Airborne Particles of the California Central Valley Alter the Lungs of Healthy Adult Rats

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Epidemiologic studies have shown that airborne particulate matter (PM) with a mass median aerodynamic diameter < 10 µm (PM_{10}) is associated with an increase in respiratory-related disease. However, there is a growing consensus that particles < 2.5 µm ($PM_{2.5}$), including many in the ultrafine (< 0.1 µm) size range, may elicit greater adverse effects. PM is a complex mixture of organic and inorganic compounds; however, those components or properties responsible for biologic effects on the respiratory system have yet to be determined. During the fall and winter of 2000–2001, healthy adult Sprague-Dawley rats were exposed in six separate experiments to filtered air or combined fine ($PM_{2.5}$) and ultrafine portions of ambient PM in Fresno, California, enhanced approximately 20-fold above outdoor levels. The intent of these studies was to determine if concentrated fine/ultrafine fractions of PM are cytotoxic and/or proinflammatory in the lungs of healthy adult rats. Exposures were for 4 hr/day for 3 consecutive days. The mean mass concentration of particles ranged from 190 to 847 µg/m^3. PM was enriched primarily with ammonium nitrate, organic and elemental carbon, and metals. Viability of cells recovered by bronchoalveolar lavage (BAL) from rats exposed to concentrated PM was significantly decreased during 4 of 6 weeks, compared with rats exposed to filtered air ($p < 0.05$). Total numbers of BAL cells were increased during 1 week, and neutrophil numbers were increased during 2 weeks. These observations strongly suggest exposure to enhanced concentrations of ambient fine/ultrafine particles in Fresno is associated with mild, but significant, cellular effects in the lungs of healthy adult rats. *Key words:* concentrated ambient particles, fine particles, particulate matter, pulmonary inflammation, ultrafine particles. *Environ Health Perspect* 111:902–908 (2003). doi:10.1289/ehp.5964 available via http://dx.doi.org/ [Online 4 March 2003]

Epidemiologic studies have shown a relationship between increased levels of particulate matter (PM) with a mass median aerodynamic diameter < 10 µm (PM_{10}) and an increase in respiratory-related morbidity and mortality (Pope et al. 1995). Recent epidemiologic studies have also suggested an even stronger correlation between particle-induced health effects and particles in the fine (< 2.5 µm) (Dockery et al. 1993; Pope et al. 2002; Schwartz et al. 1996; Schwartz and Neas 2000) or ultrafine (< 0.1 µm) (Peters et al. 1997) fractions of PM_{10}. Particle mass, composition, size, and number have all been implicated in the toxicity of inhaled PM in animal studies.

Although a wealth of epidemiologic information suggests that PM is involved in adverse health effects, the physical or chemical properties responsible remain unknown. Part of the difficulty in identifying such properties lies in the fact that creating "real-world" particles to study their effects in animals or humans in a controlled setting is difficult to do. Several studies have used particles from individual sources that contribute to the ambient PM. However, the disadvantage of exposure to particles collected from specific emission sources, including residual oil fly ash, coal fly ash, or diesel exhaust PM, is that these sources make a small contribution to

the overall PM mix and are not likely to be representative of ambient particle exposures. In addition, such studies cannot effectively reproduce the ultrafine fraction of particles originally present at the time of particle formation, thus making it impossible to study their effects on biologic systems.

Recent advances in engineering have made it possible to concentrate particles in real time from the ambient environment to study health effects. These systems have facilitated the concentration of particles ranging in size from 0.1 to 2.5 µm; however, concentrating ultrafine particles (< 0.1 µm) has been more problematic (Sioutas et al. 1997). Original particle concentrators included the Harvard Fine Particle Concentrator (Sioutas et al. 1995) and a concentrator that uses centrifugal force (Gordon et al. 1999). However, these concentrators do not enhance the concentration of particles below approximately 0.1 µm (i.e., the ultrafine particles). The concentrator used for this study is designed to concentrate both fine and ultrafine PM (Kim et al. 2000). The system uses hydration to enlarge particles to supermicrometer droplets with supersaturation and condensation. The particles are then separated by size using a virtual impactor and returned to their original (ambient) size by passing through a diffusion dryer to remove particle-bound water.

Normal rats (Clarke et al. 1999; Gordon et al. 1998; Kodavanti et al. 2000), as well as rats with chronic bronchitis (Clarke et al. 1999; Kodavanti et al. 2000) or pulmonary hypertension (Gordon et al. 1998), have been used to evaluate the effects of concentrated ambient particles (CAPs) on pulmonary function and inflammation. However, these studies have produced contrasting results. A potential deficiency in these studies was the inability to concentrate ultrafine (< 0.1 µm) particles from the environment.

The Central Valley of California has some of the highest ambient particle concentrations found in the United States, especially in the $PM_{2.5}$ range. Contributors to ambient PM in the Central Valley include agricultural and ranching activities, fires, wind-blown dust, diesel and gasoline engine exhaust, power plant emissions, and home heating (Watson et al. 2000). Pinkerton et al. (2000) recently examined the lungs of deceased young Hispanic males from the California Central Valley (Fresno, CA) who had been healthy and died of nonrespiratory causes. They found principal sites of particle deposition and/or retention in the lungs to be terminal bronchioles and first-generation respiratory bronchioles associated with thickened walls and alveolar septa along with dust-laden macrophages (Pinkerton et al. 2000). Such observations strongly suggest that particles may be retained in the lungs and result in structural alterations over time.

