inorganic ions (sulfate and nitrate) were measured using ion chromatography. Elemental carbon (EC) and organic carbon (OC) were determined by collecting particles on 37-mm pre-baked quartz filters (Pallflex Corp., Putnam, CT) at a sampling flow of 1 LPM. A circular section of the quartz filter was removed from the center of the filter with a punch of diameter approximately 1 cm^2. The punch sample was analyzed by thermo-analysis to determine the EC and OC content using thermal desorption. The remainder of the filter was frozen and stored for subsequent analyses for specific organic components. UF CAPs number concentrations were measured continuously throughout the exposures with a TSI 3022 Condensation Particle Counter (CPC) sampling at a flow rate of 0.3 LPM. The mass concentration of the F CAPs was measured continually using a DataRAM nephelometer (MIE, Inc., Billerica, MA).

Exposure Sites

The exposures were performed at two sites: one that was 50 m downwind and another that was 150 m downwind from the State Road CA 60 and Interstate 5 freeways, both of which ran in a general northwest to southeast direction at the exposure location. Approximately 30-40% of the total vehicles on these freeways were heavy-duty diesel trucks (Zhu *et al.*, 2002). Exposures were conducted between the hours of 10 AM and 2 PM. The average wind direction was relatively stable, with winds from the SW to W directions during the exposure period. The terrain in the immediate area of the sampling location was relatively flat and there were no major obstructions between the roadway and the exposure sites. There was little street traffic near the sites during the exposures.

Animal model and OVA-Instillation Protocol

For several reasons we chose to use ovalbumin (OVA)-sensitized Balb/c mice to assess the effects of PM exposure on asthma-like responses. First and foremost, the OVA-sensitized Balb/c exhibits several of the hallmarks of allergic asthma and this strain has been widely used in asthma studies. These mice show increased levels of ovalbumin-specific IgE and IgG1, and they exhibit non-specific airway hyper-responsiveness (Wilder et al., 1999), and eosinophil influx of the airways (Gruenig, et al., 1998). These changes appear to be mediated via the T-helper type 2 cell (Th2). Some studies have suggested that other mouse strains could be considered. Moroka et al. (1999) reported that serum IgE and IgG1 levels were higher in C57BL/6 mice than in Balb/c mice following OVA sensitization and a single challenge. However, Wang et al (1999) reported that in a study that used repeated antigen injections with mosquito proteins to examine Th2-related responses, the Balb/c mouse responded better for IgE production than the C57BL/6 mouse. Other groups have reported particle-induced or diesel-induced inflammatory effects in OVA-sensitized AJ mice (Harkema et al., 1999) and ICR mice (Takano et al., 1998). However, the Balb/c mouse shows airway hyper-responsiveness following challenges which are not seen in C57BL/6 or B6DF1 mice (Wilder et al., 1999). The Balb/c mouse presents a better model of allergic asthma than other strains, and is a good responder for Th2-dependent allergic and inflammatory effects.

All of the mice used in this study, including the purified air-exposed controls, were sensitized to OVA by nasal instillation. On the morning of each exposure, the mice were lightly anesthetized with isofluorane. Ovalbumin (OVA; 50 μg in 5 μL of saline; Sigma Chemical) was administered by nasal instillation and the mice were allowed to recover. The mice were placed into exposure chambers and transported to the freeway exposure site while breathing purified air.

Exposure Protocol

The mice were instilled with OVA and exposed to CAPs or purified air at a given site for 4 hr per day on five consecutive days per week for two consecutive weeks. Exposures at the sites were alternated and three sets of exposures were conducted at each site. For each 2-week exposure series, twenty-seven 6 to 8 week-old BALB/c mice were randomly assigned to one of three groups, each consisting of 9 animals. The two PM-exposure groups received UF CAPs or F CAPs and the control group (C) received purified air. All three groups were exposed at the same time. Three sets of exposures were conducted at each exposure site and at each site a total of 27 mice each were

exposed to purified air or F CAPs and 18 mice were exposed to UF CAPs. These studies were begun in 2001 and completed in 2004.

Bioassays

Immediately after completion of each series of OVA/CAPs exposures the mice were housed under purified air conditions in the UCI vivarium. One week later, the mice were challenged for 1-hour with OVA (30 mg/m3), via inhalation. A second, identical challenge was performed one week after the first challenge. The mice were euthanized 24 hr after the second challenge and bioassays were performed.

Bronchoalveolar lavage and blood sample collection

The mice were injected with a lethal dose of sodium pentobarbital (65 mg/kg, i.p.). After a surgical plane of anesthesia was achieved, lung tissue, lung fluids and blood were removed for determination of levels of cytokines, inflammatory enzymes and markers of allergic responses (pulmonary infiltration of eosinophils (EOS) and ovalbumin-specific antibodies) and inflammation (pulmonary infiltration of polymorphonuclear leucocytes, PMN). Blood was withdrawn by cardiac puncture and centrifuged to isolate the plasma. The plasma was frozen for later immunoglobulin analyses. The animal's abdominal aortas were severed, and their tracheas were exposed. A catheter was inserted into the trachea and tied in place. The lungs were lavaged with HEPES-buffered (pH 7.2) Hank's Balanced Salt Solution (HBSS) without Ca2+ or Mg2+ (GIBCO, BRL, Gaithersburg, MD). The lavage volume was 0.8 ml and it was instilled and aspirated three times at a rate of about 0.05 mL/second. The lavage was repeated three times per animal and the recovered fluid from each lavage was placed on ice. The lavage fluid from each animal was centrifuged at 800 x g for 5 minutes. The fluid from the first lavage was reserved for protein and biochemical assays. The cell pellets from all three lavages were pooled and resuspended in 1 mL HBSS with Ca^{2+} and Mg^{2+}.

Cell counts and differentials on BAL sample:

The cell pellets from the BAL were resuspended and introduced into a bright line hemocytometer to determine total and viable cells. Viability was assessed by trypan blue exclusion. The total cell yield was typically 10^5 cells per mouse, of which more than 95% were mononuclear cells. Average cell viability was greater than 90%. A 0.1 ml aliquot of cells was plated onto a glass microscope slide using a cytocentrifuge (StatsSpin Cytofuge 2, Norwood, MA). The cells were stained with Wright-Giemsa stain and a differential cell count was made to determine the percentages of EOS, mononuclear cells and PMNs. Differential cell counts were determined from the products of total cell yield and percents of each cellular component x 100.

BAL fluid analysis for IL-5 and IL-13:

IL-5 and IL-13 were analyzed using Quantikine® (R&D Systems) quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits that were specific for the mouse cytokines. Standards, controls, and samples were pipetted into the wells of microtiter plates coated with monoclonal capture antibodies specific for mouse IL-5 or IL-13. Each sample and control well was spiked with 50 pg/mL of recombinant mouse

IL-5 or IL-13 so that all controls and samples were read in a linear portion of the standard curve. Analyses were conducted according to the manufacturer's instructions.

Blood samples analyzed for OVA-specific immunoglobulins:

OVA-specific IgE and IgG1 antibodies were detected using ELISA. Plastic microplates (Nunc-Immuno MaxiSorp Plate, Fisher Scientific) were coated with 100 µL/well of 0.05% OVA (Grade V; Sigma, St. Louis, MO) in 0.1 M carbonate buffer, pH 9.5, by overnight incubation at 4 ºC. The plates were blocked by incubation for 1 h at 37 ºC with 3% bovine serum albumin (BSA; Sigma) in PBS with 5% Tween 20 (PBST). Mouse plasma samples diluted 1:5000 in PBS for IgG1, undiluted mouse plasma samples for IgE, and serial dilutions of OVA-positive mouse serum (for the calibration curve) were added to the wells of the plates, which were incubated for 1 h at 37 °C for IgE and incubated overnight at 4 ºC for IgG1. Peroxidase-conjugated rat-anti-mouse-IgE (PharMingen, San Diego, CA) was diluted to 8 µg/mL in blocking buffer, or peroxidase-conjugated rat monoclonal antimouse IgG1 (PharMingen) diluted to 2 µg/mL with blocking buffer. Between each incubation, the plates were washed with PBST Enzyme substrate (TMB substrate reagents, PharMingen), and the reaction stopped after 30 min by the addition of 2 N H_2SO_4. Absorption was measured spectrophotometrically and compared to the calibration curve to determine the concentrations of IgE and IgG1.

Statistical Analyses

To confirm the findings of our previous publication that CAPs exposure caused allergic airway responses and that effects diminished with distance downwind from the source we analyzed the data from this study using a partial factorial design with factors for Location (50 m or 150 m) and Treatment (C, UF, F). Each endpoint was analyzed separately. For most of the endpoints, we considered that the normality assumptions for analyses of variance (ANOVA) were met. The exception was EOS, which is discussed later in this section. Of primary interest for these analyses were: 1) Treatment Effect and 2) Treatment by Location Interactions. Statistical significance was assessed using the usual F statistics. The EOS data had many zero values with a skewed distribution. We therefore transformed the EOS data into a logarithmic form, i.e. log (EOS+1), before performing the ANOVA. A null hypothesis was rejected when the probability of the null hypothesis being true was ≤ 0.05.

Regression analyses were used to test hypotheses of the dependence of biological responses on concentrations of specific CAPs constituents for those biomarkers that were significant in the ANOVA. Linear and non-linear associations were tested. However, although non-linear models in some instances provided a better fit to the data than did the linear model, only the results of the linear models are discussed in this manuscript.

NCER Assistance Agreement Annual Report

Date of Report: 12/22/04

EPA Agreement Number: R-82921601

Title: Effects of Airborne Particles on Allergic Airway Disease

Investigators: Jack R. Harkema, DVM, PhD (PI); Costantinos Sioutas, PhD (Co-PI)

Institution: Michigan State University, East Lansing, MI, and the Southern California Particulate Center and Supersite, Los Angeles, CA

Research Category: STAR-B1

Project Period: 10/31/2001-10/30/2004 (with a one year, no-cost extension, ending 10/30/2005)

Objective of Research

The overall objective of this project is to conduct atmospheric and toxicologic research designed to understand the adverse effects of airborne particulate matter (PM) of various size fractions (coarse, fine, and ultrafine particles) on pulmonary airways with pre-existing allergic airway disease. The aims of this project have not changed from our original proposal. We will test the following hypotheses: 1) that PM exposure exacerbates the airway injury associated with allergic airway disease; 2) that the magnitude of PM-induced airway toxicity is dependent on particle size; 3) that PM in transported ("aged") air pollution is more toxic to airways than that in locally generated air pollution; and 4) that PM-induced airway toxicity is most severe during periods of intense photochemical activity.

Work Progress & Preliminary Data Results

For three consecutive years we have conducted inhalation toxicology studies in the Los Angeles Basin (LAB) at two different locations to distinguish the effects of locally generated versus transported particulate matter. In October 2001 and January 2002, we conducted studies using our mobile laboratory in a residential community in Claremont, CA in the northeast LAB, which served as our receptor site for transported PM. In following years, we conducted similar autumn and winter exposures in urban Los Angeles in the central LAB near the University of Southern California campus, which served as our source site of locally generated particulate air pollution. The potential health effects of co-exposures to urban air pollutants and airborne allergens have not been thoroughly investigated. The purpose of our studies was to determine the effects of inhalation exposure of various size fractions of concentrated ambient particles (CAPs) on the lungs of rats that were concurrently exposed to a pulmonary allergen (ovalbumin; OVA). A state-of-the-art mobile air research laboratory, equipped with inhalation exposure chambers and ambient particle concentrators, was used to conduct the inhalation toxicology studies. Our mobile laboratory was moved from its home site at the Michigan State University Engine Research Laboratory in Okemos, MI to a residential site in Claremont, CA, or near the University of Southern California campus in Los Angeles, CA to these community-based inhalation toxicology studies in the early fall and winter months, as mentioned above.

Year 1: Studies in Claremont, CA (PM Receptor Site)

Study 1 Exposures: In Claremont, OVA-sensitized, male, Brown Norway rats (10-12 wks of age) were exposed to filtered air (controls), concentrated ambient coarse (2.5–10 μm; CCAPs), fine (0.1–2.5 μm; FCAPs) or ultrafine (0.01–0.15 μm; UFCAPs) particles, 5 h/day (11am - 4pm), for three consecutive days. Concentrated particle mass

and number concentrations and chemical speciation during the animal inhalation exposures are presented in Table 1. Immediately prior to each daily inhalation exposure, the rats were intranasally challenged with saline alone or a 0.5% solution of OVA in saline. Rats were exposed to average mass concentrations of 554, 515 and 45μg/m^3 for CCAPs, FCAPs, and UFCAPs, respectively. Twenty-four hours after the end of the exposures, rats were sacrificed, their pulmonary airways lavaged with saline, and their lung lobes processed for light microscopic or mRNA analyses. All of the animal sacrifices and necropsies were conducted by the laboratory staff of Dr. Harkema (Michigan State University) in the laboratories of Dr. Michael Kleinman (member of the Southern California Particle Center and Supersite) at the University of California in Irvine, CA.

Results: OVA-instilled rats had an allergic bronchiolitis with mucous cell hyperplasia (increase in the number of mucus-producing secretory cells lining the pulmonary airways) and an allergic alveolitis with marked increases in eosinophils in the bronchoalveolar lavage fluid (BALF). OVA-instilled and air-exposed rats had 538% more eosinophils in the BALF, 104% more stored mucosubstances in the bronchiolar epithelium, and a 6-fold increase in mucin-specific gene expression in bronchiolar airways than saline-instilled/air-exposed controls. Using this specific exposure regime of daily allergen and CAPs exposure we observed a marked particle-induced suppression, rather than enhancement, of the pulmonary inflammatory and epithelial responses to the inhaled allergen. Exposures to FCAPs (100% inhibition) or UFCAPs (by 66%), but not CCAPs, caused a marked attenuation of the OVA-induced allergic mucous cell hyperplasia. FCAPs also inhibited OVA-induced alveolitis (42%), and both FCAPS (65%) and UFCAPs (82%) blocked mucin-specific gene expression in bronchiolar epithelium (Figures 1 – 4).

