SESSION III

Health Risks from Diesel Engines Emissions

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RISK ASSESSMENTS OF DIESEL ENGINE EMISSIONS: CURRENT ISSUES

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INTRODUCTION

Diesel engine emissions are highly complex mixtures. They consist of a wide range of organic and inorganic compounds distributed among the gaseous and particulate phases. The potential health effects of long term exposure to diesel exhaust include cancer; cardiovascular disease and chronic lung disease. More recently there have been concerns about premature death in sensitive populations due to short-term exposure to particulate matter to which diesel emissions are important contributors. This presentation provides a brief overview of the potential health effects of exposure to diesel exhaust (reviewed by Health Effects Institute [HEI] in 1995) and discusses the approaches to cancer risk assessment taken by international, national, and state organizations.

EXPOSURE

It has been difficult to obtain accurate estimates of human exposure to diesel engine emissions because of their complexity, the contribution of other pollutants to the ambient air, and the changes in diesel emissions due to improved engine technology and fuel composition. In some occupations where equipment powered by diesel engines is used in enclosed spaces, the levels of diesel particulate matter have been estimated to range from 100 to 1,700 $\mu g/m^3$ (eight-hour averages). The estimates for most workplaces are from 1 to 100 $\mu g/m^3$; ambient exposures in the Untied States generally range from 1 to 3 $\mu g/m^3$, with occasional short-term peaks of 10 to 30 $\mu g/m^3$.

CARCINOGENICITY OF DIESEL ENGINE EMISSIONS

A number of constituents of diesel engine emissions have the potential to cause cancer. Diesel exhaust contains more than 35 chemicals that have been demonstrated to be mutagenic in bacterial and mammalian cells. At least 15 of these chemicals have also been shown to be animal carcinogens. Moreover, diesel engine emissions contain fine respirable particles and other irritants that could act as promoters.

The carcinogenic activity of diesel emissions has been convincingly demonstrated in rats. Nearly lifetime exposure for 35 hours or more per week to high concentrations of diesel exhaust particulate matter (2,000 to 10,000 $\mu g/m^3$) causes an exposure-dependent increase in the incidence of lung tumors in rats. Prolonged exposure to diesel emissions does not produce lung tumors in hamsters; the results in mice are equivocal. These findings suggest that species-specific factors play a critical role in the induction of lung tumors by diesel emissions.

Surprisingly, the particle-associated organic chemicals in diesel exhaust play little or no role in the development of lung tumors in the rat. Diesel exhaust acts like a number of other poorly soluble nonfibrous particles that produce lung tumors in rats by a mechanism involving lung overload. There is currently an active debate about the relevance of the rat as a model for hazard and risk characterization of such particulate materials. The International Life Sciences Institute - Risk Sciences Institute is preparing a report on this topic.

The epidemiologic data are consistent in showing a 1.2- to a 1.5-fold increase in the relative risk of lung cancer in workers exposed to diesel exhaust from older, uncontrolled engines. However, using the results of these studies to estimate human cancer risk is limited by a lack of quantitative exposure data. Exposures to diesel engine exhaust were generally assessed qualitatively based on job titles or years of vehicle use, rather than actual measurements of diesel exhaust.

CANCER RISK ESTIMATES

Several organizations have reviewed the epidemiologic, toxicologic, and experimental data on diesel engine exhaust and have classified (or proposed to classify) the mixture as a potential or probable human carcinogen. There is, however, considerable disagreement on the magnitude of that risk and how to characterize it. Much of the controversy revolves around whether either the animal or existing epidemiologic data can be used to predict risks in the general population.

In the 1980s qualitative risk assessments of diesel exhaust issued by the National Institute for

Occupational Safety and Health (1988) and the International Agency for Research on Cancer (1989) respectively classified diesel engine exhaust as either a potential occupational carcinogen or a probable human carcinogen. In 1996, the World Health Organization's International Programme on Chemical Safety (IPCS) also classified diesel exhaust as probably carcinogenic to humans. The IPCS noted that, in its view, no quantitative epidemiologic data were available for estimating human risk and developed a unit cancer risk estimate (3.4×10^{-5} for 1 $\mu g/m^3$) using the rat bioassay data. (This value, which gives the upper limit of risk, means that it is estimated that for every 100,000 people exposed to 1 ug/m^3 of diesel exhaust over a 70-year lifetime, 3 to 4 will develop lung cancer.)

More recently, the State of California Environmental Protection Agency has completed its Diesel Risk Assessment (California EPA, 1998). The agency concluded that "a reasonable and likely explanation for the increased rates of lung cancer observed in the epidemiological studies is a causal association between diesel exhaust exposure and lung cancer." The California EPA relied on epidemiologic data for railroad workers to develop cancer risk estimates and reported a range of unit cancer risk estimates (1.3×10^{-4} to 2.4×10^{-3}) with the "more scientifically valid unit risk values . . . near the lower end of this range." Using data on the annual ambient exposures to diesel exhaust (2 to 3 ug/m^3), staff estimated that the upper limit of potential additional lung cancer cases over a 70-year lifetime was 200 to 3,600 for every one million Californians. Based on this risk assessment, the California Air Resources Board identified diesel exhaust particulate matter as a Toxic Air Contaminant.

The U.S. Environmental Protection Agency (EPA) is also assessing the risks of exposure to diesel engine exhaust. The EPA issued draft health assessment documents for public comment in 1990, 1994, and most recently in February 1998. In the 1998 version, diesel emissions were considered to be a "probable human carcinogen." Although this designation is consistent with the Agency's earlier evaluations, the quantitative risk estimates and the procedures for developing them have evolved in response to emerging data on the mechanisms of diesel exhaust-induced lung tumors in rats. The EPA presented cancer risk estimates ranging from 1×10^{-5} (based on animal data) to 2×10^{-3} (based on epidemiologic data). The EPA Clean Air Science Advisory Committee (which has a legislative mandate to review and approve such documents) has returned the February 1998 draft to staff for revision.

In addition to the documents cited above, an evaluation of the carcinogenicity of diesel exhaust has been published by the Mine Safety and Health Administration (1998) and others are being developed by the American Conference of Governmental Industrial Hygienists and the National Institute of Environmental Health Sciences.

NEW RESEARCH INITIATIVES

As the above discussion indicates, a large body of evidence supports the qualitative assessment that there is an increased risk of lung cancer in workers exposed to diesel emissions. However, there are still significant uncertainties in using either the existing epidemiologic or animal data to develop quantitative estimates of the magnitude of that risk and to characterize the risk of ambient exposures. We need new epidemiologic and mechanistic data to reduce these uncertainties. At the present time epidemiologic studies of workers exposed to diesel emissions are underway in the United States (the National Cancer Institute/National Institute of Occupational Health study of nonmetal miners), Germany (potash miners) and Denmark (rural and urban bus drivers). The Health Effects Institute has also initiated a Diesel Epidemiology Project. As part of that project, an Expert Panel is evaluating the strengths and limitations of using existing epidemiologic data to develop cancer risk estimates for diesel exhaust and will make recommendations for the design of new studies. HEI is also funding new research to reduce the uncertainties associated with using human data for risk assessments of diesel engine exhaust.

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STATUS OF INFORMATION ON AMBIENT LEVELS AND SOURCE EMISSIONS TO THE ULTRA FINE FRACTION OF PARTICULATE MATTER

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A fundamental concept of exposure to environmental levels of diesel emission is the following: how many times a day you breathe diesel emissions and how do these compare to the levels and exposure from other sources of particles. How you come into "contact" with PM or any other pollutant will provide an opportunity to determine the relationship between the exposure to pollutants, and actual or potential health effects. For example, an asthma attack can be triggered in many ways. You can have an urban child playing on the kitchen floor in the middle of winter. The building boiler is inoperable, and the residents must use the stove for heating. In such situations, children with asthma have been shown to suffer attacks caused by exposure nitrogen oxide emissions from the stove, e.g., gas stove.[1] In contrast, a neighborhood in Houston, Southern California or New Jersey is exposed to ozone and probably particulate matter during the summer. On any given day when there is relatively high ozone, a proportion of the asthmatics will respond to the outdoor ozone exposure, and some will go to the emergency room or doctor.[2] Thus, there is more than one component of the pollution that can irritate the lung and it is essential to determine the total or incremental exposures from one or more pollutants which can be associate with or cause a health effect.

At this time we are unable to define the acute or long term health effects caused by ultra fine particles in the environment. This is partially due to the lack of information on the location, levels and accumulation patterns of ultra fine particles, and the frequency and intensity of exposures that result from contact with ultra fine particles. The following will briefly describe the current state of knowledge about levels of ultra fine particles in the atmosphere, and the types of information needed to establish patterns of human exposure to ultra fine particles.