The composition and concentration of fine and ultrafine fractions of PM_{10} vary in the Central Valley with seasonal and atmospheric conditions. Therefore, studies described here were completed during two different times of the year to examine varied compositions of

PM and their potential effects on the respiratory system. Concentration of the ambient particles in Fresno by 20-fold allowed for controlled exposure of healthy, adult rats to elevated levels of particles. The intent of this study was to determine if concentrated fine/ultrafine fractions of ambient $PM_{2.5}$ are cytotoxic and/or proinflammatory in the lungs of healthy adult rats. After 3 days of exposure (4 hr/day), we measured broncho-alveolar lavage (BAL) cell viability and total cells, as well as the number and relative proportion of macrophages, neutrophils, and lymphocytes from rats exposed to CAPs.

Materials and Methods

Animals. We purchased 9–10-week-old male Sprague-Dawley rats, free of respiratory disease, from Harlan (San Diego, CA) and quarantined them for 1 week before exposure to CAPs. The study designation, dates of exposure, age of animals, and number of rats per group are shown in Table 1. Animals were handled in accordance with standards established by the U.S. Animal Welfare Acts as set forth in the National Institutes of Health guidelines (Institute of Laboratory Animal Resources 1996) as well as the Animal Care and Use committees of both the University of California, Davis and California State University, Fresno. The rats were housed in plastic cages with TEK-Chip pelleted paper bedding (Harlan Teklad, Madison, WI) and maintained on a 12-hr light/12-hr dark cycle. All animals had access to Laboratory Rodent Diet 5001 (LabDiet, Brentwood, MO) *ad libitum* before, during, and after exposures. All animals had access to water *ad libitum*, except during each daily exposure period because of the design of the exposure chambers.

Particle concentrator system. Animals were exposed either to room air passed through an activated carbon–impregnated filter media pad (Dayton Electric Manufacturing Co., Niles, IL) or to fine and ultrafine CAPs generated by portable concentrators developed over the past 2 years at the University of Southern California. These portable Versatile Aerosol Concentration Enrichment Systems (VACES) are based on previously described technology (Kim et al. 2000; Sioutas et al. 1999). These systems are able to enrich the concentration of particles in the complete size range of 0.01–10 µm by up to a factor of 40, depending on the output flow rate. By incorporating size-selective inlets, the VACES can provide CAPs in selected size ranges. These systems are compact in size and modular in design, thus allowing their use in mobile exposure platforms. The performance of these systems has been described in detail by Kim et al. (2001a, 2001b).

Concentration enrichment of particles with aerodynamic diameters < 2.5 µm is accomplished by first drawing air samples through two parallel lines, each sampling at 110 L/min for a total flow of 220 L/min (Figure 1). Each line has a 2.5-µm cut-point preimpactor, to remove essentially all of the particles > 2.5 µm from the flow. PM is drawn through a saturation–condensation system that grows particles to 2–3 µm droplets, which are then concentrated by virtual impaction from a total flow of 220 L/min to a flow of 10 L/min, to concentrate particles by a factor of 22. Two diffusion dryers are used downstream of the virtual impactors to remove excess water vapor and return concentrated particles to their original size, before delivering them to the exposure chambers.

Detailed laboratory characterization of individual components of the VACES has demonstrated the ability of this system to preserve particle mass, number, and chemical species during the concentration enrichment process (Kim et al. 2001b). Experimental characterization of the VACES demonstrated that concentration enrichment is accomplished with high efficiency and minimal particle loss over the range of particle sizes and chemical compositions encountered in ambient atmospheres. Field evaluation, described by Kim et al. (2001a), also demonstrated that volatile species, such as ammonium nitrate, are preserved throughout the supersaturation and concentration-enrichment processes. Ultrafine particles are concentrated without substantial changes in their compactness or density, as measured by fractal dimension analysis (Kim et al. 2001a).

The VACES was installed in the animal vivarium located on the campus of California State University, Fresno. The three-story building that houses the vivarium is located in the northeastern section of campus. This location is close to the secondary Supersite station monitored by the U.S. Environmental Protection Agency in Fresno. Ambient air for each exposure was drawn from the roof of the building, and the aerosol was delivered to the VACES via an 8-m-long, 7.62-cm-diameter duct. At a flow rate of 220 L/min, the aerosol residence time in the duct before reaching the VACES was about 10 sec, rapid enough to avoid particle loss on the duct walls from deposition by settling (for larger particles) or diffusion (for smaller particles). The concentrated aerosol was supplied to whole-body animal exposure chambers.

Fabrication and characterization of exposure chambers. Exposure chambers were constructed using standard animal housing cages made of polycarbonate measuring $20 \times 43 \times 18$ cm. The metal top of the cage that held food and water was replaced with a lid designed to deliver aerosols into the chamber (Figure 2). Air passing through the cyclone-designed orifice created a circular flow into the chamber. A perforated metal sheet consisting of 1.7-mm-diameter holes and 23% open area placed in the lid allowed airflow to be distributed along the top of the cage in a uniform manner. Air was drawn out of the chamber by two tubes located at the

Table 1. Experimental design showing study designation, exposure dates, age of animals, and number of rats used per group.

| Study designation	CAPs exposure dates	Age (weeks)[a]	No. of rats exposed Filtered air	CAPs
First week of fall	17–19 October 2000	10	6	6
Second week of fall	24–26 October 2000	9	6	6
Third week of fall	31 October–2 November 2000	9	6	6
First week of winter	30 January–1 February 2001	9	6	6
Second week of winter	6–8 February 2001	9	6	6
Third week of winter	13–15 February 2001	9	6	6