Conclusion: These results indicate that fine (or ultrafine) ambient airborne particles may significantly interfere with allergen-induced airway responses during co-exposure of these airborne agents.

Study 2 Exposures: In January 2002, we conducted our second inhalation toxicology study at the same site in Claremont, CA. The experimental design was similar to that conducted in October 2001. Like in the first study, male, Brown Norway rats (10-12 wks of age) were exposed to filtered air, CCAPs, FCAPs or UFCAPs particles, 5 h/day (11am - 4pm), for three consecutive days. Immediately prior to each daily inhalation exposure, the rats were again intranasally challenged with saline alone or a 0.5% solution of OVA in saline.

Results: However in this winter study, rats were exposed to average mass concentrations of 86, 103, and 25μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 2). These average mass concentrations were markedly lower than those to which rats were exposed in the first Claremont study in October 2001 (see above). In contrast to the results of the October study, no FCAPs-, UFCAPs- or CCAPs-related effects on OVA-induced allergic alveolitis, mucous cell metaplasia or mucin-specific gene expression were observed in these rats exposed to the much lower concentrations of CAPs.

Conclusions: These results, in relation to the first study, suggest that these lower mass concentrations were not sufficient to modulate the pulmonary responses induced by the allergen challenge. Further chemical analyses of the CAPs from the first and second studies are currently underway to determine if differences in the chemical makeup of the CAPs may also have contributed, in part, to the marked differences in the pulmonary responses observed between the studies.

Year 2: Studies in Los Angeles (PM Source Site)

Study 1 Exposures: In October 2002, we conducted our third inhalation toxicology study of CAPs-exposed Brown Norway Rats with and without OVA airway instillations. In this study the mobile research laboratory was parked in Los Angeles, CA near the main campus of the University of Southern California and highways with heavy motor vehicle traffic (i.e., our source site with locally generated particulate air pollution). We used the same experimental design and exposure regime as described above for the first and second studies in Claremont, CA.

In this Los Angeles study, rats were exposed to average mass concentrations of 310, 324, and 31μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 3).

Results: Similar to the particle-induced inhibition that we observed in the first study Claremont exposure study, we detected a trend for FCAPs-induced suppression of allergic responses. Sensitization and challenge with OVA induced significant accumulations of eosinophils (58-fold increase) and neutrophils (23-fold) in BALF (Figures 5, 6). In animals exposed to FCAPs these responses were not statistically significant with only 2.2-fold and 10-fold increases for eosinophils and neutrophils, respectively, when compared to saline-challenged rats. However, these inflammatory responses were not significantly less than air-exposed, OVA-challenged animals. Exposure to CCAPs, but not UFCAPs, also resulted in modest decreases of inflammatory cell recruitment. OVA induced a 160% increase in intraepithelial mucosubstances (p =0.06) that was inhibited by FCAPs by approximately 30% (Figure 7). Taken together, when compared to the inhibitory profile of CAPs we observed in Claremont, the present study showed a more modest effect for suppression of allergic responses. Average mass concentrations were approximately 60% of what was generated in Claremont 2001, but were 3-fold greater than in Claremont 2002 when no CAPs-related effects were documented.

Conclusion: Thus, our results describing a partial inhibition by CAPs may represent an intermediate point on a dose response curve.

Study 2 Exposures: In January 2003, we used Brown Norway and repeated the dosing and exposure protocol at the same site to assess the effects of the seasonal particle mixture and to compare to the January exposure in Claremont. In this second Los Angeles study, rats were exposed to average mass concentrations of 905, 1026, and 27μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 4). During these exposures, the weather pattern was unseasonably warm and PM concentrations unusually high for January in Los Angeles. For CCAPs and FCAPs, concentrations were 3-fold greater than the October exposure at this site, and 10-fold greater than the January exposure in Claremont.

Results: Despite higher concentrations of CAPs, we did not detect particle-related changes in BALF cellularity in either saline-challenged or OVA-challenged rats. Similar to previous findings however, OVA-induced increases in intraepithelial mucosubstances were less in animals exposed to CAPs. Specifically, OVA –induced mucus storage was increased by only 45% and 59% when exposed to UFCAPs or FCAPs, respectively, compared to a 144% increase in air-exposed animals (Figure 8). The inhibitory effects of CAPs in the current study was less pronounced than we observed with lower particle concentrations at the receptor site in Claremont (FCAPS = 515 $\mu g/m^3$), where allergic responses were blocked by 100%.

Conclusions: Thus, the degree of CAPs-induced inhibition of airway epithelial remodeling and inflammatory cell recruitment is not described with a simple dose-response relationship, and is more likely determined by a contributions of specific particle components, their individual concentrations, and their potential interactions. Furthermore these results provide support for our hypothesis that transported, or aged particles, are more toxic than newly generated particles near source sites. We are currently conducting comparative analyses of physicochemical particle characteristics from each exposure to determine associations of specific components with the inhibitory effects we have documented.

Year 3: Studies in Los Angeles (Source Site)

Study 1 Exposures: In the third year of the project we employed the same challenge and exposure protocol at the Los Angeles site but used younger Brown Norway rats (6-7 weeks old). In September 2003, rats were exposed to average mass concentrations of 587, 674, and 147μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 5). Thus, CCAPs and FCAPs were similar in concentration to the September exposure at the receptor site in Claremont in September, when the most pronounced inhibition occurred. In addition, UFCAPs was 5 to 6-fold greater than any of the previous exposures.

Results: As was observed previously, particles had no effect on BALF parameters or mucous cell hyperplasia in normal, non-allergic animals. Furthermore, significant CAPS-induced changes were not evident in OVA-challenged rats as we had seen with exposures with similar CAPs concentrations. Despite the lack of statistically significant differences, an apparent trend was nevertheless evident for the inhibitory effects by CAPs on OVA-induced BALF cellularity (e.g., eosinophils, Figure 9) and stored mucosubstances in pulmonary epithelium (Figure 10). During these exposures we also observed for the first time an effect of particles on nasal epithelium in non-allergic rats. CCAPs caused a 47% increase in stored mucosubstances in nasal respiratory epithelium, but had no effect on OVA-induced allergic rhinitis (Figure 11). Conversely, exposure to FCAPs inhibited by approximately 50% the OVA-induced increase in nasal intraepithelial mucosubstances.

Conclusion: It is notable that the higher concentrations of UFCAPs had no greater or lesser effects in normal and allergic rats than seen in previous exposures where lower concentrations were generated.

Study 2 Exposures: In February 2004, we repeated the protocols using the younger Brown Norway rats (6-7 weeks). In this study rats were exposed to average mass concentrations of 254, 505, and 114μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 6).

Results: Similar to the previous September exposure with younger animals, a distinct trend for particle-induced inhibition of allergic alveolitis emerged, however the comparisons failed to reach statistical significance. Also, particles had no effect on allergic mucous cell metaplasia during this exposure.

Summary of Research to Date

Our studies were designed to address four specific hypotheses. The first, that particles would enhance airway injury associated allergic airway disease, was not supported by our data. To the contrary, we found that particle exposure attenuated allergic bronchiolitis and alveolitis, and allergen-induced mucous cell metaplasia and the allergen-induced overexpression of mucin-specific gene (MUC5AC) in bronchiolar epithelium. This particle-induced attenuation of allergic airway disease was the greatest with exposures to transported ("age") FCAPs and UFCAPS in the October 2001 study in Claremont, CA (receptor site). In successive exposures, this inhibitory phenomenon was observed to varying degrees with changing variables of particle concentrations, exposure sites, and in younger animals.

The sum of our data supports our other hypotheses, that a) the magnitude of PM-induced effects is dependent on particle size, and b) PM in transported ("aged") air pollution is more "toxic" on airways than that in locally generated air pollution, and c) that PM-induced airway "toxicity" is most severe during periods of intense photochemical activity, were supported by our experimental results. We

consistently found that FCAPs, and with less frequency UFCAPs, were associated with particle-induced attenuation of allergic airway responses. Furthermore the effects of FCAPs were most pronounced during relatively warm weather (i.e. photochemical pollution) at the Claremont receptor site. By comparison the source-site exposures in Los Angeles during both summer and winter months produced less robust inhibitory effects of particles, but nonetheless demonstrated a trend for decreased allergic responses.

Although we predicted that exposures to CAPs would exacerbate the allergic airway responses, the inhibitory phenomenon we have described is not without precedent. Depression of inflammatory and immune cell function might be caused by oxidative stress that is measurable in pulmonary tissues of rats after FCAPs inhalation. We and others have demonstrated that exposure of alveolar macrophages to ambient particles *in vitro* induces dose-dependent cytotoxic responses, including oxidative stress, mitochondrial damage, and cytoskeletal derangement (Goldsmith et al. 1998; Moller et al. 2002; Becker et al. 2003; Kleinman et al. 2003; Li et al. 2003). Particle uptake by macrophages, lymphocytes, epithelial cells and dendritic cells may be counterproductive for normal immune responses to the presence of an airway allergen. Furthermore, ambient particles are known to induce a variety of cytokines from alveolar macrophages (e.g., IFN-γ, TNFα, IL-8,) that can interfere with allergic pathways to elicit eosinophil recruitment, lymphocyte activation, or IgE production.

Taken together, our results are reminiscent of data from allergic rodent models that test the effects of airway endotoxin during allergen challenge. In these studies, endotoxin exposures at the time of allergen challenge inhibited allergic eosinophilic inflammation and airway hyperreactivity (Gerhold et al. 2002; Tulic et al. 2002). Attenuation of these allergic responses was associated with the production of IFNγ, IL-10 and IL-12 (Tulic et al. 2001; Gerhold et al. 2002), cytokines known to oppose Th2-pathways of allergic inflammation. The paradigm of endotoxin-induced down regulation of allergic pathways is consistent with the "hygiene hypothesis", where bacterial or fungal stimuli promote development of Th1-lymphocytes over allergy-promoting Th2 lymphocytes during the postnatal development of the immune system (Matricardi et al. 2002; Yazdanbakhsh et al. 2002). Just as bacterial stimuli present in low hygienic environments (e.g., rural, agricultural) can minimize the development of an allergy-prone immune phenotype in growing children, inhalation of a Th1-stimulus such as bacterial endotoxin opposes IgE production, eosinophilic inflammation, and airway hyperreactivity that are initiated by Th2 cytokines in subjects with allergic airway disease (Matricardi et al. 2002). Similar Th1/Th2cytokine dynamics may have occurred during CAPs inhalation and allergen challenge in the present series of experiments. Others have shown that treatment of ovalbumin-sensitized Brown Norway rats with IFNγ, a Th1 cytokine, blocks allergic inflammation and Th2 cytokine production (Huang et al. 1999). Inhalation of FCAPs and UFCAPs may have induced an early Th1 stimulus that interfered with allergic cytokine pathways which led to marked attenuation of more down stream responses such as eosinophilic inflammation and the development of mucous cell metaplasia. Further studies are needed to specifically investigate the Th1/Th2 cytokine responses in the lungs at various times after exposure to inhaled CAPs.

Interestingly, there are several reported epidemiology studies that have demonstrated that residents of the former East Germany who lived in communities with high levels of industrial air pollution had lower prevalence rates of asthma and other allergic diseases compared to residents of the former West Germany who lived in communities with lower levels of air pollution. Among East-German children, lower prevalence rates of asthma and positive skin-prick tests for allergery were observed compared to West-German children who live in communities with less air pollution (Klein et al. 1992; von Mutius et

al., 1994; Trepka et al., 1996). Similarly, East-German adults have been reported to have lower specific IgE levels and lower prevalence rates of asthma, wheezing, positive methacholine-challenge tests, allergic rhinitis, and positive skin-prick tests compared to those of West-German adults (Nicolai et al., 1997; Heinrich et al., 1998). It has yet to be determined what specific factors related to the air pollution (gaseous or particulate components), or related to life style, may account for these regional differences in the prevalence of asthma and allergies.

The results of our inhalation studies do lend support to the hypothesis that exposure to particulate air pollution may attenuate the development of allergic airway diseases. Since the rats in our study did not have allergic airway disease prior to exposure to the airborne CAPs, our studies were not designed to test the hypothesis that exposure to CAPs exacerbates pre-existing allergic airway disease. However, we have recently demonstrated that acute exposure of BN rats to FCAPs in a Detroit community may exacerbate pre-existing ovalbumin-induced allergic airway disease in BN rats under the right experimental and exposure conditions (Harkema et al., 2004). Therefore, it appears that the development and severity of this experimentally induced allergic airway disease is highly dependent on the time at which the laboratory rodents are exposed to both the allergen and the airborne particles.

Key Personnel and Budget

There have been no changes in the key personnel involved in this project. Expenditures to date are in line with the amount of the work completed. There are no substantial differences in the present itemized expenses and estimated itemized costs in the approved project. We do not anticipate any major changes in the budget over the next year of the project.

Publications/Presentations to Date:

An abstract entitled, “Concentrated Ambient Particles Attenuate Allergen-Induced Airway Responses in the Lungs of Brown Norway Rats,” was presented at the 2003 annual meeting of the Society of Toxicology in Salt Lake City, Utah (March 9-13, 2003) and at the American Association for Aerosol Research-Particulate Matter Conference in Pittsburgh, PA (March 31-April 4, 2003).

Wagner, J.G., Sioutas, C., Timm, E. Kaminski, N., Keeler, G.J., Kleinman, M., Froines, J., and J.R. Harkema. Exposure to Concentrated Ambient Fine and Ultrafine Particles Inhibits Allergen-Induced Inflammation and Storage and Secretion of Mucosubstances in Rat Pulmonary Epithelium. In preparation.