Ultra Fine Particles

Ultra fine particles[1] may be produced in many microenvironments (outdoor and indoor), and can result from emissions of primary and secondary particles produced by a variety of sources. A simple characterization for such particles is the following items: 1) diameters less than 100 nanometers, 2) many thousands in the air at a given point in space and time, and 3) the largest numbers are found near the source. Included, are emissions for diesel engines and any other type of combustion sources.

However, ultra fine particles contribute little to the mass of the atmospheric aerosol.[3] Thus, we have a contradiction with respect to the new National Ambient Air Quality standard for fine particles (PM2.5).[4] It is based upon mass. Thus, if ultra fine particles cause health effects observed in urban areas of the US they will not be addressed using conventional control strategies. The basic reason is the large numbers of particles less than 0.1 um in diameter are not reflected proportionally by the measured mass. Therefore, if one is to have high exposure to ultra fine particles, the individual will inhale large numbers at locations near a source.

From the Figure 1, you can see that in contrast to number concentration, the volume (mass) concentration becomes higher in the accumulation mode, the range from 0.1 to about 1 um in diameter. What happens is that particles <0.1 um in diameter come together via number of physical and chemical processes to form particles in the accumulation mode. However, the accumulation mode particles reach a point where they stop forming larger particles because, the collision cross section becomes too small.

The deposition of ultra fine particles in the deep lung is greater than 70%, but only about 20% of the accumulation mode particles deposit in the same region of the lung.[5] The deposition of large coarse particles occurs in the nose. In addition, there is also the potential for having high deposition efficiency of ultra fine particles in the nose.[6] Now the nasal region is an important consideration because when you are close to a source, this will be one of the best opportunities for "contact" and high exposures to ultra fine particles and lead to efficient deposition in the respiratory system.

Ultra fine particles are formed by homogenous nucleation and heterogenous nucleation

processes, which lead to formation by the clustering of vapor molecules on embryo vapors, and by clustering of molecules on foreign substances or surfaces (particles), respectively.[1] Evaporation and nucleation of nanometer particles occur continuously, since clusters are formed and remain together only in favorable thermodynamic conditions: namely, a super saturated atmosphere. For heterogenous formation of ultra fine particles, a solid nucleus is at the center of the particle and vapor phase species condense on it. Species can also adsorb to a surface, and cause chemical reactions. For example, organic vapors condense and react on ultra fine particles.

Ultra fines are formed from diesel emissions and emissions form other combustion sources. A typical example would be in an urban area near a freeway with high traffic density. High exposure to ultra fine particles, can occur in situations where individuals are standing near the highway, or have a home or a school with open windows near the highway. The result would be exposure to particles that conform to the tri-modal distribution, shown in Figure 1: which contains ultra fine (nuclei) mode, the accumulation mode and the coarse mode particles. The material in the coarse mode would be derived from re-suspended road dust or erosion of soil. The coarse particles (which are greater than 2.5 um in diameter), may include materials that were deposited in the road dust from diesels, or gasoline power vehicles.[1]

Glenn Cass, et al, was one of the first groups to take ambient measurements of the ultra fine fraction.[7] The ambient ultra fine particles are composed of different organic and inorganic materials, including metals, elemental carbon, organic carbon (e.g. polycyclic aromatic hydrocarbon), and some sulfuric acid. One of the few examples of the chemical composition of ultra fine particles in the ambient atmosphere is shown in Figure 2.[7] Although it shows the general classes described above, about 25% is unknown. From the same study, Figure 3, it is also apparent that the levels of ultra fine particles can be influenced by the time of day, day of the week, and the mix of local truck and automobile traffic at a road side sampling location.

Ultra fine particles can also be emitted by sources in the home, including cooking, smoking, and space heaters (wood burning stove), Figure 4, and the room air number distribution peaks at 50 nm.[8] The amount of material emitted by sources in the home is not equivalent to roadway levels but an individual usually will be closer to these sources, and will be in "contact" for a much longer period of time. This does not discount the potential importance of diesel emissions into the ambient air, but it provides a cautionary note that other sources of ultra fine combustion particles can lead to exposure. However, we must again return to substantive issue: Is it the mass or, the number of particles, or the composition of ultra fine particles that are associated with exposures that may lead to health effects. It is a question that needs much more research.

Ultra fine particles can also be emitted by vacuum cleaners.[9] At first glance, one may think that there is little contact; However, hotel cleaning personnel may vacuum many hours a day on shift. Further about 5% of American households vacuum twice a day. Emission rates of ultra particles and fine particles are from the vacuum cleaner motors and the emissions can be high, since few vacuum cleaners have high efficiency filters located on the bag. They are caused by arching of the commutator brushes used on standard motors. These brushes are made of carbon and have organic binders. As shown in Table 1 and Table 2, the arching will lead to high ultra fine and fine particle emission rates, of greater then 10^{10} and 10^{9} particles/min., respectively. In this case, the uncertainty associated with health risks are associated the toxicity and the extent of "contact" with and exposure to the ultra fine particles. However, it's another source that is part of the equation of total exposure to the organic and inorganic components of ultra fine particles.

Fine particles will acquire ultra fine particles on their surface after coagulation. Coagulation occurs mostly between small and large particles because large particles provide a good target, and small particles will move past each other. Thus, the particles collide less frequently in the atmosphere. [1] Particles, greater than 0.2 um in diameter, collide with ultra fine and they accumulate the ultra fine particles. They will grow within the accumulation mode, and the net result is a larger particle which can remain suspended in the air for 2-5 days. Thus our concern is not just with the unattached ultra fine particles, but in many cases with the toxic constituents in the ultra fine fraction that attach themselves to a larger accumulation mode particle. The sources of the ultra fine particles can be direct combustion emissions secondary chemical reactions. As previously shown, Figure 2, the chemical composition of ultra fine particles suggests a mixture of ambient air sources, including diesel and automobile emission, and power plants.

An interesting chamber experiment conducted in our laboratory shows what happens when we

introduce ozone and reacted it with typical levels of indoor generated organic chemical. Homogenous reactions occur to form ultra fine particles which immediately coagulate to form fine particles. Ultimately, the ozone produced fresh fine particles from 0.1 to about 2 microns in diameter. Now this is a typical dark phase reaction for outdoor pollutants, but it was a simulation of emissions from a typical household air freshener with ozone levels that typically penetrate from outdoor air indoors. Therefore, outdoor air reacting with an indoor hydrocarbons can produce ultra fine and then fine particles. However, the composition then would be different from particle form by emissions from diesel engines. It is also an issue requiring further research.

Ultra fine particles exist as free entities near indoor and outdoor sources, and have highest numbers of any size fraction in the atmosphere. They have very short lifetimes, particle size, but can still have identifiable mass contributions near large sources, e.g., freeways. As shown in Figure 5, ultra fine particles emitted by diesel engines occur in the size range, from 10 to less than 100 meters in diameter.[10] This is also true for natural gas, propane gas, and wood burning stove, Figure 6 to 8.[6] Further, propane fuel used in many homes in southeastern United States for open space heating have ultra fine particle emissions in the same size range. For each household source the organic components of the ultra fine mode are unknown and need to be characterized for composition and eventually for exposure. Taking wood stove emissions, one step further, it can be seen in Figure 8 that the characteristics of ultra fine emissions change for the different phases of a wood stove burn cycle. In the start up phase and the intermediate phase, the emissions are the highest and in the final or shut down phase, the ultra fine particle emissions decrease. The emissions cycle is important for exposure because wood burning stoves leak and can be operated to release ultra fine emissions indoors and outdoors. Thus, people will be exposed to particle emissions from stoves when they're breathing indoor air and breathing outdoor air in neighborhoods surrounding their home.

In conclusion, the nature of the atmospheric levels of ultra fine particles is poorly characterized, but it is apparent that free ultra fine particles will be highest near the source; ultimately ultra fine particles will coagulate and form or attach to accumulation mode particles. Combustion processes emit primary ultra fine particles and ultra fine particles secondary condensation processes, and chemical reactions can produce ultra fine particles. Diesel engines as well as other outdoor combustion sources produce ultra fine particles, and indoor combustion sources will also produce ultra fine particles. Thus, any attempt to complete an exposure assessment for the general public or sensitive subgroups of the population will require defining the incremental contributions derived from indoor and outdoor exposures to fresh ultra fine particles or ultra fine particles attached to fine particles(accumulation mode) because of sources operated in specific types of locations, and for specific activities.