[a]Age of rats at beginning of exposure.
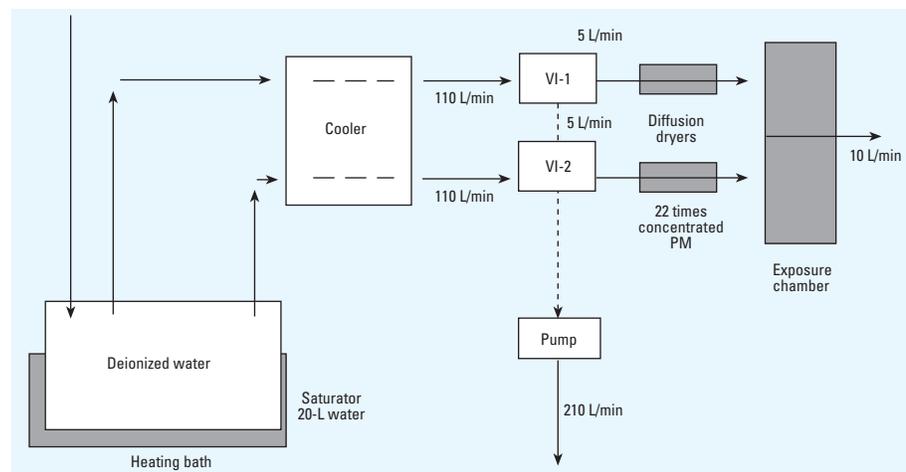
Figure 1. VACES for sampling fine/ultrafine PM. VI, virtual impactors.

bottom of the cage. Holes were placed evenly along the exit tubes to draw air out along the length of the chamber. To test airflow through the chambers, before their use in Fresno, smoke was admitted into the chamber and observed as it moved from the top to the bottom exit tubes. Repeated testing demonstrated filling and emptying of the chamber with smoke was evenly distributed from the top to bottom of each chamber. Airflow ranging from 2 L/min to 15 L/min demonstrated uniform mixing of smoke particles throughout the chamber.

During the studies in Fresno, the system was operated at a negative pressure (up to 7 inches of water) to deliver fine and ultrafine aerosols with minimal loss. Each chamber has an independent flow regulator with the total flow from the four chambers equal to the total output of the VACES.

Physicochemical characteristics of concentrated PM. Animal exposures to concentrated fine and ultrafine PM in Fresno are summarized in Table 1. Experiments were conducted during 3 consecutive weeks in the fall of 2000 and 3 consecutive weeks in the winter of 2001. Each week animals were exposed for 4 hr/day for 3 days. Of the 10 L/min of concentrated aerosol generated, 2.3 L/min was diverted into a sampling manifold to determine physical and chemical characteristics of the exposure aerosol, and the remaining 7.7 L/min was supplied to the four animal exposure chambers.

Particle mass and elemental composition were measured by collecting concentrated PM on 37-mm Teflon filters (PTEF, 2-μm pore; Gelman Science, Ann Arbor, MI) at a flow rate of 1 L/min. The retention efficiency of these filters, even for the most penetrating particles (0.2–0.3 μm), is > 99%. The Teflon filters were weighed before and after each experiment using a Mettler 5 microbalance (model MT 5; Mettler-Toledo Inc., Highstown, NJ), under controlled relative humidity (40–45%) and temperature (22–24°C) in the Aerosol Laboratory at the University of Southern California. At the end of each 3-day experiment, filters were stored at constant humidity and temperature for 24 hr before weighing to ensure removal of particle-bound water. After

Teflon filters were weighed, they were analyzed by X-ray fluorescence to determine the concentrations of particle-bound trace elements and metals.

Concentrations of inorganic ions (i.e., sulfate and nitrate), elemental carbon (EC), and organic carbon (OC) were determined by collecting particles on 37-mm prebaked quartz filters (Pallflex Corp., Putnam, CT). Particles were collected on quartz filters at a flow rate of 1 L/min, parallel to the Teflon filter used for gravimetric and metal analysis. At the end of each 3-day experiment, a 1-cm^2 circular section of the quartz filter was removed from the center of the filter and analyzed by thermoanalysis to determine EC and OC content using a process described in detail by Fung (1990). The remainder of the filter was extracted using a mixture of 0.1 mL of ethanol and 5 mL of distilled deionized water and analyzed by ion chromatography to determine the concentrations of sulfate and nitrate. Particle number concentrations were measured continuously throughout each exposure period with a TSI 3022 condensation particle counter, sampling at a flow rate of 0.3 L/min.

BAL fluid analysis. Immediately after exposure on the third day, rats were anesthetized by intraperitoneal injection of pentobarbital (100–150 mg/kg body weight) and exsanguinated via the abdominal aorta. The trachea was cannulated and the lungs lavaged with Ca^{2+}/Mg^{2+}-free phosphate-buffered saline (PBS; pH 7.4) at a volume equal to 35 mL/kg body weight. Three in-and-out lavages were performed using the same fluid. BAL fluid was centrifuged at 2,000 rpm for 10 min at 4°C. Supernatant was removed, and the cell pellet was resuspended in Ca^{2+}/Mg^{2+}-free PBS. Resuspended cells (0.1 mL) were used to determine total cell count and viability. Cell viability was measured by exclusion of 0.4% trypan blue (Sigma, St. Louis, MO), an indicator of irreversible loss of plasma membrane integrity. A second aliquot of cells was centrifuged using a Shandon Cytospin (Thermo Shandon, Inc., Pittsburgh, PA) to prepare cell differential slides. The slides were dried at room temperature and stained with HEMA 3 (Biochemical

Sciences, Inc., Swedesboro, NJ). Macrophages, neutrophils, and lymphocytes were counted using light microscopy (1,000 cells/sample).