Future Activities: Approximately 80% of the project is now complete. In 2005, we will complete our biologic and atmospheric analyses from the 2004 animal inhalation studies. We anticipate that two or three manuscripts will be submitted to scientific journals for peer-reviewed publication. In addition, a final report including the results of all of our studies will be prepared and submitted to the EPA office before the end of the project.

Table 1. Concentrated Particle mass and number concentrations and chemical speciation during the animal inhalation exposures of October 8-10, 2001.

PM Mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	2.0E+03	0.078	4.39	553.5	2.6	28.2	115.7	21.8	374.3
Fine	4.5E+04	0.078	0.88	515.4	19.4	132.0	207.5	113.8	23.3
Ultrafine	2.8E+04	0.050	0.08	45.2	6.2	33.7	0.0	2.0	1.5

Table 2. Concentrated Particle mass and number concentrations and chemical speciation during the animal inhalation exposures of January 28-30, 2002.

PM Mode	Number (particles/cm^3)	Number geometric mean diameter(μm)	Mass median diameter(μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	2.3E+03	0.034	3.78	86.4	0.2	4.8	4.2	2.9	55.7
Fine	5.2E+04	0.034	0.47	102.7	4.3	75.5	33.0	7.5	5.6
Ultrafine	4.7E+04	0.023	0.04	24.7	2.1	22.2	0.0	0.0	2.3

Table 3. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of September 30-October 1, 2002.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	310.0	0.0	28.8	83.6	23.8	233.1
Fine	1.2E+05	ND	ND	323.5	12.8	122.7	34.2	67.8	121.7
Ultrafine	9.6E+04	ND	ND	31.0	6.1	50.8	0.0	3.1	2.9

ND = Not yet determined.

Table 4. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of January 2003.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	904.6	1.4	147.4	100.8	24.2	303.4
Fine	4.3E+05	ND	ND	1025.9	46.9	367.3	459.7	89.3	90.9
Ultrafine	3.8E+04	ND	ND	132.1	11.7	93.3	13.6	6.8	6.7

ND = Not yet determined.

Table 5. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of September 2003.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	586.7	3.2	41.1	148	43	167.8
Fine	ND	ND	ND	674.7	22.6	175.2	121.8	192.8	70.2
Ultrafine	2.6E+05	ND	ND	147.5	8.1	70.2	0.0	20	0.6

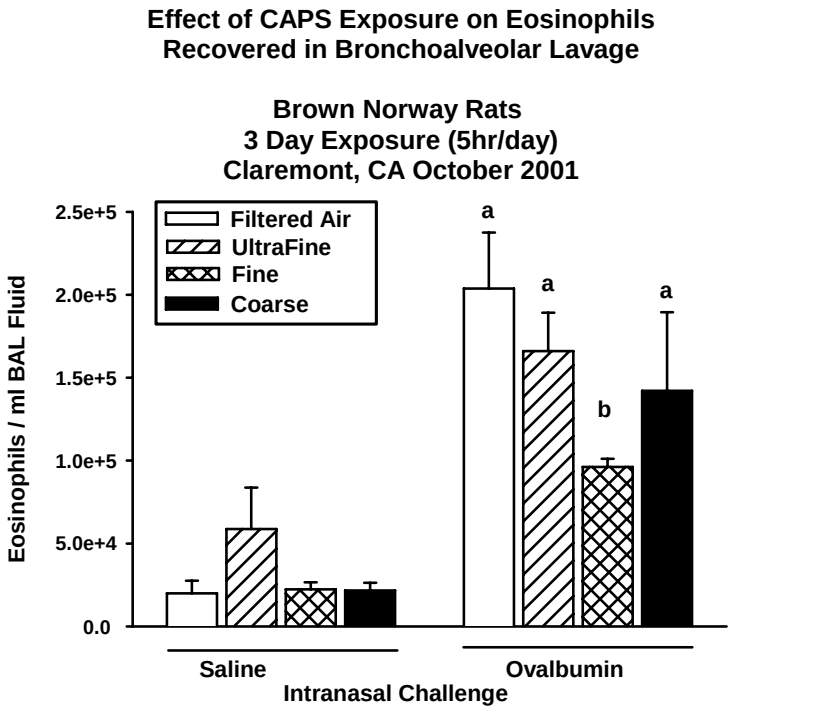
ND = Not yet determined.

Table 6. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of February 2004.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	253.6	0.5	29.1	80.3	19.6	81.4
Fine	ND	ND	ND	505.2	15.7	121.3	68.4	27.4	36.7
Ultrafine	5.9E+04	ND	ND	114.1	14.3	62.9	4.4	13.5	1.3

ND = Not yet determined.

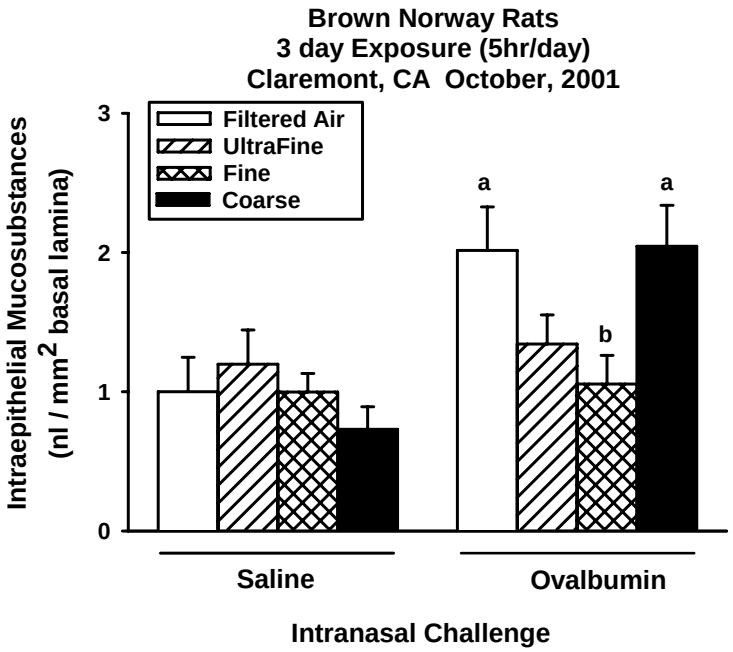
Figure 1:



a = significantly different from respective group in Normal Airways, ($p \leq .05$)

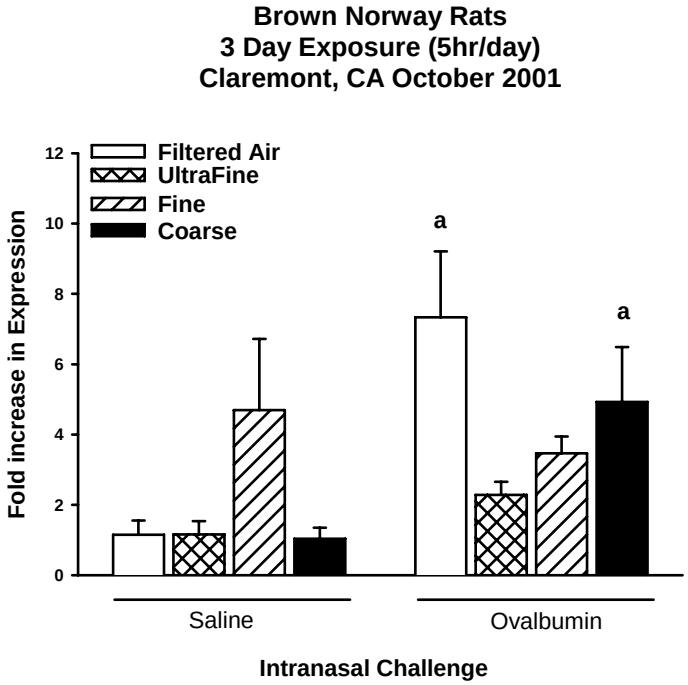
b = significantly different from respective group exposed to Filtered Air, ($p \leq 0.05$)

Figure 2. **Effect of CAPs Exposure on Intraepithelial Mucosubstances (Axial Airway, Left Lung Lobe, Generation 5)**



a = Significantly different from respective group instilled with Saline, ($p \leq 0.05$).
b = Significantly different from respective group breathing Filtered Air, ($p \leq 0.05$).

Figure 3. **Muc5AC mRNA in the Axial Airway of Right Caudal Lobe**



a = Significantly different from respective group instilled with Saline, ($p \leq 0.05$).

Figure 4. Light photomicrographs of pulmonary tissue sections taken from rats exposed to filtered air and saline (A); filtered air and ovalbumin (OVA; sensitized and challenged) (B); coarse concentrated air particles (CCAPs) and OVA (C); or fine concentrated air particles (FCAPs) and OVA (D). No allergic alveolitis/bronchiolitis is present in A. Marked allergic alveolitis/bronchiolitis is present in B and C, but minimal pulmonary lesions are present in D. All tissues are stained with hematoxylin and eosin. Bar = 200 microns. Ba = bronchiolar airway; ap = alveolar parenchyma.

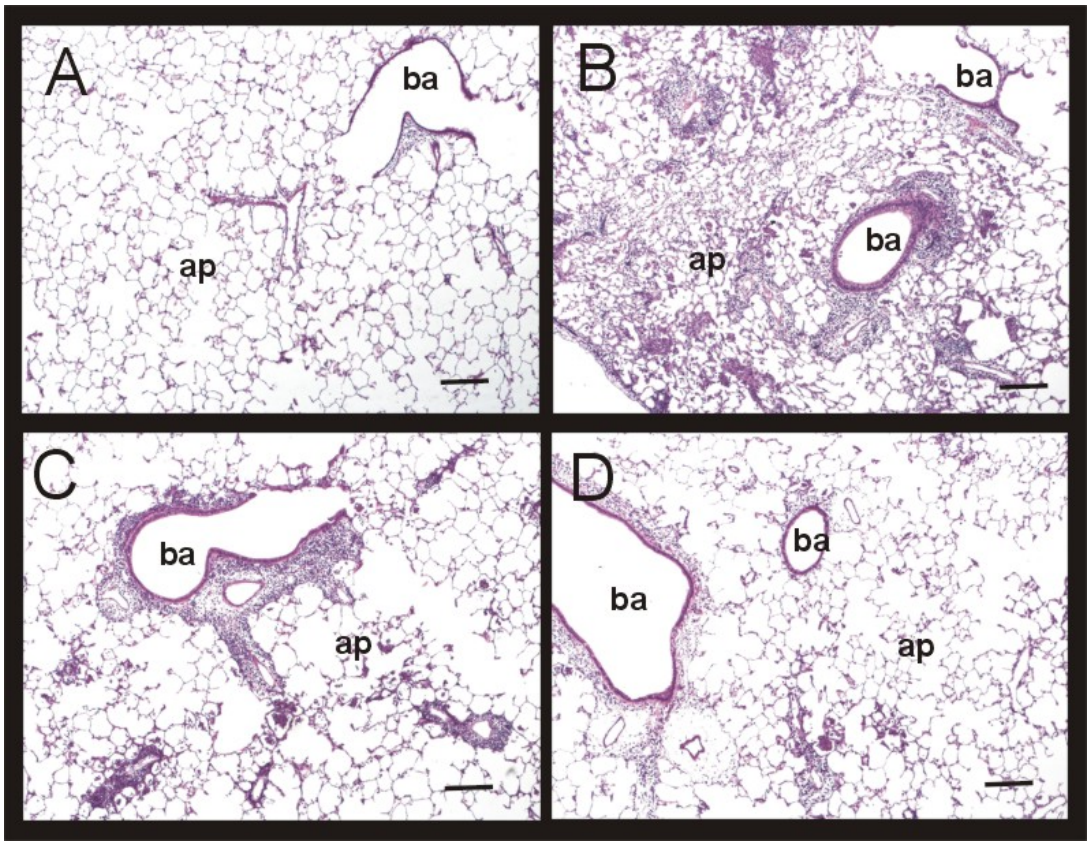
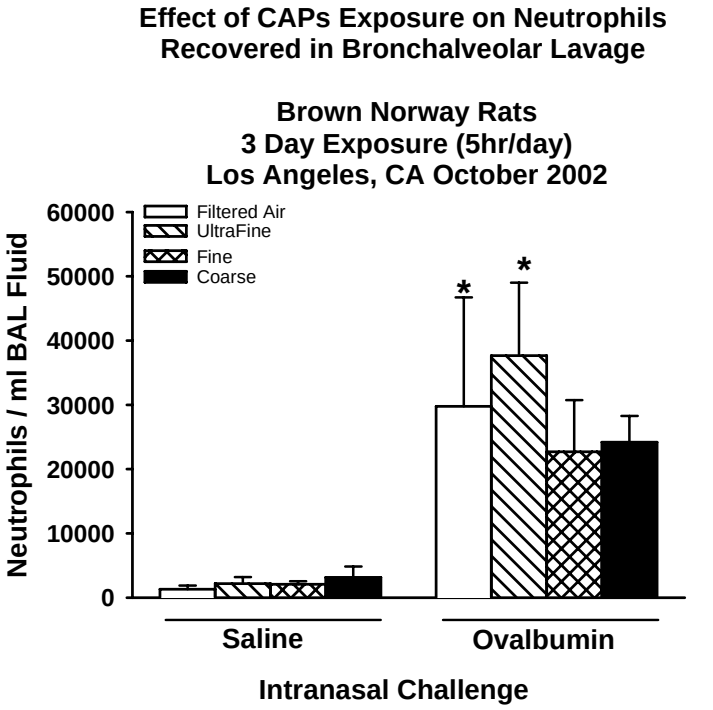
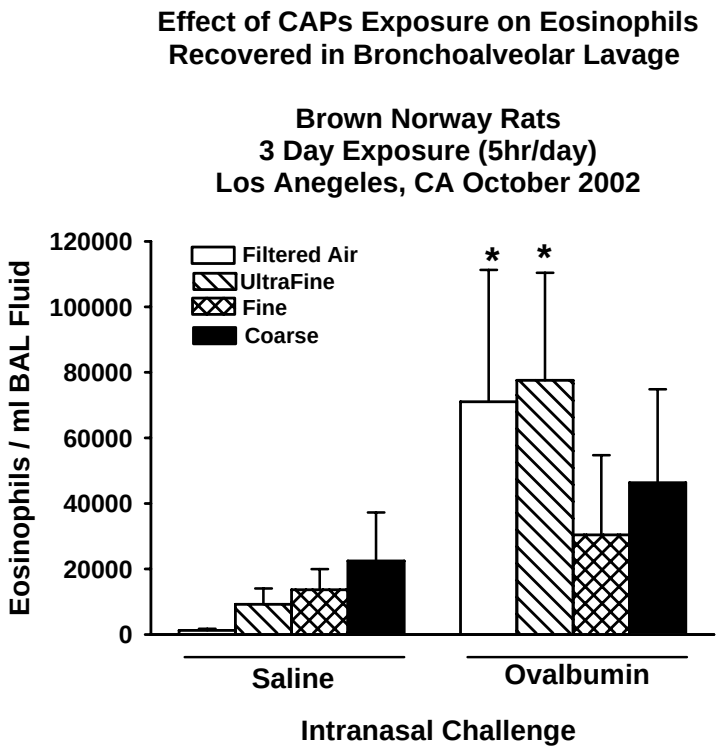