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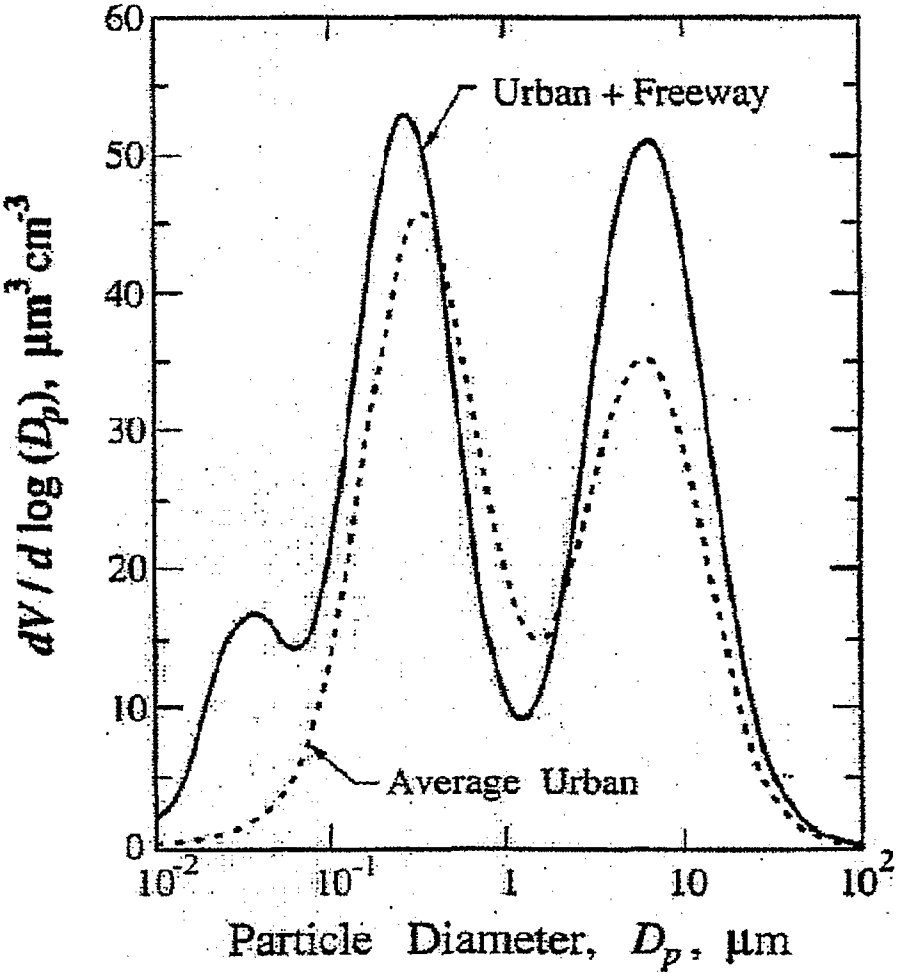


Figure 1 Aerosol volume distributions next to a source (freeway) and for average urban conditions.

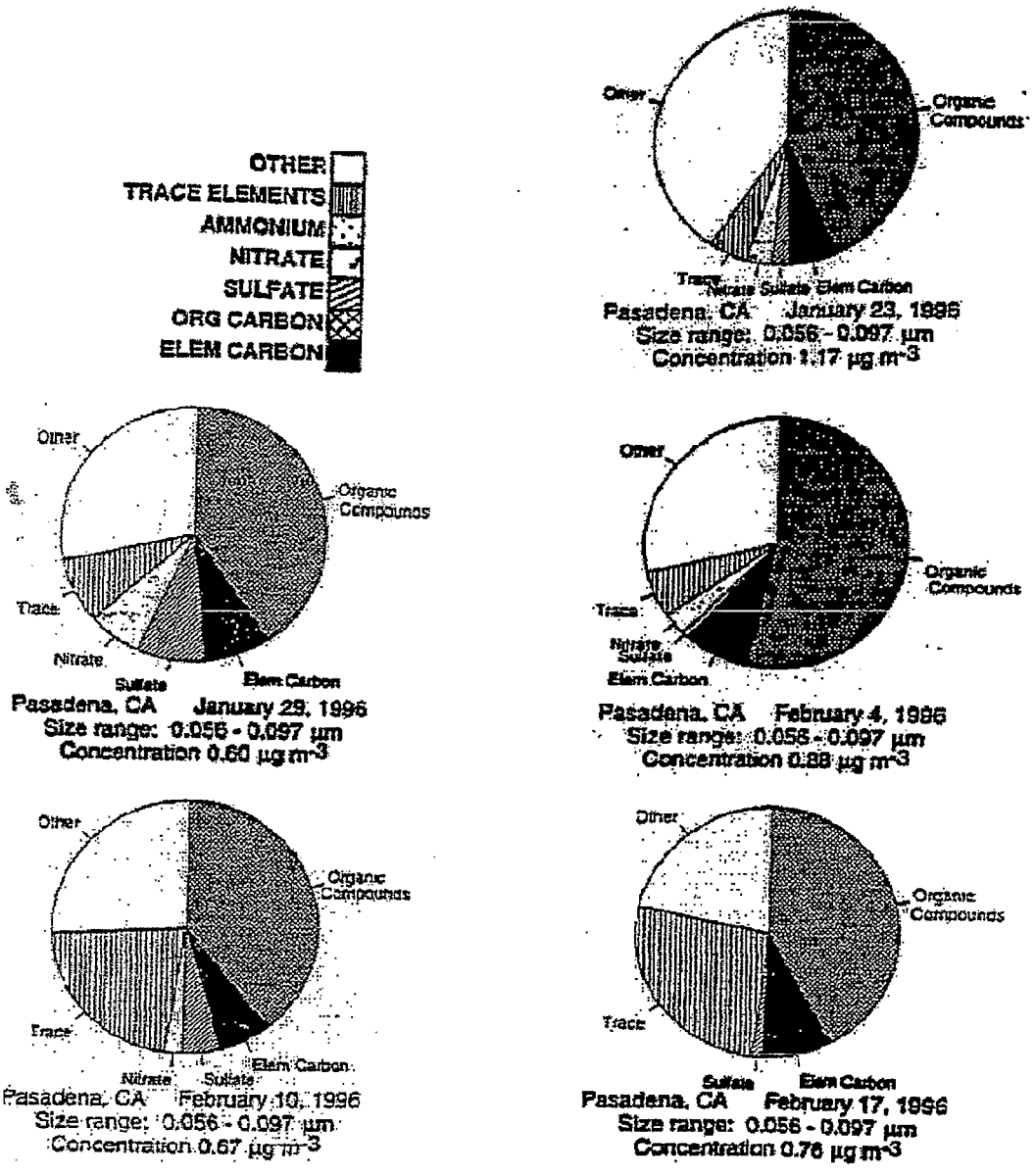


Figure 2 The chemical composition of wintertime ultra fine particles of size 0.056 <d_p <0.097 μm measured at Pasadena, CA. The most abundant trace elements other than NA and Mg are shown at the molecular weight of their common oxides.