Statistics. Each experiment consisted of a filtered air–exposed group and a CAPs-exposed group for all 6 weeks. The data were analyzed by analysis of variance. Comparisons among exposure groups were evaluated by Fisher's protected least significant difference test. Comparisons were considered significant if a value of $p < 0.05$ was observed. Statistical analysis was performed with StatView 5.0.1 (SAS Institute Inc., Cary, NC).

Results

Characterization of exposure chambers. To test the exposure chambers, the flow rate through each exposure chamber was set at 2.5 L/min, and fluorescein particles were generated with a nebulizer. Particles were heated and passed through a diffusion dryer to remove water vapor. Particles were collected by placing round microscope cover slips on six cooled brass rods. The microscope slides were placed in pH 10 water and counted in a fluorometer. The large particles [3.1 μm mass median aerodynamic diameter (MMAD)] were collected on the cover slips and showed 13% variability. Smaller particles, with a MMAD of 0.56 μm, were generated and showed 10% variability. Cages were swabbed, and the swabs were analyzed in a fluorometer but exhibited no activity over background. This demonstrated that particles were not depositing on chamber walls.

Characteristics of concentrated aerosols. Physicochemical characteristics of concentrated particles during fall 2000 and winter 2001 are shown in Figures 3–6 and in Table 2. These data represent averages over each 3-day period for each week of exposure.

Exposures during the fall of 2000 demonstrated remarkably consistent particle numbers, varying between 1.1×10^5 and 1.2×10^5 particles/cm^3. However, the average mass concentration of the concentrated aerosol varied substantially, ranging from 260 to 847 μg/m^3, as shown in Table 2. Ammonium nitrate and OC were the most prevalent constituents of concentrated fine/ultrafine PM in Fresno, accounting for approximately 60–80% of the total PM mass. The highest particle mass concentration during the fall was observed in the first week, with this week also demonstrating the highest fall concentrations for individual PM species, especially nitrates and EC. Ammonium nitrate concentration was 24% of the total PM mass during the second week and 57% of the total PM mass during the third week of fall. EC concentration during the first week of fall was 10 times higher than during the next 2 weeks of exposure. OC concentration was relatively consistent over the 3-week fall period, ranging from 80 to 141 μg/m^3. The concentration of trace metals was
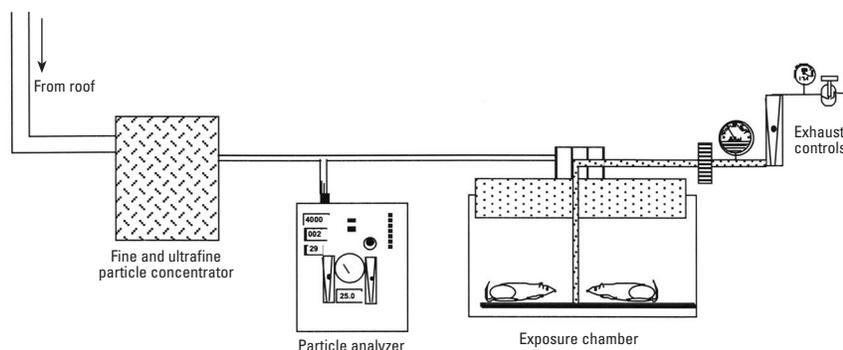

Figure 2. CAPs exposure system (see text for details).

similar during the first 2 weeks (38–42 μg/m³) but decreased to 14 μg/m³ during the third week. Figure 4 shows mass concentrations of the most predominant trace elements and metals, with Al, Si, S, Ca, and Fe having the highest concentrations. With the exception of sulfur, metal and trace element concentrations during the first 2 weeks of exposure were similar. An increase in sulfur concentration during the first week of fall may have been due to the greater ammonium sulfate concentration detected during the same week (Figure 3A),

because sulfur measured by X-ray fluorescence is also part of the ammonium sulfate measured by ion chromatography. The overall higher nitrate and sulfate concentrations for the first week of fall were most likely due to increased photochemical activity coupled with prevailing quasistagnant meteorologic conditions during this period.

The total mass and particle number concentrations measured during the winter 2001 exposures in Fresno (Table 2) were remarkably similar to those of the fall exposures. Average