Figure 5:



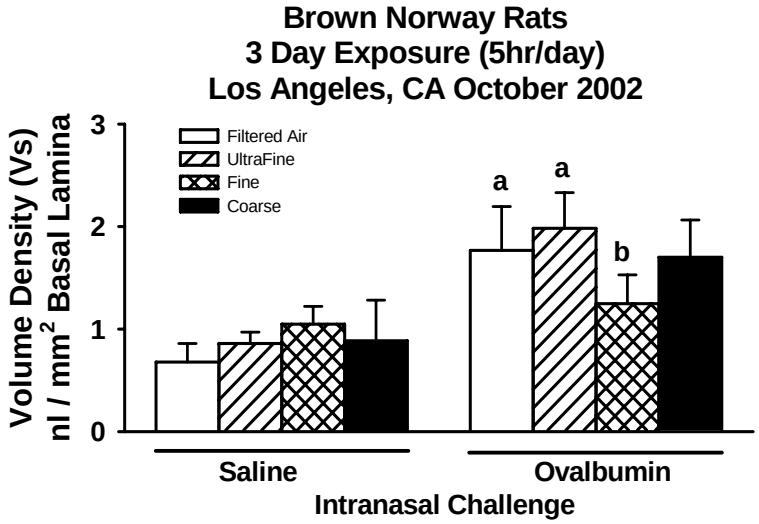
* = Significantly different from respective group receiving Saline, ($p \leq .05$).

Figure 6:



* = Significantly different from respective group receiving Saline, ($p \leq .05$).

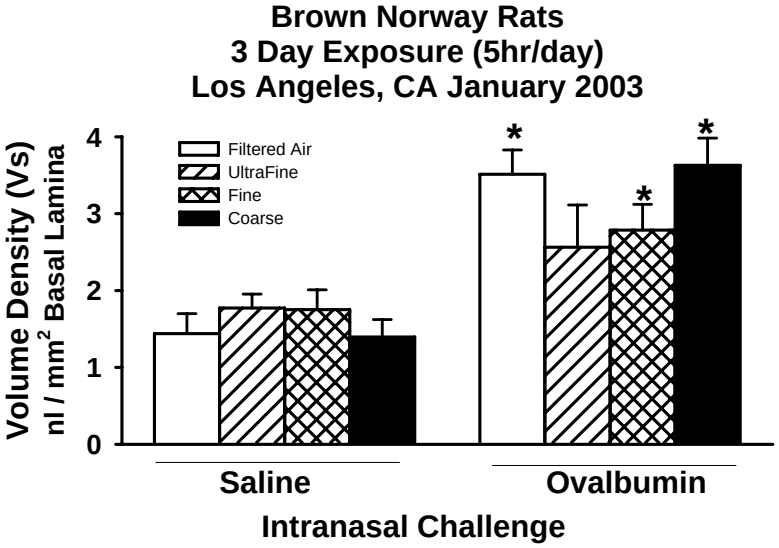
Figure 7: **Effect of CAPs Exposure on Intraepithelial Mucosubstances (Axial Airway, Left Lung, Lobe, Generation 5)**



a = Significantly different from respective group receiving Saline, ($p \leq 0.05$).
b = Significantly different from respective group exposed to Filtered Air, ($p \leq 0.05$).

Figure 8:

Effect of CAPs Exposure on Intraepithelial Mucosubstances (Axial Airway, Left Lung, Lobe, Generation 5)



*** = Significantly different from respective group recieving Saline, ($p \leq 0.05$).**

Figure 9:

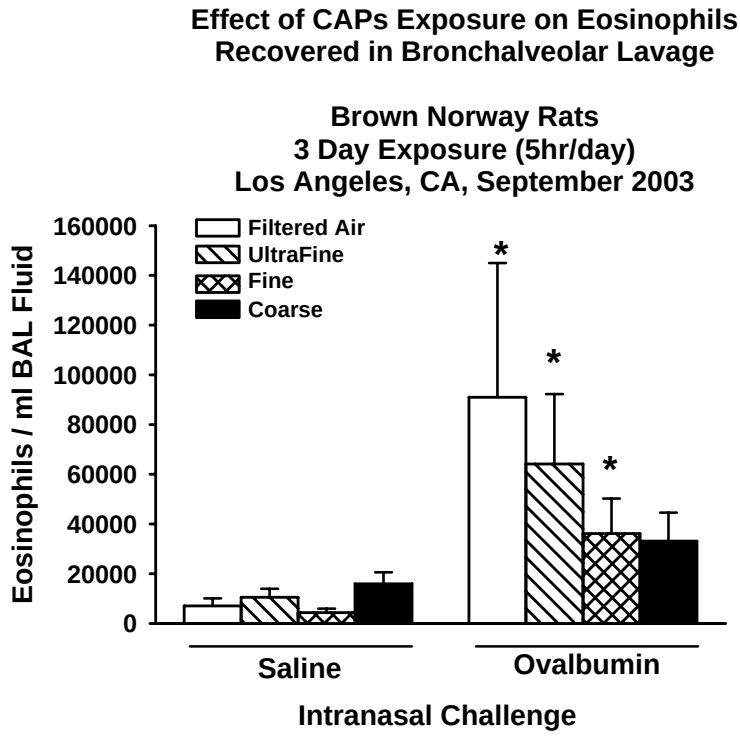
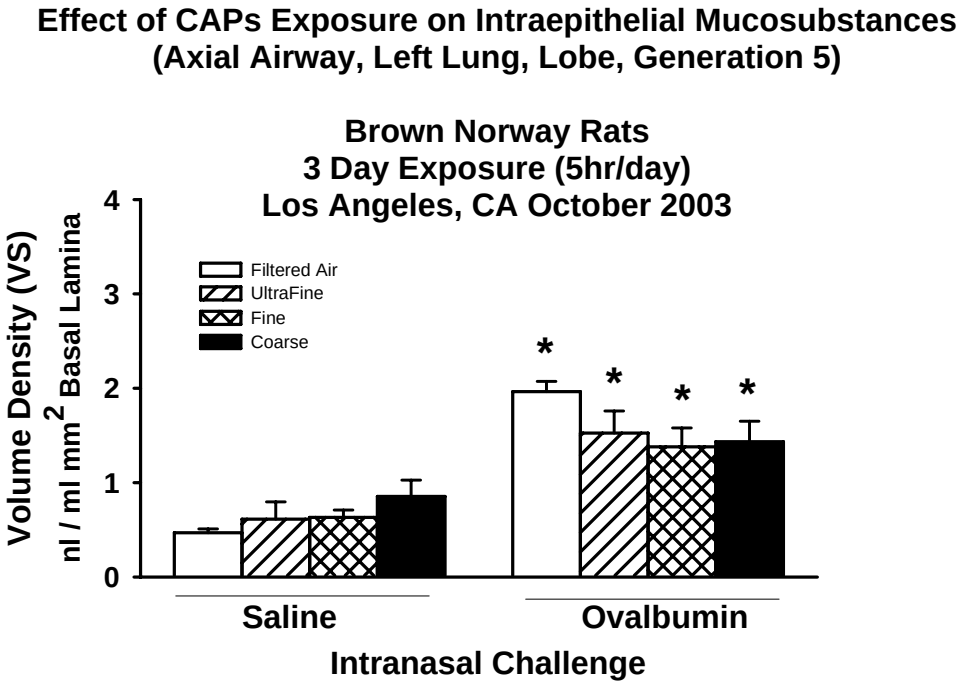


Figure 10:

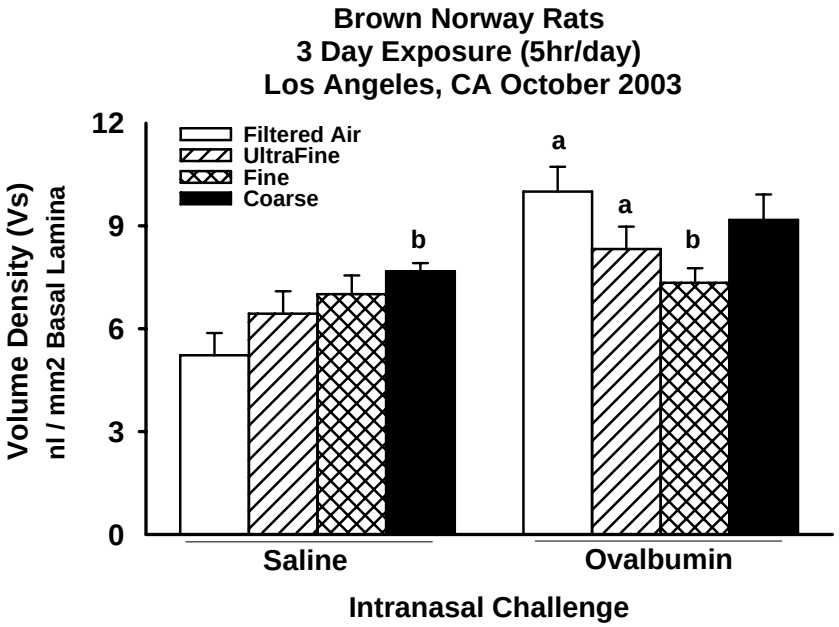
* = Significantly different from respective group receiving Saline, ($p \leq .05$).



* = Significantly different from respective group recieving Saline, ($p \leq 0.05$).

Figure 11:

Effect of CAPs Exposure on Intraepithelial Mucosubstances (Proximal Nasal Septum)



a = Significantly different from respective exposed to Filtered Air, ($p \leq 0.05$).

b = Significantly different from respective group receiving Intranasal Saline, ($p \leq 0.05$).

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APPENDIX J

Materials and methods for the animal studies reported in Tasks 2 AND 3.

Atmosphere Generation and Characterization

Fine (F; $d_p \leq 2.5$ μm) and ultrafine (UF; dp ≤ 180 nm) ambient particles were concentrated using a Versatile Aerosol Concentration Enrichment System (VACES), which has been characterized and described in detail by Kim et al. (Kim et al., 2001a; Kim et al., 2001b). The US EPA uses the term "$PM_{2.5}$" to define particles with aerodynamic diameters less than or equal to 2.5 μm. Thus the F particles used could be considered concentrated $PM_{2.5}$. Analogously, the UF could be considered concentrated $PM_{0.18}$. The VACES system is mobile and capable of enriching the concentration of particles ranging in size from 0.01 to 10 μm by up to 40 times, depending on the output flow rate. The VACES can incorporate size-selective inlets to provide concentrated ambient particles (CAPs) in a defined size range.

For this study, the VACES was installed inside a one-ton van (Dodge RAM 350 Custom), which had been internally modified for the safe handling of animals. Electric power for the VACES and all other sampling instruments was supplied by a 1.2 kW gasoline-powered portable power generator (Model EU 1000i, Honda Motor Co., LTD., Tokyo, Japan). The generator was placed approximately 18 m downwind of the sampling location of the van and PM emissions from the generator did not affect the input aerosol to the VACES (Kleinman et al., 2005). Ambient air samples for the exposures were drawn into the VACES via a 2 m long and 7.5 cm in diameter duct made of aluminum in order to avoid particle losses due to electrostatic deposition. The concentrated aerosols were delivered to whole-body animal exposure chambers (Oldham et al., 2004; Kleinman et al., 2005) as shown in Figure 2. Each exposure chamber was a sealed unit, sectioned for housing 9 mice per chamber. Temperature and airflow were controlled during the exposures to ensure adequate ventilation, minimize buildup of animal-generated contaminants (dander, ammonia, and CO_2) and to avoid thermal stresses. The animals were transported to and from the exposure site in an air conditioned vehicle and provided with purified air during transport. Between exposures the animals were housed at UCI in and AAALAC-accredited vivarium and provided with food and water, ad lib.