L. S. Hughes, et al., Environmental Science & Technology, 1998, 32, 1153-

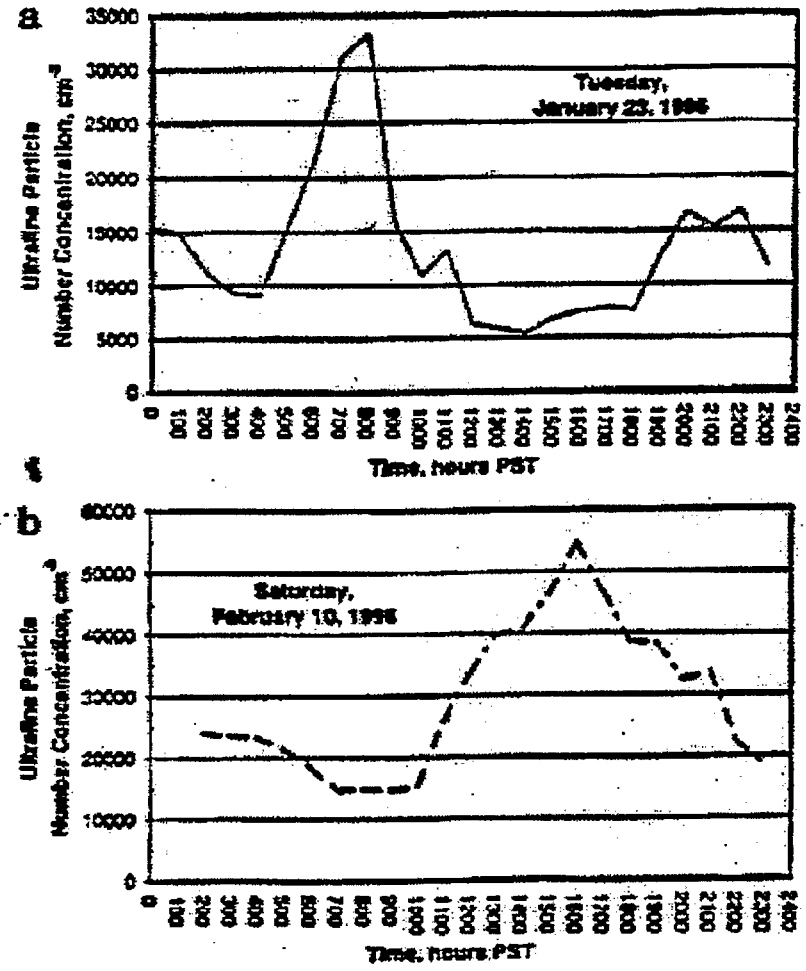
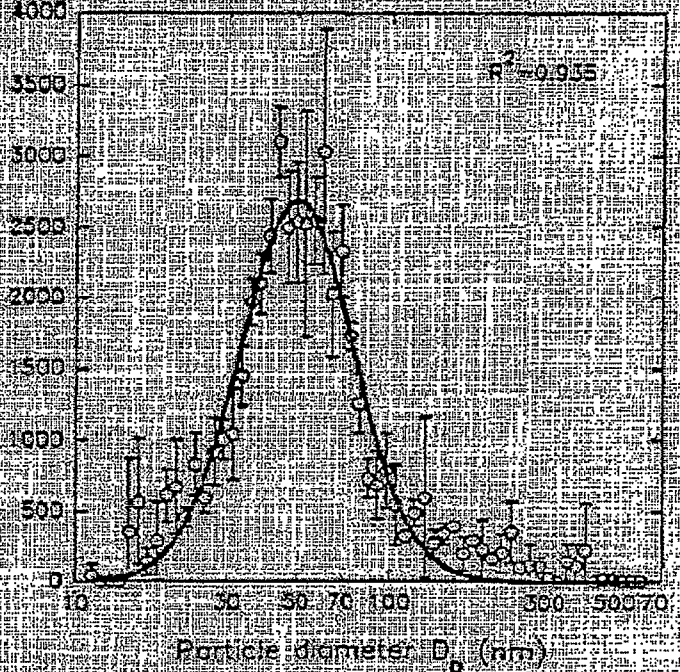


Figure 3 Time series of 24-h average ultra fine particle ($0.017 < d_p < 0.1$ μm) number concentrations, as measured by the DMA/CNC combination showing (a) Tuesday, January 23, 1996, with high concentrations near midnight and at the time of the morning traffic peak, and (b) Saturday, February 10, 1996, a day with early morning fog.

Particle source	Geometric mean (nm)	GSD
Main Stream	328.3	1.524
Side Stream	140.7	1.532
Candle Flame	37.37	1.608
Incense Smoke	198.0	1.729
Natural Gas Flame	19.46	1.363
Propane Flame	26.51	1.248

Figure(4a) Geometric Means and Geometric Standard Deviations for Indoor Combustion Aerosols.



4b) Room air particle size distribution.

W. Li and P. K. Hopke, Aerosol Science and Technology, 1993, 19, 305-318

ULTRA FINE EMISSION RATES			
Vacuum Cleaners	Total CF 0.01-0.3 μm	CF/min	UltraFines #/min
G	1.4×10^{9}	60.5	8.5×10^{10}
J_N	3.9×10^{8}	87.2	3.4×10^{10}
J_H	3.8×10^{5}	79.3	3.0×10^{7}

Figure 5a)

Lioy et al., Journal Air and Waste Management Association, 1998 (in press)

Total Emission Rates of Fine Particles (0.3 to 3.0 μm in diameter) from Motor Wear of Using Typical Operating Parameters of Vacuum Cleaners			
Vacuum Cleaners	Total Number of Fine Particles/min	Total Number of Particles $\frac{0.3-3.0\mu m}{min}$	Total Fine Mass (μg/min) (estimated) 0-3 to 3.0 μ m Diameter
A	3.34×10^{6}	2.83×10^{6}	68.2
B	1.87×10^{6}	1.61×10^{6}	74.7
C	0.48×10^{6}	0.34×10^{6}	18.7
D	0.61×10^{6}	0.50×10^{6}	6.2
E	0.12×10^{6}	0.079×10^{6}	6.5
F	0.29×10^{6}	0.26×10^{6}	3.6
G	2.88×10^{6}	2.18×10^{6}	176
H	0.48×10^{6}	0.40×10^{6}	127.1
I	0.38×10^{6}	0.29×10^{6}	128.8
$J_{N^{**}}$	0.91×10^{6}	0.74×10^{6}	31.3
$J_{H^{**}}$	0.00096×10^{6}	0.00081×10^{6}	0.028

* HEPA Filter

** (No HEPA Filter): Not Recommended operating condition

Figure 5b)

Lioy et al., Journal Air and Waste Management Association, 1998 (in press)

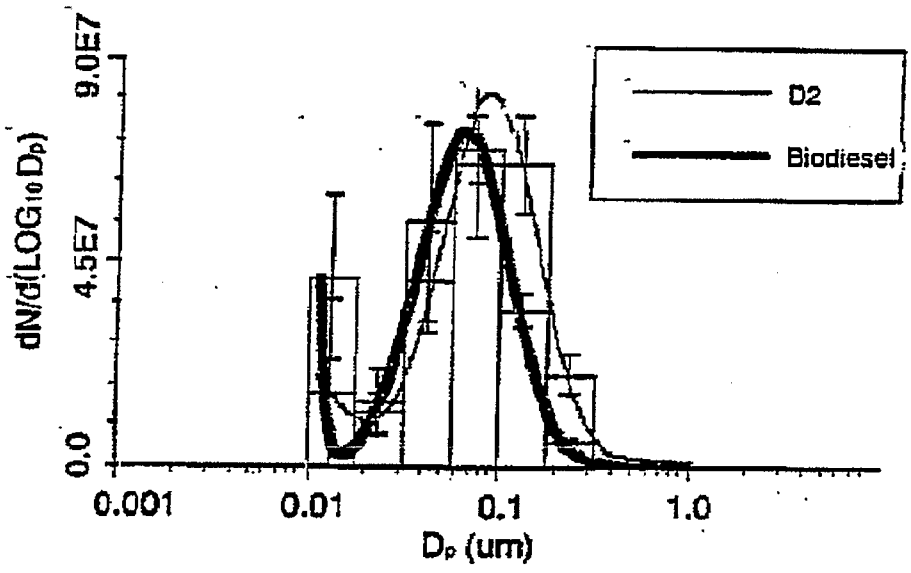
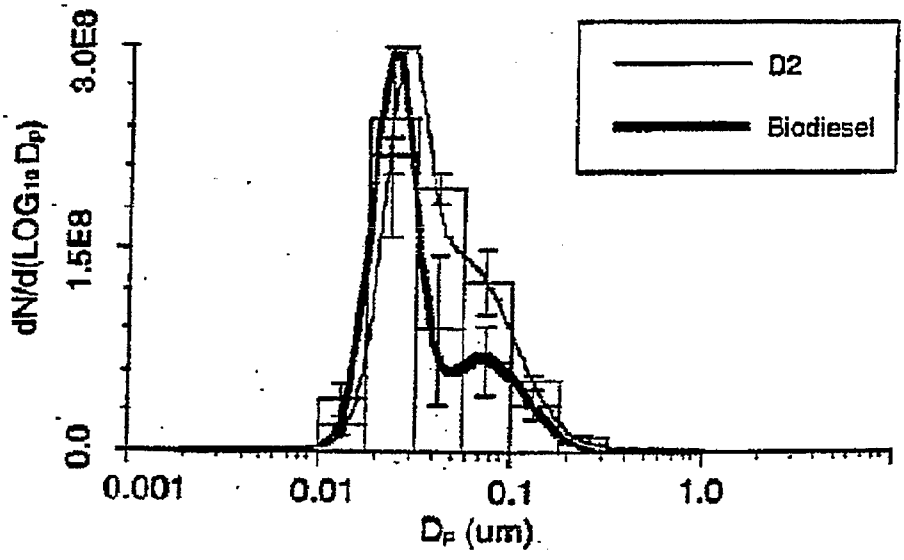


Figure 6a) Particle size number distribution for D2 and biodiesel fuels at 100% load, 1500 rpm.



6b) Particle size number distribution for the OCC with the D2 and biodiesel fuels at 100% load, 1500 rpm.

S. T. Bagley, et al., Environmental Science & Technology, 1998, 32, 1183-1191

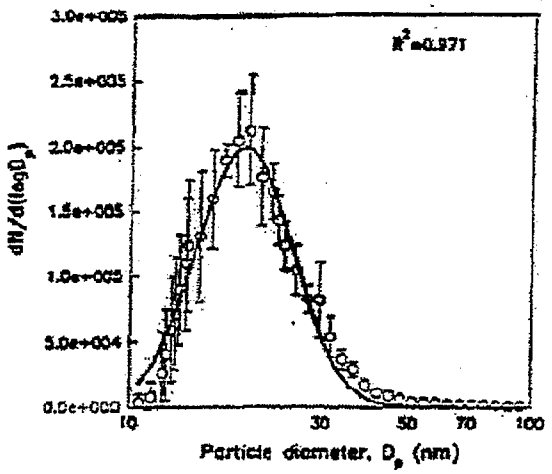


Figure 7 Natural gas flame particle size distribution.