total mass concentrations were 815, 190, and 371 μg/m³, for the first, second, and third weeks, respectively. Particle number concentration did not vary substantially for the 3 weeks of exposure, as indicated by Table 2. These concentrations were similar to those measured in the fall, varying from 0.9×10^5 to 1.2×10^5 particles/cm³. Nitrate and OC were the predominant PM constituents, accounting for 46–69% of the total CAPs mass each week. Nitrate and sulfate concentrations were substantially higher during the first week, compared with the second and third weeks of winter. The concentrations of combined trace elements and metals were 16, 14, and 8 μg/m³ for the first, second and third weeks of exposure, respectively. Sulfur concentration was appreciably higher during the first week, along with a higher ammonium sulfate concentration. CAPs concentrations for metals of primarily crustal origin, such as Al, Si, K, Ca, and Ti were similar during the first 2 weeks of exposure but lower during the third week (Figure 6). Concentrations of primarily
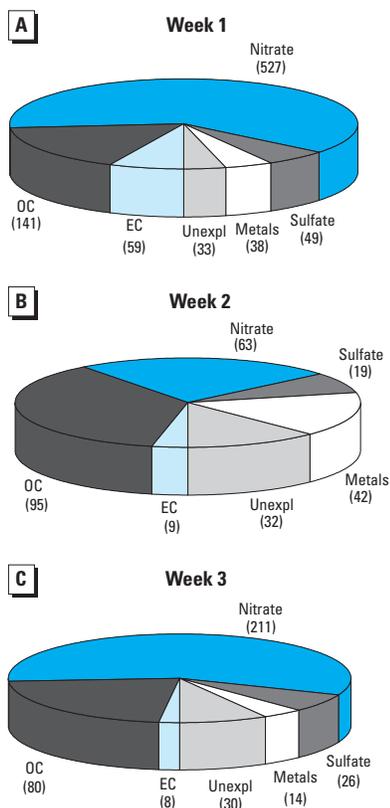

Figure 3. Chemical composition of CAPs during the first (*A*), second (*B*), and third (*C*) weeks of fall 2000 exposures. Numbers represent the average mass concentration (μg/m³) of each species measured over the 3-day period of exposure for each experiment. The unexplained fraction (Unexpl) represents that portion of the total particle mass not accounted for by chemical analysis.

Figure 5. Chemical composition of CAPs during the first (*A*), second (*B*), and third (*C*) weeks of winter 2001 exposures. Numbers represent the average mass concentration (μg/m³) of each species measured over the 3-day period of exposure for each experiment. The unexplained fraction (Unexpl) represents that portion of the total particle mass not accounted for by chemical analysis.
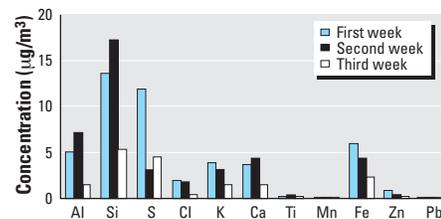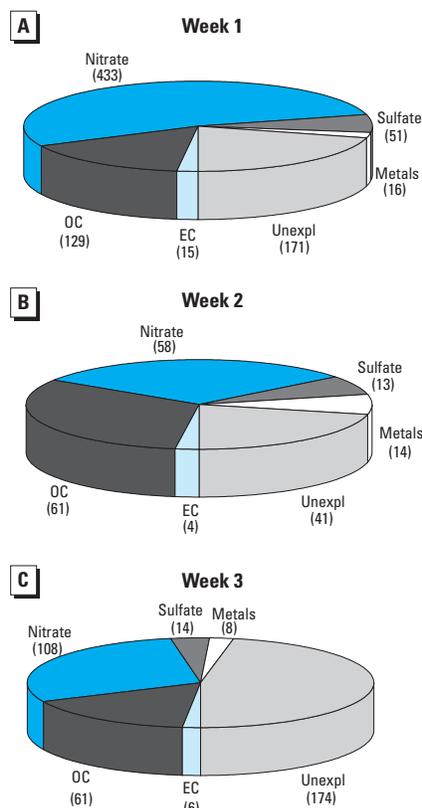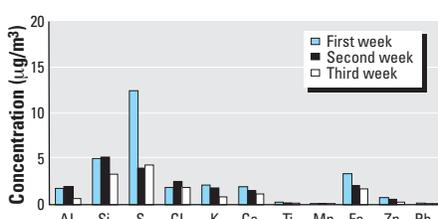

Figure 4. Average mass concentration of trace elements and metals in the exposure chamber during fall 2000 exposures. Bars represent the average mass concentration (μg/m³) of each element measured over the 3-day period of exposure for each experiment.

Figure 6. Average mass concentration of trace elements and metals in the exposure chamber during winter 2001 exposures. Bars represent the average mass concentration (μg/m³) of each element measured over the 3-day period of exposure for each experiment.

Table 2. Mass and number concentrations of CAPs.

Study	Mass concentration (μg/m³)	Number concentration (particles/cm³)
Fall 2000		
First week	847	1.2×10^5
Second week	260	1.2×10^5
Third week	369	1.1×10^5
Winter 2001		
First week	815	0.9×10^5
Second week	190	1.0×10^5
Third week	371	1.2×10^5

Table 3. CAPs means and coefficients of variation for 6 weeks of exposures in Fresno, California.

CAPs species and elements	Arithmetic mean concentration (μg/m³)[a]	Coefficient of variation
Number	1.10×10^{5b}	13
Mass	475	60
OC	94.5	36
EC	16.7	125
Nitrate	233	86
Sulfate	28.7	60
Al	3.0	83
Si	8.3	69
S	6.7	63
Cl	1.8	39
K	2.2	50
Ca	2.4	56
Ti	0.26	45
Fe	3.3	49
Zn	0.54	46
Mn	0.08	46
Ba	0.13	200
Ni	13[c]	63
Cu	130[c]	65
Cd	11.2[c]	245
Se	9.6[c]	136
Pb	91[c]	36

[a]Arithmetic mean concentrations shown are in μg/m³ except where indicated. [b]Concentrations are expressed in particles/cm³. [c]Concentrations are expressed in ng/m³.

combustion-generated metals, such as Fe, Zn, and Pb were similar during the 3-week exposure period (Figure 6).