Figure 1. Schematic Diagram of VACES and Exposure System

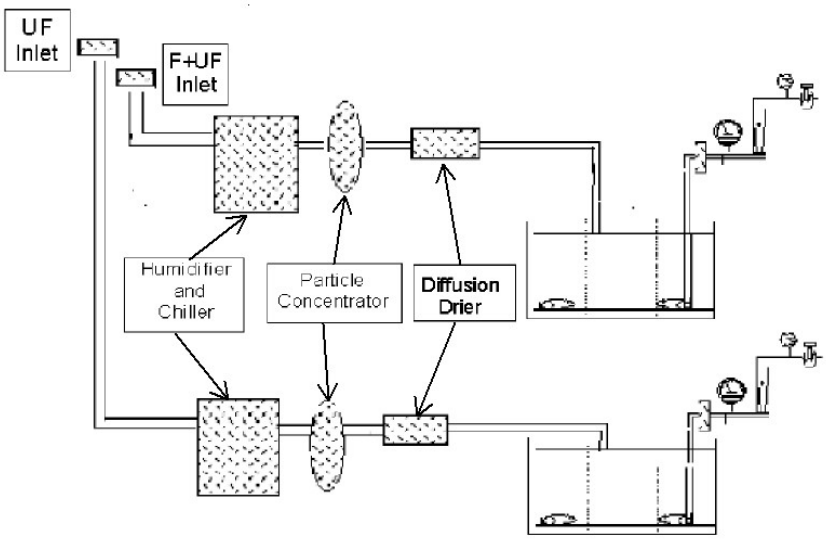


Figure 1. Schematic diagram of the VACES particle concentrator for simultaneous exposures of mice to fine + ultrafine (F+UF; $d_p < 2.5$ μm diameter) and ultrafine (UF; dp < 0.15 μm diameter) in a mobile exposure facility.

Physico-Chemical Characteristics of Concentrated PM

Samples of F and UF CAPs were collected from a port immediately upstream of the exposure chambers to determine physical and chemical characteristics of the exposure aerosols. Samples for analysis of inorganic constituents were collected on 37 mm Teflon filters (PTFE 2 μm pore, Gelman Science, Ann Arbor, MI) at a flow rate of 1 LPM and analyzed for particle masses and chemical composition. The filters were equilibrated at constant humidity and temperature overnight and weighed before and after each exposure session using a Mettler 5 Microbalance (MT 5, Mettler-Toledo Inc., Hightstown, NJ), under controlled relative humidity (40-45%) and temperature (22-24°C) conditions. The filters were analyzed by X-Ray fluorescence (XRF) to determine the concentrations of particle-bound trace elements and metals. Inorganic ions (sulfate and nitrate) were measured using ion chromatography. Elemental carbon (EC) and organic carbon (OC) were determined by collecting particles on 37-mm pre-baked quartz filters (Pallflex Corp., Putnam, CT) at a sampling flow of 1 LPM. A circular section of the quartz filter was removed from the center of the filter with a punch of diameter approximately 1 cm^2. The punch sample was analyzed by thermo-analysis to determine the EC and OC content using thermal desorption. The remainder of the filter was frozen and stored for subsequent analyses for specific organic components. UF CAPs number concentrations were measured continuously throughout the exposures with a TSI 3022 Condensation Particle Counter (CPC) sampling at a flow rate of 0.3 LPM. The mass concentration of the F CAPs was measured continually using a DataRAM nephelometer (MIE, Inc., Billerica, MA).

Exposure Sites

The exposures were performed at two sites: one that was 50 m downwind and another that was 150 m downwind from the State Road CA 60 and Interstate 5 freeways, both of which ran in a general northwest to southeast direction at the exposure location. Approximately 30-40% of the total vehicles on these freeways were heavy-duty diesel trucks (Zhu *et al.*, 2002). Exposures were conducted between the hours of 10 AM and 2 PM. The average wind direction was relatively stable, with winds from the SW to W directions during the exposure period. The terrain in the immediate area of the sampling location was relatively flat and there were no major obstructions between the roadway and the exposure sites. There was little street traffic near the sites during the exposures.

Animal model and OVA-Instillation Protocol

For several reasons we chose to use ovalbumin (OVA)-sensitized Balb/c mice to assess the effects of PM exposure on asthma-like responses. First and foremost, the OVA-sensitized Balb/c exhibits several of the hallmarks of allergic asthma and this strain has been widely used in asthma studies. These mice show increased levels of ovalbumin-specific IgE and IgG1, and they exhibit non-specific airway hyper-responsiveness (Wilder et al., 1999), and eosinophil influx of the airways (Gruenig, et al., 1998). These changes appear to be mediated via the T-helper type 2 cell (Th2). Some studies have suggested that other mouse strains could be considered. Moroka et al. (1999) reported that serum IgE and IgG1 levels were higher in C57BL/6 mice than in Balb/c mice following OVA sensitization and a single challenge. However, Wang et al (1999) reported that in a study that used repeated antigen injections with mosquito proteins to examine Th2-related responses, the Balb/c mouse responded better for IgE production than the C57BL/6 mouse. Other groups have reported particle-induced or diesel-induced inflammatory effects in OVA-sensitized AJ mice (Harkema et al., 1999) and ICR mice (Takano et al., 1998). However, the Balb/c mouse shows airway hyper-responsiveness following challenges which are not seen in C57BL/6 or B6DF1 mice (Wilder et al., 1999). The Balb/c mouse presents a better model of allergic asthma than other strains, and is a good responder for Th2-dependent allergic and inflammatory effects.

All of the mice used in this study, including the purified air-exposed controls, were sensitized to OVA by nasal instillation. On the morning of each exposure, the mice were lightly anesthetized with isofluorane. Ovalbumin (OVA; 50 μg in 5 μL of saline; Sigma Chemical) was administered by nasal instillation and the mice were allowed to recover. The mice were placed into exposure chambers and transported to the freeway exposure site while breathing purified air.

Exposure Protocol

The mice were instilled with OVA and exposed to CAPs or purified air at a given site for 4 hr per day on five consecutive days per week for two consecutive weeks. Exposures at the sites were alternated and three sets of exposures were conducted at each site. For each 2-week exposure series, twenty-seven 6 to 8 week-old BALB/c mice were randomly assigned to one of three groups, each consisting of 9 animals. The two PM-exposure groups received UF CAPs or F CAPs and the control group (C) received purified air. All three groups were exposed at the same time. Three sets of exposures were conducted at each exposure site and at each site a total of 27 mice each were

exposed to purified air or F CAPs and 18 mice were exposed to UF CAPs. These studies were begun in 2001 and completed in 2004.

Bioassays

Immediately after completion of each series of OVA/CAPs exposures the mice were housed under purified air conditions in the UCI vivarium. One week later, the mice were challenged for 1-hour with OVA (30 mg/m3), via inhalation. A second, identical challenge was performed one week after the first challenge. The mice were euthanized 24 hr after the second challenge and bioassays were performed.

Bronchoalveolar lavage and blood sample collection

The mice were injected with a lethal dose of sodium pentobarbital (65 mg/kg, i.p.). After a surgical plane of anesthesia was achieved, lung tissue, lung fluids and blood were removed for determination of levels of cytokines, inflammatory enzymes and markers of allergic responses (pulmonary infiltration of eosinophils (EOS) and ovalbumin-specific antibodies) and inflammation (pulmonary infiltration of polymorphonuclear leucocytes, PMN). Blood was withdrawn by cardiac puncture and centrifuged to isolate the plasma. The plasma was frozen for later immunoglobulin analyses. The animal's abdominal aortas were severed, and their tracheas were exposed. A catheter was inserted into the trachea and tied in place. The lungs were lavaged with HEPES-buffered (pH 7.2) Hank's Balanced Salt Solution (HBSS) without Ca2+ or Mg2+ (GIBCO, BRL, Gaithersburg, MD). The lavage volume was 0.8 ml and it was instilled and aspirated three times at a rate of about 0.05 mL/second. The lavage was repeated three times per animal and the recovered fluid from each lavage was placed on ice. The lavage fluid from each animal was centrifuged at 800 x g for 5 minutes. The fluid from the first lavage was reserved for protein and biochemical assays. The cell pellets from all three lavages were pooled and resuspended in 1 mL HBSS with Ca^{2+} and Mg^{2+}.

Cell counts and differentials on BAL sample:

The cell pellets from the BAL were resuspended and introduced into a bright line hemocytometer to determine total and viable cells. Viability was assessed by trypan blue exclusion. The total cell yield was typically 10^5 cells per mouse, of which more than 95% were mononuclear cells. Average cell viability was greater than 90%. A 0.1 ml aliquot of cells was plated onto a glass microscope slide using a cytocentrifuge (StatsSpin Cytofuge 2, Norwood, MA). The cells were stained with Wright-Giemsa stain and a differential cell count was made to determine the percentages of EOS, mononuclear cells and PMNs. Differential cell counts were determined from the products of total cell yield and percents of each cellular component x 100.

BAL fluid analysis for IL-5 and IL-13:

IL-5 and IL-13 were analyzed using Quantikine® (R&D Systems) quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits that were specific for the mouse cytokines. Standards, controls, and samples were pipetted into the wells of microtiter plates coated with monoclonal capture antibodies specific for mouse IL-5 or IL-13. Each sample and control well was spiked with 50 pg/mL of recombinant mouse

IL-5 or IL-13 so that all controls and samples were read in a linear portion of the standard curve. Analyses were conducted according to the manufacturer's instructions.

Blood samples analyzed for OVA-specific immunoglobulins:

OVA-specific IgE and IgG1 antibodies were detected using ELISA. Plastic microplates (Nunc-Immuno MaxiSorp Plate, Fisher Scientific) were coated with 100 µL/well of 0.05% OVA (Grade V; Sigma, St. Louis, MO) in 0.1 M carbonate buffer, pH 9.5, by overnight incubation at 4 ºC. The plates were blocked by incubation for 1 h at 37 ºC with 3% bovine serum albumin (BSA; Sigma) in PBS with 5% Tween 20 (PBST). Mouse plasma samples diluted 1:5000 in PBS for IgG1, undiluted mouse plasma samples for IgE, and serial dilutions of OVA-positive mouse serum (for the calibration curve) were added to the wells of the plates, which were incubated for 1 h at 37 °C for IgE and incubated overnight at 4 ºC for IgG1. Peroxidase-conjugated rat-anti-mouse-IgE (PharMingen, San Diego, CA) was diluted to 8 µg/mL in blocking buffer, or peroxidase-conjugated rat monoclonal antimouse IgG1 (PharMingen) diluted to 2 µg/mL with blocking buffer. Between each incubation, the plates were washed with PBST Enzyme substrate (TMB substrate reagents, PharMingen), and the reaction stopped after 30 min by the addition of 2 N H_2SO_4. Absorption was measured spectrophotometrically and compared to the calibration curve to determine the concentrations of IgE and IgG1.

Statistical Analyses

To confirm the findings of our previous publication that CAPs exposure caused allergic airway responses and that effects diminished with distance downwind from the source we analyzed the data from this study using a partial factorial design with factors for Location (50 m or 150 m) and Treatment (C, UF, F). Each endpoint was analyzed separately. For most of the endpoints, we considered that the normality assumptions for analyses of variance (ANOVA) were met. The exception was EOS, which is discussed later in this section. Of primary interest for these analyses were: 1) Treatment Effect and 2) Treatment by Location Interactions. Statistical significance was assessed using the usual F statistics. The EOS data had many zero values with a skewed distribution. We therefore transformed the EOS data into a logarithmic form, i.e. log (EOS+1), before performing the ANOVA. A null hypothesis was rejected when the probability of the null hypothesis being true was ≤ 0.05.

Regression analyses were used to test hypotheses of the dependence of biological responses on concentrations of specific CAPs constituents for those biomarkers that were significant in the ANOVA. Linear and non-linear associations were tested. However, although non-linear models in some instances provided a better fit to the data than did the linear model, only the results of the linear models are discussed in this manuscript.

NCER Assistance Agreement Annual Report

Date of Report: 12/22/04

EPA Agreement Number: R-82921601

Title: Effects of Airborne Particles on Allergic Airway Disease

Investigators: Jack R. Harkema, DVM, PhD (PI); Costantinos Sioutas, PhD (Co-PI)

Institution: Michigan State University, East Lansing, MI, and the Southern California Particulate Center and Supersite, Los Angeles, CA

Research Category: STAR-B1

Project Period: 10/31/2001-10/30/2004 (with a one year, no-cost extension, ending 10/30/2005)

Objective of Research

The overall objective of this project is to conduct atmospheric and toxicologic research designed to understand the adverse effects of airborne particulate matter (PM) of various size fractions (coarse, fine, and ultrafine particles) on pulmonary airways with pre-existing allergic airway disease. The aims of this project have not changed from our original proposal. We will test the following hypotheses: 1) that PM exposure exacerbates the airway injury associated with allergic airway disease; 2) that the magnitude of PM-induced airway toxicity is dependent on particle size; 3) that PM in transported ("aged") air pollution is more toxic to airways than that in locally generated air pollution; and 4) that PM-induced airway toxicity is most severe during periods of intense photochemical activity.

Work Progress & Preliminary Data Results

For three consecutive years we have conducted inhalation toxicology studies in the Los Angeles Basin (LAB) at two different locations to distinguish the effects of locally generated versus transported particulate matter. In October 2001 and January 2002, we conducted studies using our mobile laboratory in a residential community in Claremont, CA in the northeast LAB, which served as our receptor site for transported PM. In following years, we conducted similar autumn and winter exposures in urban Los Angeles in the central LAB near the University of Southern California campus, which served as our source site of locally generated particulate air pollution. The potential health effects of co-exposures to urban air pollutants and airborne allergens have not been thoroughly investigated. The purpose of our studies was to determine the effects of inhalation exposure of various size fractions of concentrated ambient particles (CAPs) on the lungs of rats that were concurrently exposed to a pulmonary allergen (ovalbumin; OVA). A state-of-the-art mobile air research laboratory, equipped with inhalation exposure chambers and ambient particle concentrators, was used to conduct the inhalation toxicology studies. Our mobile laboratory was moved from its home site at the Michigan State University Engine Research Laboratory in Okemos, MI to a residential site in Claremont, CA, or near the University of Southern California campus in Los Angeles, CA to these community-based inhalation toxicology studies in the early fall and winter months, as mentioned above.