W. Li and P. K. Hopke, Aerosol Science and Technology, 1993, 19, 305-316

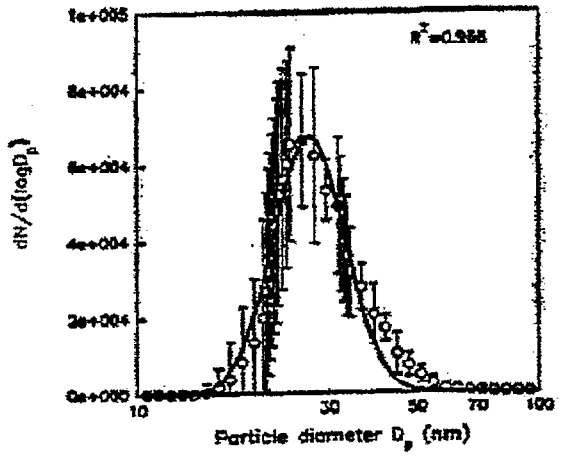


Figure 8 Propane fuel flame particle size distribution.

W. Li and P. K. Hopke, Aerosol Science and Technology, 1993, 19, 305-316

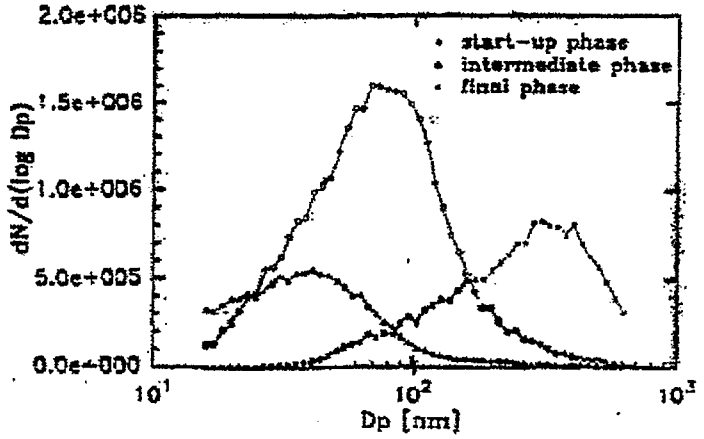


Figure 9 Particle size distribution in different phases of a wood stove burn cycle.

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RECENT EPIDEMIOLOGICAL EVIDENCE FOR HEALTH EFFECTS OF FINE AND ULTRAFINE PARTICLES

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Our quantitative knowledge of the health effects of particulate air pollution exposures can be traced back to the London fog episode of 1952. During the first week of December, a high pressure system settled over London, trapping coal emissions from domestic heating. Particle levels increased dramatically, reaching approximately 4500 $\mu g/m^3$ on the 7th (Figure 1). Particulate air pollution concentrations remained high until the 9th when a cold front passage brought concentrations back towards normal (although still high by today=s standards. Counts of daily deaths showed a similar rise and fall matching the air pollution exposures. This episode left no doubt that air pollution at high concentrations could be associated with acute increases in deaths and other health outcomes. In response, restrictions were placed on coal combustion in London, which ultimately led to

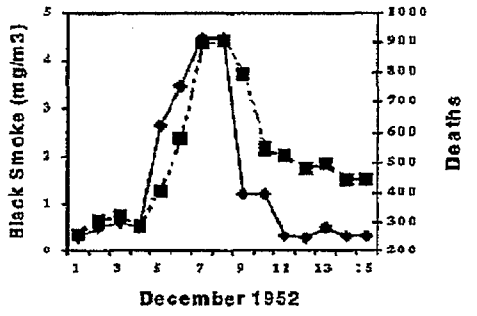

Figure 1. Black Smoke (◆) and deaths (■) by day for the first two weeks of December 1952, London, England.

much lower sulfur oxide and particulate air pollution exposures in the city.

In December 1991, there was another air pollution episode in London. As in the 1952 episode, an anticyclone had settled over southern England. Air pollution levels rose dramatically from the 12th through the 18th. Levels were considerably lower than the 1952 episode, and the mix of pollutants pointed to motor vehicle emission rather than coal burning. NO_2 concentrations reached a maximum 24-hour average of 198 ppb, more the twice the World Health Organization guideline of 90 ppb. Black smoke concentrations reached 148 $\mu g/m^3$ for 24 hours, also above the WHO guideline of 125 $\mu g/m^3$. Anderson and colleagues (1995) reported that daily deaths increased by 10% (95% CI 2% to 19%) during this episode, respiratory mortality increased by 22%, and cardiovascular mortality increased by 14%. Hospital admission of the elderly during the episode increased by 19% for all respiratory diagnoses, 36% for chronic obstructive pulmonary disease, and 97% for asthma. These results showed that traffic related air pollution can produce the same types of health effects observed with coal emissions earlier in this century.

Why are diesel emissions of specific concern as a potential health problem? Compared to automobiles, diesel engines produce much higher emission of particles and nitrogen oxides. The gas phase components of diesel emissions contains many irritants and toxins. Recent advances have reduced the mass of particles emitted from diesel engines, but have increased the numbers of particles being emitted. Hundreds of chemicals (including many known carcinogens and mutagens) are adsorbed onto the surface of these particles. If diesel particle controls are to be designed which most effectively reduce the potential for adverse health effects, information is needed regarding the specific characteristics of particles (mass, surface area, or number concentrations) most directly related to observed health effects.

The epidemiologic evidence for health effects of particles is largely based on measures of the mass concentration. This simply reflects the fact that almost all of the available particle data are mass measurements as required by Environmental Protection Agency regulations. However, the mass of particles is only one measure of the concentration of particles in the air, and may not reflect the concentration of the underlying toxic component of the particles. It may be that the important characteristic of particles in determining their toxicity is not the mass, but the surface area or the total number of particles. This has important implications for the size of the particles associated with health effects.

Particle number concentrations are dominated by the smallest size ranges, less than 50 to 100 nm (0.05 to 0.1 μm), frequently referred to as the *ultrafine* particles. Particle surface area is dominated by sub-micron size particles, 100 to 500 nm (0.1 to 0.5 μm). Particle mass is dominated by the larger particles, greater than 500 nm (>0.5 μm). Alternatively, particle toxicity may be defined by the composition, *e.g.* sulfate, acidity, or carbon concentrations.

In this paper we review the recent epidemiologic literature on the acute effects of particles with specific attention to indicators of particles most directly relevant to diesel emissions. In addressing the effects of acute exposures, we will not consider the carcinogenic effects, or the effects on chronic disease. No studies have direct measures of diesel exposure, but insights can be gained from analyses of studies of exposure to indicators of diesel emissions including studies of inhalable and fine particle mass, Black Smoke, ultrafine particle number, and traffic volume.

INHALABLE PARTICLES

Recent epidemiologic evidence has clearly implicated particulate air pollution as being associated with increased mortality, hospital admissions, emergency department visits, and symptom. Episodes of particulate air pollution are associated with exacerbation of chronic respiratory diseases such as asthma and chronic obstructive pulmonary disease (emphysema and bronchitis). Dockery and Pope (1994) summarized the observed associations of acute particulate matter (PM_{10}) exposures on various classes of respiratory illness (Table 1). More recent work has shown that particulate air pollution episodes are also associated with increased cardiovascular events including hospital admissions for ischemia, dysrhythmias, and heart failure (Burnett et al, 1995; Schwartz and Morris, 1995; Schwartz 1997).

TABLE 1. Estimated % change in health indicator for each 10 μg/m³ increase in PM_{10}. (after Dockery and Pope, 1994)

Increase in Daily Mortality	
Total Deaths	1.0%
Respiratory Deaths	3.4%
Cardiovascular Deaths	1.4%
Increase in Hospital Usage (Respiratory)	
Admissions	0.8%
Emergency Visits	1.0%
Exacerbation of Asthma	
Asthmatic Attacks	3.0%
Bronchodilator use	2.9%
Emergency Visits	3.4%
Hospital Admissions	1.9%
Increase in Respiratory Symptoms	
Lower Respiratory	3.0%
Upper Respiratory	0.7%
Cough	1.2%
Decrease in Lung Function	
Forced expired volume	0.15%
Peak expiratory flow	0.08%

Limited data are available on the effects of the mass concentration of smaller particles. Schwartz, Dockery, and Neas (1996) reported that daily mortality in six US cities was specifically associated with the mass concentration of particles less than 2.5 μm aerodynamic diameter ($PM_{2.5}$), rather than larger particles. Several studies have shown that lung function of children measured as peak expiratory flow rate is also specifically related to these smaller size ranges. Thus while most of the epidemiological studies have been based on PM_{10} measurements, limited data suggests these effects are specifically related to the smaller size ranges.