Table 3 provides a summary of the CAPs mass and chemical and elemental composition during all 6 weeks of exposures. Each species (including particle number and mass) is represented by its arithmetic mean and coefficient of variation.

CAPs-induced BAL changes. Total BAL cells for each exposure week are shown in Figure 7A. Exposure to CAPs during the first week of winter was associated with a significant increase in the total number of cells recovered by lavage (1.2×10^5 cells/mL) compared with filtered air controls (0.8×10^5 cells/mL) (Figure 7A). The percentage of macrophages in the BAL fluid after exposure to filtered air or CAPs was greater than 99.3% or 98.8%, respectively (Table 4). The percentage of macrophages with CAPs exposure was only significantly reduced in the second week of the fall season (Table 4). Rats exposed to CAPs in the first week of the winter season had a significantly higher number of BAL macrophages compared with rats exposed only to filtered air (Figure 7B). There was not a significant change in macrophage number during the other 5 exposure weeks. In 5 of 6 exposure weeks, rats exposed to CAPs had an apparent increase in the number of neutrophils compared with rats exposed only to filtered air (Figure 7C). However, statistically

significant increases in the number of neutrophils were noted only in rats exposed to CAPs during the first week of fall (2.3-fold increase) and the first week of winter (3.0-fold increase) (Figure 7C). During these same 2 weeks, the highest levels of PM mass, nitrate, and OC were also observed. The percentage of BAL neutrophils was apparently increased in animals exposed to CAPs during five of six exposures in fall and winter, although statistical significance was achieved only in the first week of the winter season (Table 4). We also noted that lymphocytes during 3 of 6 exposure weeks displayed a trend of increased proportions, although statistically significant changes between control and CAPs groups were not attained (Table 4). CAPs exposure was also associated with a trend toward an increase in the total number of BAL lymphocytes in all 6 weeks (Figure 7D), although statistical significance was not achieved.

BAL cell permeability, an indicator of decreased membrane integrity, was measured for all 6 weeks of particle exposure (Figure 8). Permeable BAL cells from rats exposed to filtered air ranged from 6% to almost 11% (Figure 8). With CAPs exposure, the proportion of nonviable BAL cells increased significantly over matched control animals in all 3 weeks of the fall and in the third week of winter (Figure 8). As much as a 242% increase in nonviable cells over control values was

observed (Figure 8). Although the percentage of nonviable cells in the second week of winter was increased by exposure to CAPs, this did not attain a level of statistical significance.

Discussion

The intent of this study was to determine if CAPs, including both fine (< 2.5 μm) and ultrafine (< 0.1 μm) particles, would be cytotoxic and/or proinflammatory in the lungs of healthy adult rats. In this study we used a unique particle concentrator, which is able to concentrate particles in both fine and ultrafine sizes to identify specific biologic responses. During 6 weeks of particle exposure in Fresno, California, particle numbers were remarkably consistent, whereas the average mass of CAPs and nitrate, sulfate, and EC concentrations varied substantially.

The most striking findings of this study were consistent cytotoxic and inflammatory responses associated with exposure to CAPs. Although a clear dose-dependent response cannot be established to explain cellular changes such as neutrophil influx in the lungs as measured by BAL, the two highest mass concentrations observed during the course of these exposures were associated with a significant increase in numbers of total cells and/or neutrophils recovered from the lungs by BAL. Lung cytotoxicity measured in the BAL fluid of animals exposed to CAPs was also significantly increased during 4 of 6 exposure weeks, but this measure of cytotoxicity was not dependent upon particle mass. These CAPs exposures were for only 4 hr/day for 3 days, whereas humans are exposed to PM for more extensive periods of time, albeit at lower concentrations. All exposures were to the ambient mixture of particles present in Fresno.

Prior experiments with CAPs in other regions of the United States have also shown variable results in the induction of pulmonary inflammation. Rats exposed to concentrations of CAPs as high as 350 μg/m³ for 3 hr in Tuxedo, New York, did not show an increase in lung inflammation, either 3 hr or 24 hr after exposure (Gordon et al. 1998). However,
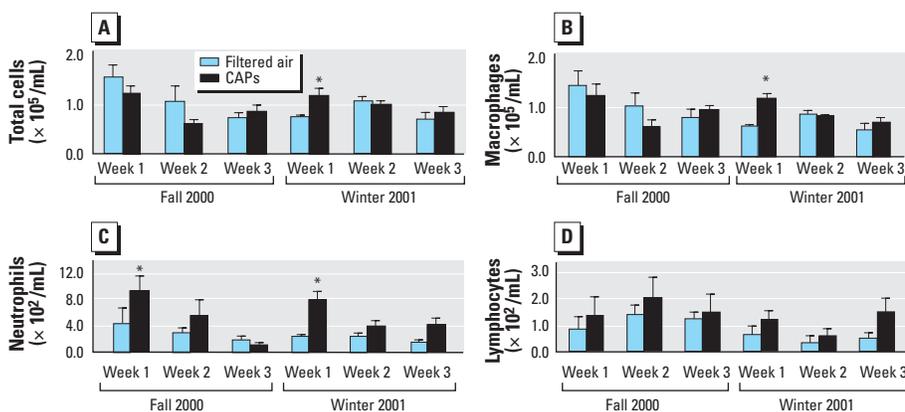

Figure 7. Total cells (*A*), macrophages (*B*), neutrophils (*C*), and lymphocytes (*D*) in BAL fluid for 6 weeks of exposures in Fresno. Error bars indicate SE.
*$p < 0.05$, compared with filtered air.