Year 1: Studies in Claremont, CA (PM Receptor Site)

Study 1 Exposures: In Claremont, OVA-sensitized, male, Brown Norway rats (10-12 wks of age) were exposed to filtered air (controls), concentrated ambient coarse (2.5–10 μm; CCAPs), fine (0.1–2.5 μm; FCAPs) or ultrafine (0.01–0.15 μm; UFCAPs) particles, 5 h/day (11am - 4pm), for three consecutive days. Concentrated particle mass

and number concentrations and chemical speciation during the animal inhalation exposures are presented in Table 1. Immediately prior to each daily inhalation exposure, the rats were intranasally challenged with saline alone or a 0.5% solution of OVA in saline. Rats were exposed to average mass concentrations of 554, 515 and 45μg/m^3 for CCAPs, FCAPs, and UFCAPs, respectively. Twenty-four hours after the end of the exposures, rats were sacrificed, their pulmonary airways lavaged with saline, and their lung lobes processed for light microscopic or mRNA analyses. All of the animal sacrifices and necropsies were conducted by the laboratory staff of Dr. Harkema (Michigan State University) in the laboratories of Dr. Michael Kleinman (member of the Southern California Particle Center and Supersite) at the University of California in Irvine, CA.

Results: OVA-instilled rats had an allergic bronchiolitis with mucous cell hyperplasia (increase in the number of mucus-producing secretory cells lining the pulmonary airways) and an allergic alveolitis with marked increases in eosinophils in the bronchoalveolar lavage fluid (BALF). OVA-instilled and air-exposed rats had 538% more eosinophils in the BALF, 104% more stored mucosubstances in the bronchiolar epithelium, and a 6-fold increase in mucin-specific gene expression in bronchiolar airways than saline-instilled/air-exposed controls. Using this specific exposure regime of daily allergen and CAPs exposure we observed a marked particle-induced suppression, rather than enhancement, of the pulmonary inflammatory and epithelial responses to the inhaled allergen. Exposures to FCAPs (100% inhibition) or UFCAPs (by 66%), but not CCAPs, caused a marked attenuation of the OVA-induced allergic mucous cell hyperplasia. FCAPs also inhibited OVA-induced alveolitis (42%), and both FCAPS (65%) and UFCAPs (82%) blocked mucin-specific gene expression in bronchiolar epithelium (Figures 1 – 4).

Conclusion: These results indicate that fine (or ultrafine) ambient airborne particles may significantly interfere with allergen-induced airway responses during co-exposure of these airborne agents.

Study 2 Exposures: In January 2002, we conducted our second inhalation toxicology study at the same site in Claremont, CA. The experimental design was similar to that conducted in October 2001. Like in the first study, male, Brown Norway rats (10-12 wks of age) were exposed to filtered air, CCAPs, FCAPs or UFCAPs particles, 5 h/day (11am - 4pm), for three consecutive days. Immediately prior to each daily inhalation exposure, the rats were again intranasally challenged with saline alone or a 0.5% solution of OVA in saline.

Results: However in this winter study, rats were exposed to average mass concentrations of 86, 103, and 25μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 2). These average mass concentrations were markedly lower than those to which rats were exposed in the first Claremont study in October 2001 (see above). In contrast to the results of the October study, no FCAPs-, UFCAPs- or CCAPs-related effects on OVA-induced allergic alveolitis, mucous cell metaplasia or mucin-specific gene expression were observed in these rats exposed to the much lower concentrations of CAPs.

Conclusions: These results, in relation to the first study, suggest that these lower mass concentrations were not sufficient to modulate the pulmonary responses induced by the allergen challenge. Further chemical analyses of the CAPs from the first and second studies are currently underway to determine if differences in the chemical makeup of the CAPs may also have contributed, in part, to the marked differences in the pulmonary responses observed between the studies.

Year 2: Studies in Los Angeles (PM Source Site)

Study 1 Exposures: In October 2002, we conducted our third inhalation toxicology study of CAPs-exposed Brown Norway Rats with and without OVA airway instillations. In this study the mobile research laboratory was parked in Los Angeles, CA near the main campus of the University of Southern California and highways with heavy motor vehicle traffic (i.e., our source site with locally generated particulate air pollution). We used the same experimental design and exposure regime as described above for the first and second studies in Claremont, CA.

In this Los Angeles study, rats were exposed to average mass concentrations of 310, 324, and 31μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 3).

Results: Similar to the particle-induced inhibition that we observed in the first study Claremont exposure study, we detected a trend for FCAPs-induced suppression of allergic responses. Sensitization and challenge with OVA induced significant accumulations of eosinophils (58-fold increase) and neutrophils (23-fold) in BALF (Figures 5, 6). In animals exposed to FCAPs these responses were not statistically significant with only 2.2-fold and 10-fold increases for eosinophils and neutrophils, respectively, when compared to saline-challenged rats. However, these inflammatory responses were not significantly less than air-exposed, OVA-challenged animals. Exposure to CCAPs, but not UFCAPs, also resulted in modest decreases of inflammatory cell recruitment. OVA induced a 160% increase in intraepithelial mucosubstances (p =0.06) that was inhibited by FCAPs by approximately 30% (Figure 7). Taken together, when compared to the inhibitory profile of CAPs we observed in Claremont, the present study showed a more modest effect for suppression of allergic responses. Average mass concentrations were approximately 60% of what was generated in Claremont 2001, but were 3-fold greater than in Claremont 2002 when no CAPs-related effects were documented.

Conclusion: Thus, our results describing a partial inhibition by CAPs may represent an intermediate point on a dose response curve.

Study 2 Exposures: In January 2003, we used Brown Norway and repeated the dosing and exposure protocol at the same site to assess the effects of the seasonal particle mixture and to compare to the January exposure in Claremont. In this second Los Angeles study, rats were exposed to average mass concentrations of 905, 1026, and 27μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 4). During these exposures, the weather pattern was unseasonably warm and PM concentrations unusually high for January in Los Angeles. For CCAPs and FCAPs, concentrations were 3-fold greater than the October exposure at this site, and 10-fold greater than the January exposure in Claremont.

Results: Despite higher concentrations of CAPs, we did not detect particle-related changes in BALF cellularity in either saline-challenged or OVA-challenged rats. Similar to previous findings however, OVA-induced increases in intraepithelial mucosubstances were less in animals exposed to CAPs. Specifically, OVA –induced mucus storage was increased by only 45% and 59% when exposed to UFCAPs or FCAPs, respectively, compared to a 144% increase in air-exposed animals (Figure 8). The inhibitory effects of CAPs in the current study was less pronounced than we observed with lower particle concentrations at the receptor site in Claremont (FCAPS = 515 $\mu g/m^3$), where allergic responses were blocked by 100%.

Conclusions: Thus, the degree of CAPs-induced inhibition of airway epithelial remodeling and inflammatory cell recruitment is not described with a simple dose-response relationship, and is more likely determined by a contributions of specific particle components, their individual concentrations, and their potential interactions. Furthermore these results provide support for our hypothesis that transported, or aged particles, are more toxic than newly generated particles near source sites. We are currently conducting comparative analyses of physicochemical particle characteristics from each exposure to determine associations of specific components with the inhibitory effects we have documented.

Year 3: Studies in Los Angeles (Source Site)

Study 1 Exposures: In the third year of the project we employed the same challenge and exposure protocol at the Los Angeles site but used younger Brown Norway rats (6-7 weeks old). In September 2003, rats were exposed to average mass concentrations of 587, 674, and 147μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 5). Thus, CCAPs and FCAPs were similar in concentration to the September exposure at the receptor site in Claremont in September, when the most pronounced inhibition occurred. In addition, UFCAPs was 5 to 6-fold greater than any of the previous exposures.

Results: As was observed previously, particles had no effect on BALF parameters or mucous cell hyperplasia in normal, non-allergic animals. Furthermore, significant CAPS-induced changes were not evident in OVA-challenged rats as we had seen with exposures with similar CAPs concentrations. Despite the lack of statistically significant differences, an apparent trend was nevertheless evident for the inhibitory effects by CAPs on OVA-induced BALF cellularity (e.g., eosinophils, Figure 9) and stored mucosubstances in pulmonary epithelium (Figure 10). During these exposures we also observed for the first time an effect of particles on nasal epithelium in non-allergic rats. CCAPs caused a 47% increase in stored mucosubstances in nasal respiratory epithelium, but had no effect on OVA-induced allergic rhinitis (Figure 11). Conversely, exposure to FCAPs inhibited by approximately 50% the OVA-induced increase in nasal intraepithelial mucosubstances.

Conclusion: It is notable that the higher concentrations of UFCAPs had no greater or lesser effects in normal and allergic rats than seen in previous exposures where lower concentrations were generated.

Study 2 Exposures: In February 2004, we repeated the protocols using the younger Brown Norway rats (6-7 weeks). In this study rats were exposed to average mass concentrations of 254, 505, and 114μg/m3 for CCAPs, FCAPs, and UFCAPs, respectively (Table 6).

Results: Similar to the previous September exposure with younger animals, a distinct trend for particle-induced inhibition of allergic alveolitis emerged, however the comparisons failed to reach statistical significance. Also, particles had no effect on allergic mucous cell metaplasia during this exposure.

Summary of Research to Date

Our studies were designed to address four specific hypotheses. The first, that particles would enhance airway injury associated allergic airway disease, was not supported by our data. To the contrary, we found that particle exposure attenuated allergic bronchiolitis and alveolitis, and allergen-induced mucous cell metaplasia and the allergen-induced overexpression of mucin-specific gene (MUC5AC) in bronchiolar epithelium. This particle-induced attenuation of allergic airway disease was the greatest with exposures to transported ("age") FCAPs and UFCAPS in the October 2001 study in Claremont, CA (receptor site). In successive exposures, this inhibitory phenomenon was observed to varying degrees with changing variables of particle concentrations, exposure sites, and in younger animals.

The sum of our data supports our other hypotheses, that a) the magnitude of PM-induced effects is dependent on particle size, and b) PM in transported ("aged") air pollution is more "toxic" on airways than that in locally generated air pollution, and c) that PM-induced airway "toxicity" is most severe during periods of intense photochemical activity, were supported by our experimental results. We

consistently found that FCAPs, and with less frequency UFCAPs, were associated with particle-induced attenuation of allergic airway responses. Furthermore the effects of FCAPs were most pronounced during relatively warm weather (i.e. photochemical pollution) at the Claremont receptor site. By comparison the source-site exposures in Los Angeles during both summer and winter months produced less robust inhibitory effects of particles, but nonetheless demonstrated a trend for decreased allergic responses.

Although we predicted that exposures to CAPs would exacerbate the allergic airway responses, the inhibitory phenomenon we have described is not without precedent. Depression of inflammatory and immune cell function might be caused by oxidative stress that is measurable in pulmonary tissues of rats after FCAPs inhalation. We and others have demonstrated that exposure of alveolar macrophages to ambient particles *in vitro* induces dose-dependent cytotoxic responses, including oxidative stress, mitochondrial damage, and cytoskeletal derangement (Goldsmith et al. 1998; Moller et al. 2002; Becker et al. 2003; Kleinman et al. 2003; Li et al. 2003). Particle uptake by macrophages, lymphocytes, epithelial cells and dendritic cells may be counterproductive for normal immune responses to the presence of an airway allergen. Furthermore, ambient particles are known to induce a variety of cytokines from alveolar macrophages (e.g., IFN-γ, TNFα, IL-8,) that can interfere with allergic pathways to elicit eosinophil recruitment, lymphocyte activation, or IgE production.

Taken together, our results are reminiscent of data from allergic rodent models that test the effects of airway endotoxin during allergen challenge. In these studies, endotoxin exposures at the time of allergen challenge inhibited allergic eosinophilic inflammation and airway hyperreactivity (Gerhold et al. 2002; Tulic et al. 2002). Attenuation of these allergic responses was associated with the production of IFNγ, IL-10 and IL-12 (Tulic et al. 2001; Gerhold et al. 2002), cytokines known to oppose Th2-pathways of allergic inflammation. The paradigm of endotoxin-induced down regulation of allergic pathways is consistent with the "hygiene hypothesis", where bacterial or fungal stimuli promote development of Th1-lymphocytes over allergy-promoting Th2 lymphocytes during the postnatal development of the immune system (Matricardi et al. 2002; Yazdanbakhsh et al. 2002). Just as bacterial stimuli present in low hygienic environments (e.g., rural, agricultural) can minimize the development of an allergy-prone immune phenotype in growing children, inhalation of a Th1-stimulus such as bacterial endotoxin opposes IgE production, eosinophilic inflammation, and airway hyperreactivity that are initiated by Th2 cytokines in subjects with allergic airway disease (Matricardi et al. 2002). Similar Th1/Th2cytokine dynamics may have occurred during CAPs inhalation and allergen challenge in the present series of experiments. Others have shown that treatment of ovalbumin-sensitized Brown Norway rats with IFNγ, a Th1 cytokine, blocks allergic inflammation and Th2 cytokine production (Huang et al. 1999). Inhalation of FCAPs and UFCAPs may have induced an early Th1 stimulus that interfered with allergic cytokine pathways which led to marked attenuation of more down stream responses such as eosinophilic inflammation and the development of mucous cell metaplasia. Further studies are needed to specifically investigate the Th1/Th2 cytokine responses in the lungs at various times after exposure to inhaled CAPs.