BLACK SMOKE

Black smoke is the most common measure of particulate air pollution in Europe. Particles are collected on a filter strip, and the "blackness" of collected particles is measured by optical reflectance. Historically, it was considered an indicator of coal combustion emissions, but today it is considered to be an indicator of diesel emissions. Indeed, diesel exhaust particles contain large amounts of elemental carbon, and

it is the elemental carbon which is the primary determinant of the blackness of the particles. Sampling near busy roads has shown that there is a high correlation between elemental carbon concentrations and the traditional Black Smoke measurements.

The APHEA (Air Pollution and Health: a European Approach) Study is a multi-center European collaborative assessment of the short-term effects of air pollution on health using time series methods (Katsouyanni et al, 1997). Daily mortality and hospital admissions data were analyzed in fifteen cities in ten European countries. In the western European cities (Athens, Barcelona, London, and Paris), daily mortality was associated with Black Smoke and SO_2 concentrations (Katsouyanni et al, 1997). Black Smoke and O_3 concentrations were associated with adults= respiratory hospital admissions in Amsterdam, Rotterdam, London, Paris and Barcelona (Spix et al 1998). Black Smoke and NO_2 were associated with hospital admissions for asthma in three cities (Barcelona, London, and Paris) (Sunyer et al, 1997). Table 2 shows the results for the analysis of particulate air pollution measured as Black Smoke.

Table 2. Estimated effect of each 10 μg/m³ increase in Black Smoke on health in APHEA Study.

	Increase (95% CI)
Daily Mortality	0.6% (0.4%, 0.7%)
Respiratory Hospital Admissions	0.7% (0.2%, 1.2%)
Asthma Emergency Visits	0.4% (-0.3%, 1.2%)

ULTRAFINE PARTICLES

Rudell and colleagues (1996) have reported the results of a controlled human exposure study in which human volunteers were exposed to filtered and unfiltered diesel exhaust. A particle trap removed 46% of the particle numbers, reducing the number concentration from 2.6 million to 1.4 million particles per cm³. Despite this halving of number concentration, there was not detectable reduction in the response to the diesel exposure as measured by symptom reports or measures of broncho constriction. This suggests that particle number concentration is not involved in these effects.

To date, there have been only two epidemiologic studies of the health effects of ultrafine particles as indicated by particle number concentrations. Both are winter studies of panels of subjects who performed daily measures of lung function (peak flow) and respiratory symptoms in Europe.

Peters and colleagues (1997) studied 27 adult nonsmoking asthmatics in Erfurt, Germany during the winter of 1991/92. Particle concentrations were measured as PM_{10}, estimated $PM_{2.5}$, and number concentrations in various size ranges. Evening peak flow measurements were significantly decreased in association with PM_{10}, $PM_{2.5}$, and number concentration less than 0.1 μm ($NC_{<0.1}$) (Figure 2). The authors interpreted these results as showing that ultrafine particle mass was most strongly associated with peak flow. However, there is no statistically significant difference in effect size for any of these particle exposure measures.

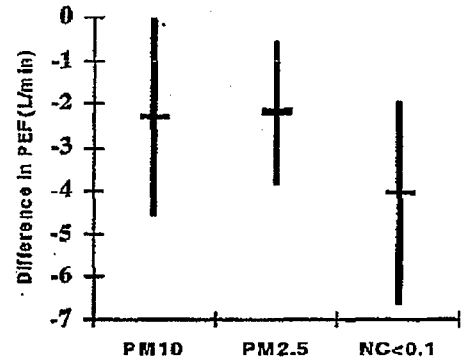

Figure 2. Estimated change in evening peak flow among asthmatics in Erfurt, Germany associated with a change equal to mean in PM_{10} (59 μg/N³, $PM_{2.5}$ (51 μg/m³, and $NC_{<0.1}$ (11,230 cm⁻³). (Peters et al, 1997)

Pekkanen and colleagues (1997) studied a panel of 39 asthmatic children in Kuopio, Finland during the winter of 1993/94. Daily peak flow and respiratory symptom reports were compared to particles concentrations measured as PM_{10}, Black Smoke, and number concentrations in various size ranges. Morning peak flow was significantly associated with PM_{10}, but not with Black Smoke nor either of the measures of ultrafine number concentrations ($NC_{<0.1}$ or $NC_{<0.32}$) (Figure 3).

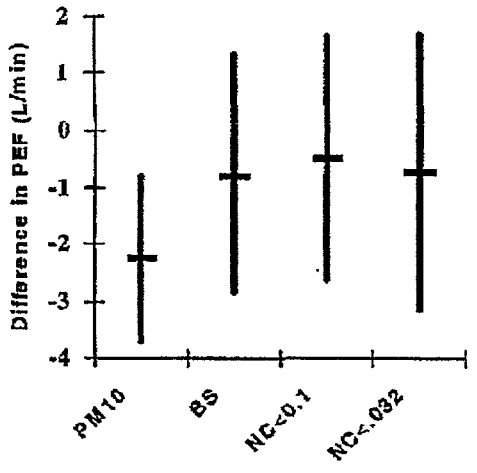

Figure 3. Estimated change in evening peak flow among asthmatic children in Kuopio, Finland associated with an interquartile range change in PM_{10} (13 $\mu g/m^3$), Black Smoke (10 $\mu g/m^3$), $NC_{<0.1}$ (20,700 cm^{-3}), or $NC_{<0.032}$ (13,170 cm^{-3}). (Pekkanen et al, 1997)

Thus while the authors of these studies come to apparently different conclusions with respect to the association of number concentrations of ultrafine particles with decreased peak flow in asthmatics, they both found significantly lower peak flow associated with PM_{10}.

Interest in the effects of ultrafine particles remains high in Europe, and there are three major studies currently underway.

- Analysis of daily mortality and ultrafine particle concentrations in Erfurt, Germany for period 1995-98 (funded by Health Effects Institute).
- Measurement of ultrafine exposures in Erfurt, Germany; Alkmaar, the Netherlands; and Helsinki, Finland in 1996/97 (funded by the European Union).
- Effects of ultrafine particle exposures on acute cardiovascular and respiratory endpoint in elderly patients in Finland, Germany, and the Netherlands during winter of 1998/99 (funded by European Union).

TRAFFIC VOLUME

Edwards and colleagues (1994) compared the traffic volume exposures of children admitted to hospital for asthma with those of patients admitted for other causes (and also community controls) in Birmingham, England. The relative odds for asthmatic patients increased with proximity to a major road and also with the amount of traffic on that road (Figure 4)

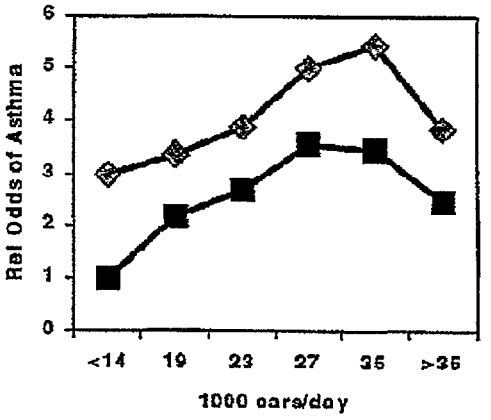

Figure 4. Relative odds for asthma hospital admission versus traffic volume on nearest major road by distance to road - ◆ <200 m from road, ■ >200 m from road. (After Edwards et al, 1994).

In 1993, Wjst and colleagues reported a study of the respiratory health of fourth grad school children in Munich compared to traffic volume. Counts of cars were determined for the street with the highest volume (7,000 to 125,000 cars per day) in each of the 117 school districts in Munich. Average lung function and respiratory symptom rates of fourth grade school children were compared to the district specific traffic volume, adjusting for parental asthma, smoking and education, plus the use of gas or coal for cooking and/or heating. Lung function was reported to decrease with increasing traffic volume (Figure 5).