Table 4. BAL fluid cell differentials.[a]

Study	Percent macrophages		Percent neutrophils		Percent lymphocytes	
	Filtered air	CAPs	Filtered air	CAPs	Filtered air	CAPs
Fall 2000						
First week	99.7 ± 0.2	99.2 ± 0.1	0.3 ± 0.2	0.7 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
Second week	99.5 ± 0.1	98.8 ± 0.5[b]	0.4 ± 0.1	0.9 ± 0.4	0.2 ± 0.0	0.3 ± 0.1
Third week	99.6 ± 0.1	99.7 ± 0.1	0.3 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
Winter 2001						
First week	99.3 ± 0.3	99.3 ± 0.1	0.3 ± 0.0	0.6 ± 0.1[b]	0.1 ± 0.0	0.1 ± 0.0
Second week	99.7 ± 0.1	99.6 ± 0.1	0.2 ± 0.1	0.4 ± 0.1	0.0 ± 0.0	0.1 ± 0.0
Third week	99.6 ± 0.1	99.3 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.1 ± 0.1	0.2 ± 0.1

[a]Data are presented as mean ± SE (*n* = 6). [b]Significantly different compared with filtered air (*p* < 0.05).
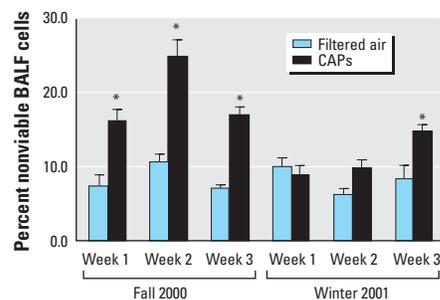

Figure 8. Percentage of nonviable cells in BAL fluid for 6 weeks of exposures in Fresno. Error bars indicate SE.
*$p < 0.05$, compared with filtered air.

a recent study described by Saldiva et al. (2002) demonstrated that short-term exposure to Boston, Massachusetts, CAPs induced an inflammatory response in the lungs of normal rats and in rats with chronic bronchitis. A modest increase in BAL neutrophils was reported in dogs exposed to CAPs in Boston (Clarke et al. 2000). CAPs have also been shown to induce mild pulmonary inflammation in healthy humans at concentrations as high as 311 µg/m^3 in Chapel Hill, North Carolina (Ghio et al. 2000). Preliminary studies in Los Angeles demonstrated that elderly humans exposed to CAPs had slight effects on blood oxygenation, cardiac arrhythmia, and airway inflammation (Gong et al. 2002a). Further studies demonstrated that exposure of healthy and asthmatic adult humans to fine CAPs in Los Angeles resulted in small acute effects (Gong et al. 2002b). The differences in response to CAPs by humans, dogs, and rats may be due to differences in composition of the particles inhaled in the different regions of the United States, differences in species sensitivity, and/or differences in ventilation rates. However, in each of these reported studies, ultrafine particles were not concentrated. Therefore, the entire range of particle sizes found in an ambient mixture was not concentrated in these previous studies.

Fresno is located in the San Joaquin Valley and has some of the highest PM$_{10}$ concentrations in the United States. Chow et al. (1992, 1993) has described the seasonal variability and physicochemical characteristics of PM in Fresno. During the winter, PM$_{10}$ has a dominant PM$_{2.5}$ fraction that consists primarily of carbonaceous constituents, ammonium nitrate, and ammonium sulfate. During the summer and early fall, PM$_{10}$ is elevated by windblown dust. These particles are dominated by fugitive mineral dusts primarily associated with coarse (2.5–10 µm) particles but also found in the fine fraction. Nighttime transport of particles via a jet stream from the San Francisco Bay area into the San Joaquin Valley during spring, summer, and fall further contribute to the makeup of pollutants found in the region (Smith et al. 1981a, 1981b). Pollutants can also accumulate during the winter because of periods of stagnation accompanied by strong inversions lasting as long as 20 days (Chow et al. 1992).

The mechanisms by which ultrafine particles may be more toxic than fine particles remain unknown, although a number of studies have provided insight into this problem. Ferin et al. (1992) demonstrated that when rats were exposed to ultrafine titanium dioxide (TiO$_2$) particles, pulmonary inflammation was induced. However, rats exposed to a mass of fine TiO$_2$ equal to that used in the ultrafine TiO$_2$ exposures did not develop lung inflammation (Ferin et al. 1992). In a recent study

(Donaldson et al. 2002) rats were exposed to ultrafine or fine carbon black particles, and only those rats exposed to the ultrafine particles exhibited an increase in BAL neutrophils. This increase in pulmonary inflammation in rats exposed to ultrafine carbon black was not due to trace amounts of redox-active transition metals present in the particles (Donaldson et al. 2002). Additional studies described by Donaldson et al. (2002) demonstrated a linear correlation between particle surface area and extent of lung inflammation. However, studies described by Smith et al. (2000) showed that induction of the proinflammatory cytokine interleukin-8 in human lung epithelial cells was dependent upon the amount of bioavailable iron in coal fly ash and not on surface area of the ash particles. Current theories on how particles, including those in the ultrafine size range, induce an inflammatory response in the lungs include either an oxidative stress or particle interactions with cell-surface receptors. Either of these possibilities may activate signaling in macrophages or epithelial cells, resulting in proinflammatory cytokine and chemokine production. Other possible explanations for the greater toxicity of ultrafine particles relative to fine particles include decreased ability of macrophages to phagocytose ultrafine particles (Kreyling and Scheuch 2000) or inhibition of phagocytosis by ultrafine particles (Donaldson et al. 2001; Lundborg et al. 2001). Renwick et al. (2001) demonstrated that ultrafine TiO$_2$ particles impaired alveolar macrophage phagocytosis to a greater extent than did fine TiO$_2$ particles.