Interestingly, there are several reported epidemiology studies that have demonstrated that residents of the former East Germany who lived in communities with high levels of industrial air pollution had lower prevalence rates of asthma and other allergic diseases compared to residents of the former West Germany who lived in communities with lower levels of air pollution. Among East-German children, lower prevalence rates of asthma and positive skin-prick tests for allergery were observed compared to West-German children who live in communities with less air pollution (Klein et al. 1992; von Mutius et

al., 1994; Trepka et al., 1996). Similarly, East-German adults have been reported to have lower specific IgE levels and lower prevalence rates of asthma, wheezing, positive methacholine-challenge tests, allergic rhinitis, and positive skin-prick tests compared to those of West-German adults (Nicolai et al., 1997; Heinrich et al., 1998). It has yet to be determined what specific factors related to the air pollution (gaseous or particulate components), or related to life style, may account for these regional differences in the prevalence of asthma and allergies.

The results of our inhalation studies do lend support to the hypothesis that exposure to particulate air pollution may attenuate the development of allergic airway diseases. Since the rats in our study did not have allergic airway disease prior to exposure to the airborne CAPs, our studies were not designed to test the hypothesis that exposure to CAPs exacerbates pre-existing allergic airway disease. However, we have recently demonstrated that acute exposure of BN rats to FCAPs in a Detroit community may exacerbate pre-existing ovalbumin-induced allergic airway disease in BN rats under the right experimental and exposure conditions (Harkema et al., 2004). Therefore, it appears that the development and severity of this experimentally induced allergic airway disease is highly dependent on the time at which the laboratory rodents are exposed to both the allergen and the airborne particles.

Key Personnel and Budget

There have been no changes in the key personnel involved in this project. Expenditures to date are in line with the amount of the work completed. There are no substantial differences in the present itemized expenses and estimated itemized costs in the approved project. We do not anticipate any major changes in the budget over the next year of the project.

Publications/Presentations to Date:

An abstract entitled, “Concentrated Ambient Particles Attenuate Allergen-Induced Airway Responses in the Lungs of Brown Norway Rats,” was presented at the 2003 annual meeting of the Society of Toxicology in Salt Lake City, Utah (March 9-13, 2003) and at the American Association for Aerosol Research-Particulate Matter Conference in Pittsburgh, PA (March 31-April 4, 2003).

Wagner, J.G., Sioutas, C., Timm, E. Kaminski, N., Keeler, G.J., Kleinman, M., Froines, J., and J.R. Harkema. Exposure to Concentrated Ambient Fine and Ultrafine Particles Inhibits Allergen-Induced Inflammation and Storage and Secretion of Mucosubstances in Rat Pulmonary Epithelium. In preparation.

Future Activities: Approximately 80% of the project is now complete. In 2005, we will complete our biologic and atmospheric analyses from the 2004 animal inhalation studies. We anticipate that two or three manuscripts will be submitted to scientific journals for peer-reviewed publication. In addition, a final report including the results of all of our studies will be prepared and submitted to the EPA office before the end of the project.

Table 1. Concentrated Particle mass and number concentrations and chemical speciation during the animal inhalation exposures of October 8-10, 2001.

PM Mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	2.0E+03	0.078	4.39	553.5	2.6	28.2	115.7	21.8	374.3
Fine	4.5E+04	0.078	0.88	515.4	19.4	132.0	207.5	113.8	23.3
Ultrafine	2.8E+04	0.050	0.08	45.2	6.2	33.7	0.0	2.0	1.5

Table 2. Concentrated Particle mass and number concentrations and chemical speciation during the animal inhalation exposures of January 28-30, 2002.

PM Mode	Number (particles/cm^3)	Number geometric mean diameter(μm)	Mass median diameter(μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	2.3E+03	0.034	3.78	86.4	0.2	4.8	4.2	2.9	55.7
Fine	5.2E+04	0.034	0.47	102.7	4.3	75.5	33.0	7.5	5.6
Ultrafine	4.7E+04	0.023	0.04	24.7	2.1	22.2	0.0	0.0	2.3

Table 3. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of September 30-October 1, 2002.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	310.0	0.0	28.8	83.6	23.8	233.1
Fine	1.2E+05	ND	ND	323.5	12.8	122.7	34.2	67.8	121.7
Ultrafine	9.6E+04	ND	ND	31.0	6.1	50.8	0.0	3.1	2.9

ND = Not yet determined.

Table 4. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of January 2003.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	904.6	1.4	147.4	100.8	24.2	303.4
Fine	4.3E+05	ND	ND	1025.9	46.9	367.3	459.7	89.3	90.9
Ultrafine	3.8E+04	ND	ND	132.1	11.7	93.3	13.6	6.8	6.7

ND = Not yet determined.

Table 5. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of September 2003.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	586.7	3.2	41.1	148	43	167.8
Fine	ND	ND	ND	674.7	22.6	175.2	121.8	192.8	70.2
Ultrafine	2.6E+05	ND	ND	147.5	8.1	70.2	0.0	20	0.6

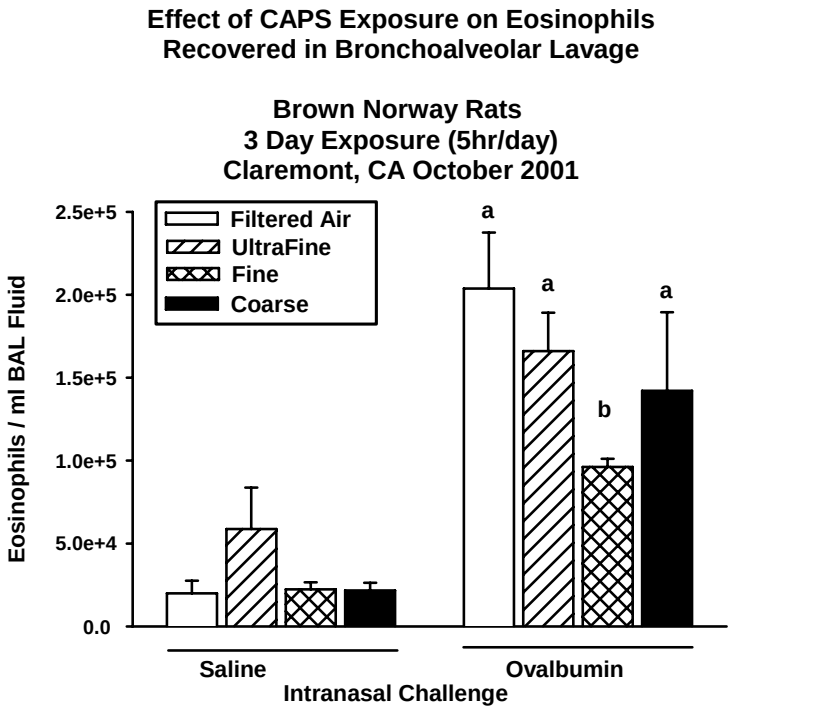
ND = Not yet determined.

Table 6. Concentrated particle mass and number concentrations and chemical speciation during the animal inhalation exposures of February 2004.

PM mode	Number (particles /cm^3)	Number geometric mean diameter (μm)	Mass median diameter (μm)	Mass (μg/m^3)	EC (μg/m^3)	OC (μg/m^3)	Nitrate (μg/m^3)	Sulfate (μg/m^3)	Metals (μg/m^3)
Coarse	ND	ND	ND	253.6	0.5	29.1	80.3	19.6	81.4
Fine	ND	ND	ND	505.2	15.7	121.3	68.4	27.4	36.7
Ultrafine	5.9E+04	ND	ND	114.1	14.3	62.9	4.4	13.5	1.3

ND = Not yet determined.

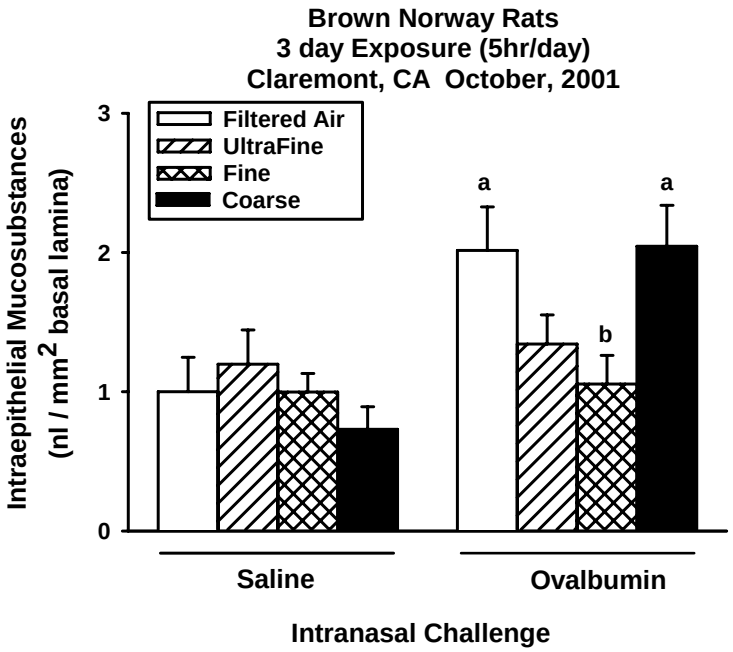
Figure 1:



a = significantly different from respective group in Normal Airways, ($p \leq .05$)

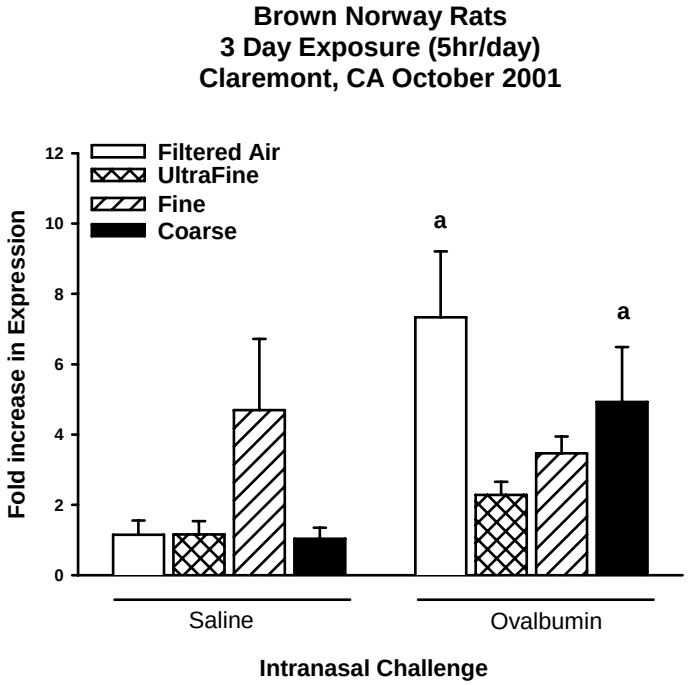
b = significantly different from respective group exposed to Filtered Air, ($p \leq 0.05$)

Figure 2. **Effect of CAPs Exposure on Intraepithelial Mucosubstances (Axial Airway, Left Lung Lobe, Generation 5)**



a = Significantly different from respective group instilled with Saline, ($p \leq 0.05$).
b = Significantly different from respective group breathing Filtered Air, ($p \leq 0.05$).

Figure 3. **Muc5AC mRNA in the Axial Airway of Right Caudal Lobe**



a = Significantly different from respective group instilled with Saline, ($p \leq 0.05$).

Figure 4. Light photomicrographs of pulmonary tissue sections taken from rats exposed to filtered air and saline (A); filtered air and ovalbumin (OVA; sensitized and challenged) (B); coarse concentrated air particles (CCAPs) and OVA (C); or fine concentrated air particles (FCAPs) and OVA (D). No allergic alveolitis/bronchiolitis is present in A. Marked allergic alveolitis/bronchiolitis is present in B and C, but minimal pulmonary lesions are present in D. All tissues are stained with hematoxylin and eosin. Bar = 200 microns. Ba = bronchiolar airway; ap = alveolar parenchyma.