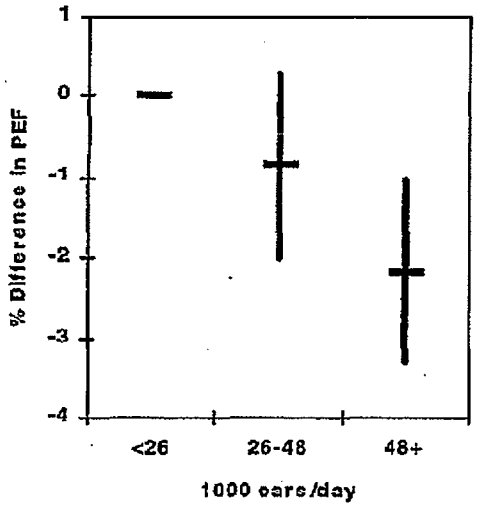

Figure 5. Percent difference in Peak Expiratory Flow (PEF) versus traffic volume within the school district in Munich (after Wjst et al, 1993).

Brunekreef et al (1997) reported a study of 877 school children, 7-12 years of age, living in six communities in the Netherlands. All children lived within one kilometer of a major roadway. Questionnaires and lung function tests were administered to all the children. Black smoke and NO_2 concentrations were measured in the schools. Average truck and automobile traffic volume on the nearby road was determined. The mean lung function of the children was found to decrease with Black Smoke and NO_2 concentrations. Mean lung function also decreased with truck traffic volume (Figure 6), but not with automobile traffic volume.

CONCLUSIONS

Short-term exposures to inhalable particles are consistently associated with increased daily deaths, hospital admissions, emergency room visits, exacerbation of asthma and chronic obstructive pulmonary disease, respiratory symptoms and decrease lung function. Black Smoke is a potential marker for diesel emissions and has been associated with increased daily deaths, hospital admissions, respiratory symptoms, and decreased lung function in epidemiological studies in Europe. The epidemiological data for effects of ultrafine particles is limited at present to two winter studies from Europe. One study suggests decreased lung function more strongly associated with number count than particle mass, while the other study suggests decreased peak flow more strongly associated with particle mass. European studies consistently suggest adverse health effects associated with measured traffic volume in the vicinity of subjects. The newest studies are pointing to a specific association with truck traffic.

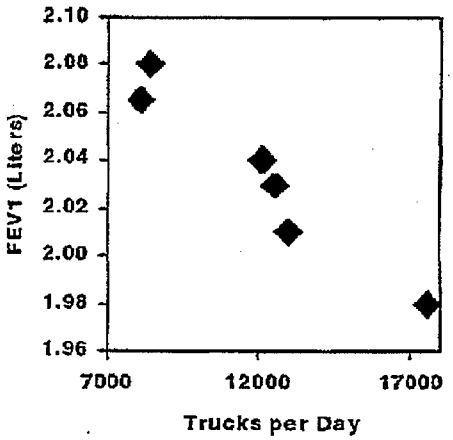

Figure 6. Adjusted lung function (FEV1) of school children in six communities in the Netherlands versus truck traffic on nearby roadways (after Brunekreef et al, 1997).

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TOXICITY OF ULTRAFINE PARTICLES

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Particles below 100 nm in diameter are regular constituents of the urban atmosphere. They are derived from a number of different combustion processes and from gas to particle conversions. Particles of this size range are defined as ultrafine particles, and such particles also occur in some occupational settings at the workplace. While workplace ultrafine particles such as fumes can be highly irritating and acutely toxic, ultrafine particles in the urban atmosphere seem to be more benign and potentially may cause effects only in susceptible subpopulations, but will not cause effects in the healthy organism at the existing low concentrations. However, although ultrafine particles occurring at the workplace are not appropriate surrogates for ambient ultrafine particles, the study of their behavior in terms of deposition, disposition and potentially also their effects may give some clues as to the potential of ambient ultrafine particles to cause effects in the compromised respiratory tract. Thus, this paper will describe toxicological findings of an extremely toxic ultrafine particle at the workplace, *i.e.*, polytetrafluoroethylene (PTFE, Teflon®) fumes, and results from studies with benign ultrafine particles, *i.e.*, carbon, as surrogates for urban particles.

Figure 1 shows the size distribution of a typical PTFE fume with a count median diameter of ~16 nm and a geometric standard deviation of 1.43. Rats exposed to these fumes at a concentration of ~50 $\mu g/m^3$ for 10-15 mins. develop within 4 hrs. post-exposure severe breathing difficulties with marked pulmonary edema and influx of a large number of polymorphonuclear cells into the alveolar space as well as protein exudation and red cell extravasation (Seidel *et al.*, 1991; Warheit *et al.*, 1990; Oberdörster *et al.*, 1995). Since PTFE fumes in addition to particles also contain gas phase compounds such as HF, the question as to whether ultrafine particles alone can cause these effects was explored in studies in which groups of 10-wk. old rats were exposed either to the gas phase alone or the particles alone, or the total fume (gas + particle phase) (Oberdörster *et al.*, 1997). Sham-exposed animals served as controls. As can be seen in Figure 2, gas phase components alone or ultrafine PTFE particles alone did not cause significant inflammatory responses as determined by the absence of increased neutrophils and protein in lung lavage 4 hrs. after exposure, only the total fumes caused the previously described very high inflammatory response in the lungs. Measurements of fluoride in the gas phase showed that both in the group exposed to the total fume and the group exposed to the gas phase only about 4 μg F/liter could be measured, whereas in the particle only group fluoride levels were near background. This result indicates that both the gas phase compounds as well as the ultrafine particles are necessary to cause the extreme toxicity of PTFE fumes. This may suggest that the ultrafine particles due to their high surface area can act as a carrier for gas phase constituents, which otherwise are absorbed in the upper respiratory tract and do not reach the deep lung, or reach the lower respiratory tract only when inhaled at 100-fold higher concentrations (Stavert *et al.*, 1991).

Obviously, PTFE fumes are not a surrogate for ambient ultrafine particles, yet certain characteristics are common to all ultrafine particles. One is the coagulation behavior of ultrafine particles which present an important mechanism for particle number reduction. Figure 3 shows a typical trimodal particle size distribution of the urban aerosol, with a nucleation (ultrafine) mode, an accumulation mode and a coarse particle mode. Ultrafine particles tend to coagulate onto themselves *via* homogeneous coagulation if a certain number concentration has been reached (Hinds, 1982) but a more important mechanism is heterogeneous coagulation of ultrafine particles onto accumulation mode particles which is a 10-100 times faster process than homogeneous accumulation (NRC, 1979). This mechanism of heterogeneous coagulation is extremely important for keeping ambient ultrafine particles at a low level. A reduction of accumulation mode particles by specific measures to clean the urban air may in fact increase the persistence and, thereby, number concentration of ultrafine particles. Such an increase in ultrafine particle concentrations has been reported for the city of Erfurt, Germany, following successful clean-up measures to reduce fine particle mass (Tuch *et al.*, 1997). Our results with laboratory-generated ultrafine carbon

particles demonstrate the homogeneous coagulation of these particles when they are left at a high initial concentration of $1x10^7$ particles/cm^3 in an animal exposure chamber with no flow (Fig. 4). Within minutes the initially ~30 nm particles coagulate to a larger size above 100 nm.

Coagulation of ultrafine particles and formation of larger particles may also impact on the health effects of these particles. Schwartz and Marcus (1990) summarized daily mortality data from the London pollution episodes from 1958 to 1972 and found a curvilinear relationship between particle mass concentration and daily mortality: At low concentrations, below ~130 μg/m^3, the slope of the exposure-response curve was steeper, whereas above this concentration the response curve flattened, seemingly showing a lower toxicity rate per particle mass at higher exposure concentrations (Fig. 5). This result, however, is consistent with a greater potential for ambient ultrafine particles to cause adverse effects per given mass since there can be many more ultrafine particles present at low total mass concentrations, whereas at higher mass concentrations ultrafine particle numbers are quickly reduced by the aforementioned mechanism of heterogeneous coagulation. Although this phenomenon provides a plausible explanation for the curvilinear exposure-response relationship, other interpretations of these exposure-response data may exist as well.

Generally, the mass concentration of ultrafine urban particles is very low at about 2-5 μg/m^3 but with a high number concentration of $2\text{-}4x10^4$ particles/cm^3 (Brand *et al.*, 1992; Hughes *et al.*, 1998). However, during episodic events, ultrafine particles can reach extremely high values, up to $3x10^5$ particles/cm^3, with a concomitant mass concentration approaching 40-50 μg/m^3 (Brand *et al.*, 1992). Figure 5 shows the result of measurements of ambient aerosol particles of the Brand *et al.* (1992) study in Frankfurt, Germany, before, during and after an episodic event, demonstrating that the mass concentration of the ultrafine and accumulation mode particles were about the same during the event with an average total mass concentration of ~100 μg/m^3.