In vitro investigations using human monocytes exposed to fine or coarse fractions of PM found an increased proinflammatory cytokine response with the coarse fraction but not with the fine fraction (Monn and Becker 1999). This cytokine induction was attributed to endotoxin present only in coarse particles. In the present study, the increase in neutrophils in the lungs of animals exposed to CAPs is not likely due to endotoxins because they are not typically found at significant levels in ultrafine or fine particle size fractions.

Loss of membrane integrity is a late event in the process leading to decreased viability and cell death (Tyson and Green 1987). Cell viability has not been routinely measured to date in studies to examine the effects of PM on the respiratory system. However, one study reported viability of human monocytes after treatment with extracts of PM (Monn and Becker 1999). Cytotoxicity was increased in cells treated with the coarse fraction of PM. This cytotoxicity was attributed to transition metals. However, the water-insoluble fraction of PM was not used to treat monocytes in this study; therefore, cytotoxicity due to insoluble metals or other components was not determined. The decreased viability of BAL cells in

our Fresno-based study may also be due to soluble or insoluble metals from ultrafine or fine CAPs. Transition metals in PM, such as iron, have been shown to catalyze the production of free radicals and lead to damage of biomolecules (Donaldson et al 1997; Prahalad et al. 2001; Smith and Aust 1997).

The total concentration of individual chemical PM species during the fall season exposures was 88–96% of the total mass concentration measured gravimetrically, indicating a mass balance near 100%. During the last week of the winter season, the combined PM chemical species concentrations accounted for only 53% of the total PM mass, leaving 47% of the CAPs mass unexplained. The discrepancy in the mass balance during this week may have been due to the uncertainty associated with conversion of the measured OC concentration to the actual OC concentration. OC concentrations measured by thermal desorption must be multiplied by a conversion factor to obtain the mass of organic particulate compounds. A value of 1.4 has typically been accepted with this factor stemming from limited theoretical and laboratory studies (White and Roberts 1977). However, additional studies suggest that this factor may be as high as 2.6 (Schauer 1998; Turpin and Lim 2001). In all 6 weeks studied, measured OC concentrations were multiplied by a factor of 1.4 to maintain consistency. However, it is likely that seasonal changes in Fresno may have affected the value of this conversion factor. If the remaining unexplained portion of the mass concentration is attributable to OC, we believe that the actual OC concentration during the third week of the winter season could have been as high as 115 µg/m^3. This difficulty in accurately measuring constituents of PM makes correlating particle components with health effects more problematic. Because OC has been associated with significant health effects (Nel et al. 2001), this uncertainty in OC measurement will require further research to improve its accuracy in determination.

Associations between OC or endotoxin and biologic effects are only two of many associations made between particle components and health effects. Increased pulmonary neutrophils in dogs exposed to CAPs have been correlated with Al and Si in CAPs using factorial analysis (Clarke et al. 2000). Smith et al. (2000) used three different types of coal fly ash, with the same mean aerodynamic diameter but different concentrations of iron, to demonstrate that bioavailability, rather than concentration of iron, was a predictor of proinflammatory cytokine induction in cultured human lung epithelial cells. Veranth et al. (2000) later determined that ferric iron associated with the aluminosilicate phase was the source of bioavailable iron in coal fly ash. It was further determined that bioavailable iron

was associated with combustion particles but not with crustal dust derived from soil minerals (Veranth et al. 2000). These findings emphasize that knowledge of particle composition may not always allow accurate prediction of resultant health effects.

Table 3 suggests that the most consistent particle characteristic for six separate experiments in Fresno was particle number concentration. Concentrations of OC, Cl, Ti, Fe, Zn, Mn, and Pb were also moderately consistent throughout the exposure period, with coefficients of variation less than 50%. In contrast, particle mass, as well as nitrate, sulfate, and EC concentrations, varied substantially over the 6-week period of exposure, with coefficients of variation ranging from 60% (for mass and sulfate) to 125% for EC. The concentrations of the remaining trace elements and metals (i.e., Al, Si, S, K, Ca, Ba, Ni, Cu, Se, Cd) also varied considerably, with coefficients of variation ranging from 50 to 250%.

Although each 3-day exposure over the 6 weeks of study varied in particle mass and composition, we were able to identify significant CAPs-induced respiratory effects in healthy adult rats produced by ambient particles in the Fresno area during both fall and winter seasons. It remains a challenge to assign specific biologic effects to a particular component or number of particles. However, additional studies in Fresno should help to determine if a consistent pattern of injury to the lung emerges as a result of exposure to well-characterized ambient particles based on particle size, composition, and season.

In summary, this study demonstrated that *a*) fine and ultrafine particles can be concentrated to study health effects; *b*) CAPs in Fresno produce respiratory change in healthy adult rats; *c*) respiratory changes could be measured during both the fall and winter seasons in Fresno; and *d*) respiratory changes based on cell viability are independent of mean particle mass concentration during these 3-day periods of exposure.

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