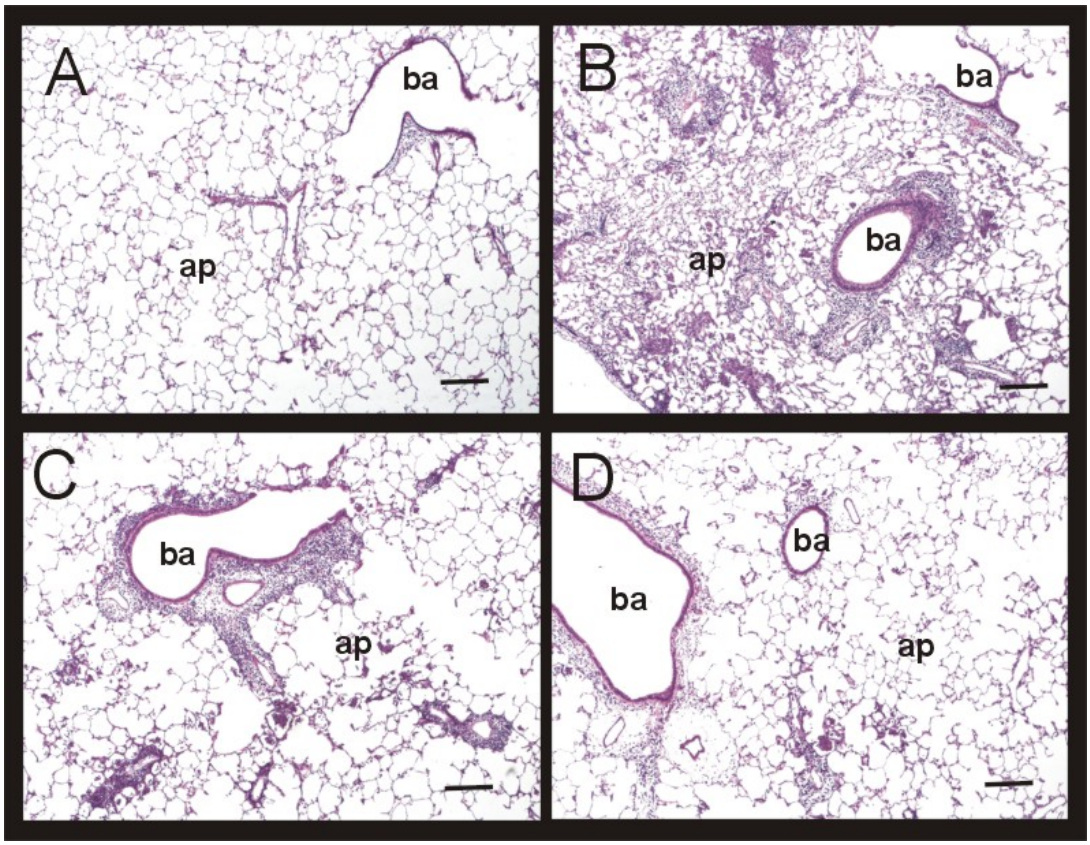
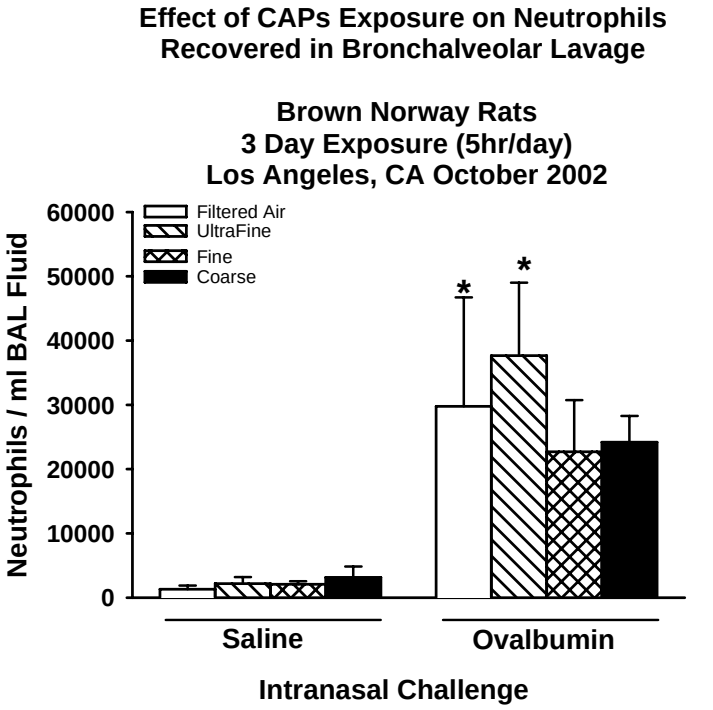
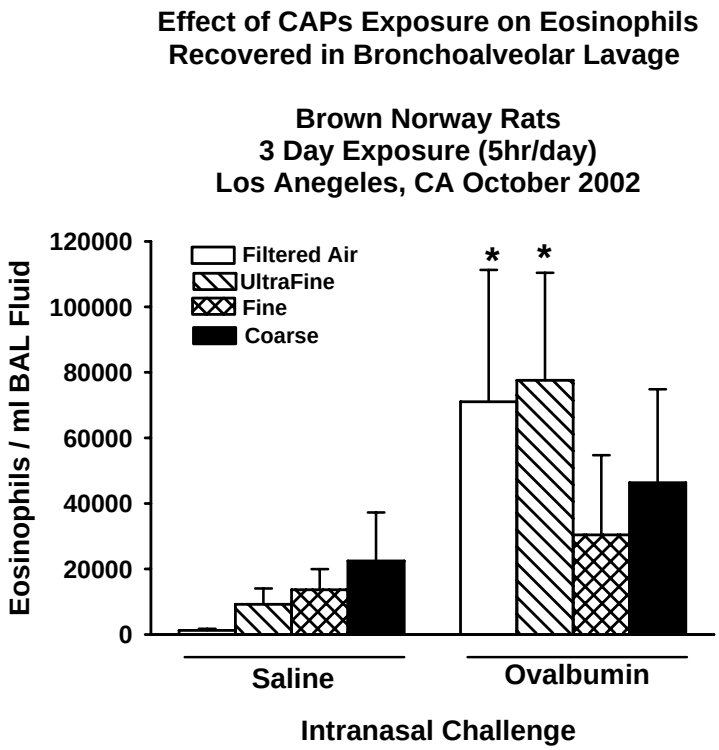


Figure 5:



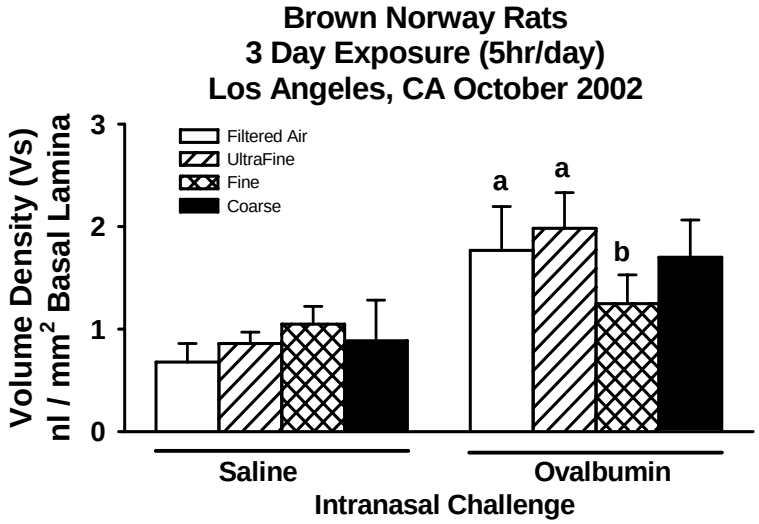
* = Significantly different from respective group receiving Saline, ($p \leq .05$).

Figure 6:



* = Significantly different from respective group receiving Saline, ($p \leq .05$).

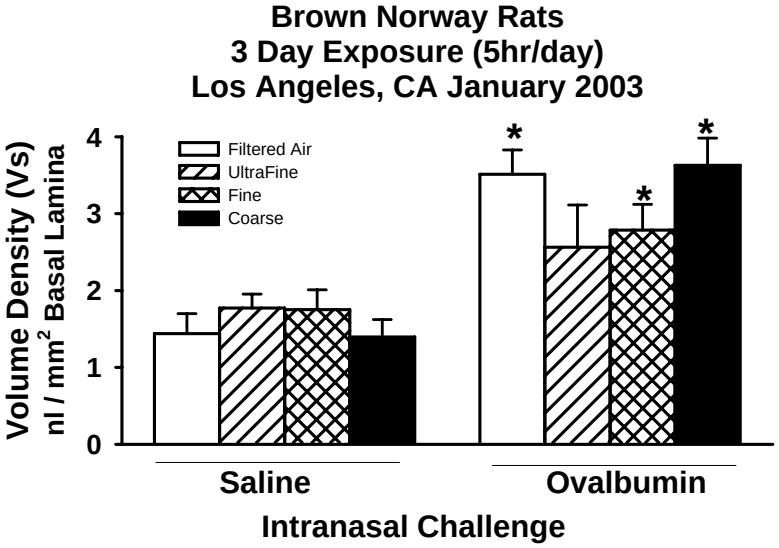
Figure 7: **Effect of CAPs Exposure on Intraepithelial Mucosubstances (Axial Airway, Left Lung, Lobe, Generation 5)**



a = Significantly different from respective group receiving Saline, ($p \leq 0.05$).
b = Significantly different from respective group exposed to Filtered Air, ($p \leq 0.05$).

Figure 8:

Effect of CAPs Exposure on Intraepithelial Mucosubstances (Axial Airway, Left Lung, Lobe, Generation 5)



*** = Significantly different from respective group recieving Saline, ($p \leq 0.05$).**

Figure 9:

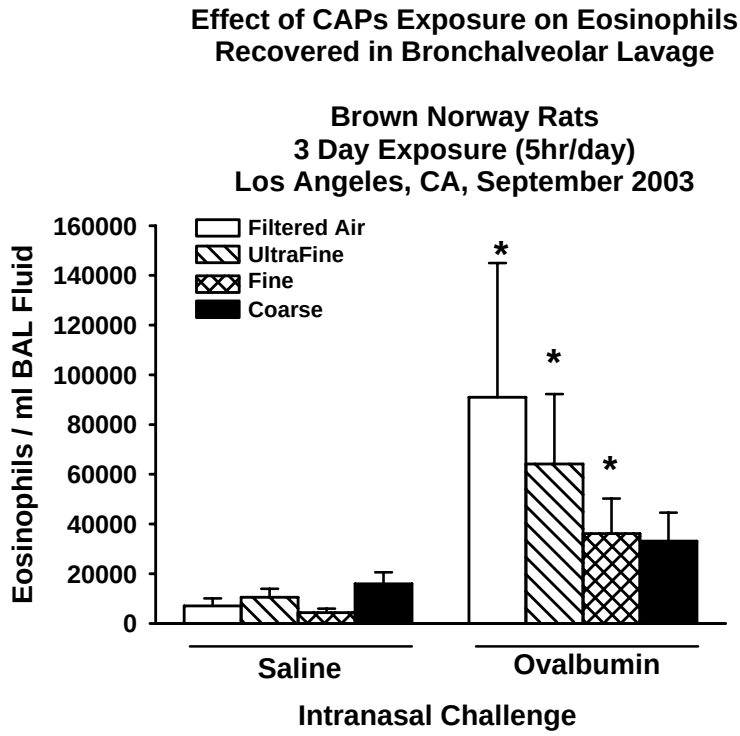
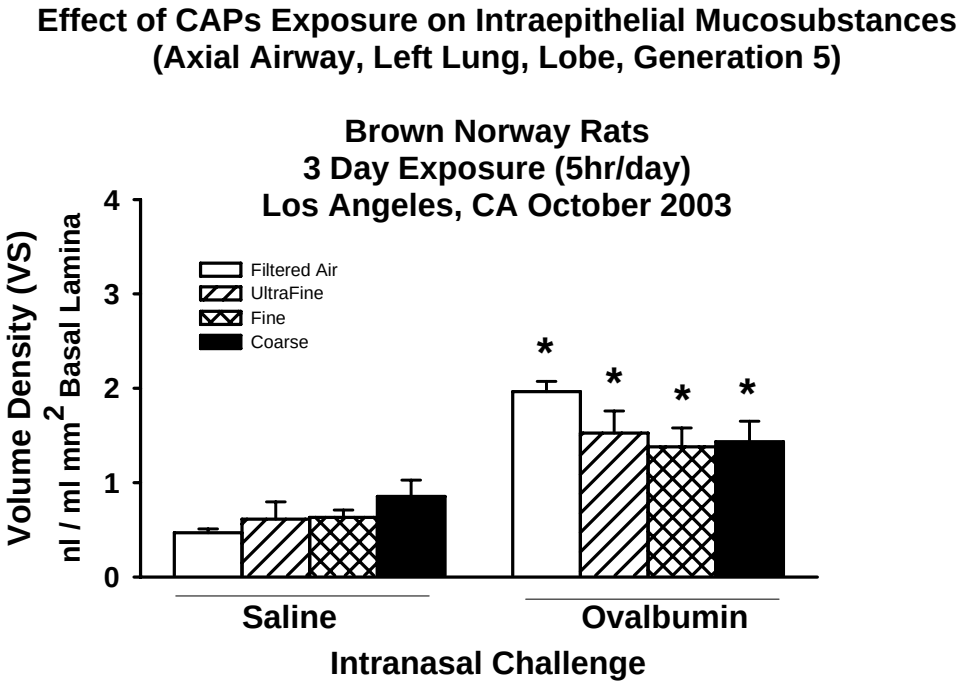


Figure 10:

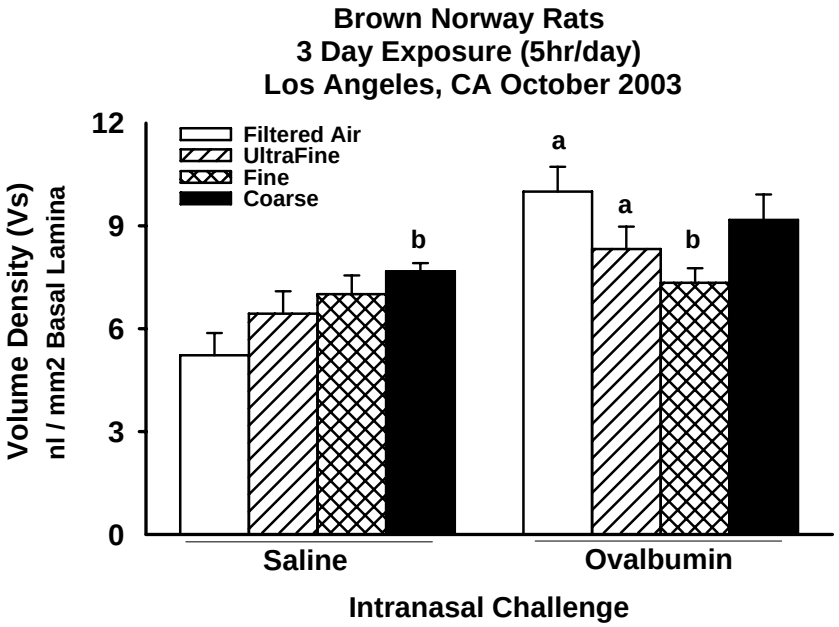
* = Significantly different from respective group receiving Saline, ($p \leq .05$).



* = Significantly different from respective group recieving Saline, ($p \leq 0.05$).

Figure 11:

Effect of CAPs Exposure on Intraepithelial Mucosubstances (Proximal Nasal Septum)



a = Significantly different from respective exposed to Filtered Air, ($p \leq 0.05$).

b = Significantly different from respective group receiving Intranasal Saline, ($p \leq 0.05$).

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