Evidence that ambient ultrafine particles or surrogate ultrafine particles may have significant adverse effects comes from both epidemiological and toxicological studies. Peters *et al.* (1997) studied the association between fine and ultrafine particles and respiratory health in 27 non-smoking asthmatics in the city of Erfurt, Germany. They found that the effects showed a stronger association with ultrafine particles than with particulate matter smaller than 10 μm. Their study coincided with the aforementioned increase in ultrafine particle number concentration in the city of Erfurt after initiation of clean-up measures. However, another epidemiological study (Pekkanen *et al.*, 1997) did not find a stronger effect of ultrafine particles than larger-sized particles on respiratory parameters in asthmatic children. Thus, epidemiological studies are not yet conclusive regarding the potential adverse effects of urban ultrafine particles.

There are a number of potential mechanisms that can contribute to an increased toxicological potency of ultrafine particles compared to larger-sized particles. These mechanisms relate largely to dosimetry and include a high particle number per mass for ultrafine particles as well as a large surface area per given particle mass (Table 1). Specifically, the larger surface area can act as a carrier for radicals or reactive groups and gases to be administered to the deep lung. Furthermore, deposition of the inhaled singlet ultrafine particles is very high in the lower respiratory tract. As shown in Figure 6, predicted deposition of 20 nm particles can be up to 50% in the alveolar region of the human respiratory tract, and deposition in the tracheobronchial region can also be very high. In fact, if the large difference in surface areas of the conducting airways and of the alveolar region is taken into account, deposited dose per unit surface area is several-fold higher in the tracheobronchial region compared to the alveolar region for inhaled ultrafine particles. After deposition, the disposition of ultrafine particles also appears to be different from larger-sized particles in that a fast penetration to epithelial and interstitial sites occurs (Stearns *et al.*, 1994). This fast penetration of ultrafine particles can even reach endothelial sites and thus the vascular component so that some ultrafine particle translocation after inhalation exposure to remote organs such as the liver has been observed (Kanapilly and Diel, 1980).

These dosimetry-related characteristics predict a higher toxicity per given mass for inhaled ultrafine particles. Indeed, our studies with ultrafine (~20 nm) and fine (~250 nm) TiO_2 particles confirmed the greater toxicity per given mass for the ultrafine particles. Figure 7 shows dose-response curves with respect to lavagable neutrophils in rats 24 hrs. after intratracheal instillation of doses ranging from 32 μg to 500 μg for ultrafine TiO_2 particles and 125 – 2,000 μg for fine TiO_2 particles. It is evident from Figure 7 that the dose-response relationship for the ultrafine particles is much steeper than that for the fine particles, a finding which might be compared to the different slopes

Table 1. Toxicological Importance of Ultrafine Particles

NUMBERS AND SURFACE OF MONODISPERSE PARTICLES OF UNIT DENSITY OF DIFFERENT SIZES AT A MASS CONCENTRATION OF 10 $\mu g/m^3$

Particle Diamter μm	Particle Number per cm^3 air	Particle Surface Area, μm^2 per cm^3 air
0.02	2,400,000	3,016
0.1	19,100	600
0.5	153	120
1.0	19	60
2.5	1.2	24

of the exposure-response relationship of the epidemiological data shown in Figure 5. However, if the dose of the administered TiO_2 particles is expressed as particle surface area, the dose-response relationship of both the ultrafine and fine TiO_2 fall on the same curve as shown in Figure 8. Similar results have been observed by Li *et al.* (1996) who found significantly greater effects of ultrafine compared to fine carbon particles upon instillation of 100 μg into rats.

Given these results, it is tempting to predict the pulmonary inflammatory potential of nucleation mode particles relative to accumulation mode particles in humans. If one assumes that the composition of the two particle types is the same and, furthermore, that the resulting toxicity is proportional to the deposited dose when expressed as particle surface area (as shown with fine and ultrafine TiO_2 and carbon particles), one can deduce that the relative predicted toxicity of the ultrafine particles is more than 30-fold greater per given mass than that of accumulation mode particles (Table 2). This toxicity factor is composed of a factor for alveolar deposition and a factor for increased surface area of ultrafines. Expressed in terms of ambient particle mass concentration this means that 10 $\mu g/m^3$ of ambient ultrafine particles are toxicologically equivalent to 360 $\mu g/m^3$ of accumulation mode particles. Further studies are needed to test this prediction of the relative toxicities of inhaled ambient nucleation and accumulation mode particles.

Several factors can modify responses to inhaled ultrafine particles. These include age, disease, co-pollutant exposure, and pre-exposure history. We have performed several studies to evaluate these factors with laboratory-generated ultrafine carbon particles. The size distribution of these particles generated by electric arc discharge is shown in Figure 9, with a count median diameter of 24 nm and a geometric standard deviation of 1.86. We have also generated ultrafine platinum particles by the same methodology and exposed 18-month old and 8-week old mice with moderate pulmonary emphysema (induced by instillation of elastase) for 6 hrs. at a concentration of ~115 $\mu g/m^3$ to both particle types. A small, yet significant, increase in leukocytes in lavage fluid 24 hrs. after exposure was seen only in the old mice, but not in the young mice (Fig. 10). Healthy, non-emphysematous old mice, however, did not show any response to these types and concentrations of ultrafine particles. In another model of genetic emphysema in mice (Tsk mice, developing mild to moderate emphysema by about 12-months of age), preliminary results indicate that these mice also show a slight increase of inflammatory cells in lung lavage, whereas 18-month old healthy mice do not (Fig. 12) (Oberdörster *et al.*, 1998). Further studies are necessary to validate this mouse strain as a useful model for people with chronic obstructive pulmonary disease.

Epidemiological studies have shown that increased morbidity and mortality can occur in the elderly population with a compromised respiratory tract such as pneumonia. Using inhalation of endotoxin as a model for inflammatory events induced by a bacterial infection in the lung, we could show that such endotoxin-priming of the

Table 2. Relative Toxicity of Ultrafines vs. Accumulation Model

Accumulation- *vs.* Nucleation-(ultrafine) Mode Particles: Pulmonary Inflammatory Potential in Humans

Assumption:

- ***Composition of 2 particle types is the same***
- ***Toxicity is proportional to deposited dose, expressed as particle surface area***

(example: fine and ultrafine TiO_2)

	Accumulation Mode Particle (~250 nm)	*Ultrafine Particle (~20 nm)*
Rel. alveolar deposition	*1*	*3.6*
Rel. particle surface area	*1*	*10*
Rel. predicted toxicity	**1**	**36***

10 µg/m³ u.f. ⟶ 360 µg/m³ accum. mode

*Additional factors need to be considered:

Increased interstitial translocation ⟶ extrapulmonary effects

respiratory tract caused a significant increase of pulmonary inflammatory parameters by instillation of 50 μg of ultrafine TiO_2, but not after instillation of 50 μg of fine TiO_2. Furthermore, using the same endotoxin model to sensitize the respiratory tract, we observed in rats that co-exposure of ultrafine carbon particles with an oxidant pollutant, ozone, caused a significant increase of the inflammatory response. Statistical analysis of the results confirmed that, indeed, inhaled singlet ultrafine carbon particles do have a significant effect at a concentration of ~105 $\mu g/m^3$ inhaled over a 6-hr. period in rats. Although this concentration appears high, 100 $\mu g/m^3$ for the rat is approximately equivalent to 50 $\mu g/m^3$ for humans in terms of the deposited dose in the alveolar region. The increased effect of a combined ultrafine particle/ozone exposure may be due to the aforementioned carrier mechanisms of ozone absorbed onto the large surface area of even low mass concentrations of ultrafine carbon particles.

Finally, the importance of pre-exposure history on the effects of ultrafine particles needs to be addressed. This can be demonstrated with the highly toxic ultrafine particles of PTFE fumes introduced at the beginning of this paper. A 15-min. exposure of rats at an ultrafine PTFE particle concentration of 50 $\mu g/m^3$ results in severe damage and high mortality by 4 hrs. post-exposure. However, animals receiving for three days a 5-min. exposure each day of the same PTFE fume concentration developed complete tolerance and showed no pulmonary inflammatory response on day 4 after a 15-min. normally lethal exposure (Oberdörster *et al.*, 1995).

In summary, urban ultrafine particles occur in high number but generally low mass concentrations, they coagulate onto accumulation mode particles which is an important mechanism to reduce their number concentration. Results from toxicological studies show that ultrafine particles per given mass induce significantly greater inflammatory responses than accumulation mode particles; their large surface area also may act as a carrier for ambient gaseous pollutants. Deposition models predict that ultrafine particles have a very high deposition efficiency in the lower respiratory tract, and available data indicate that after deposition rapid translocation to epithelial and interstitial sites can occur. At low mass concentrations these ultrafine particles are unlikely to cause effects in the healthy organism, either young or old. However, they may elicit an increase in alveolar inflammatory responses in the compromised aged organism, in the sensitized respiratory tract and in combination with gaseous pollutant co-exposures such as

ozone. The response to ultrafine particles may also highly depend on pre-exposure history which may confer resistance to those particles.

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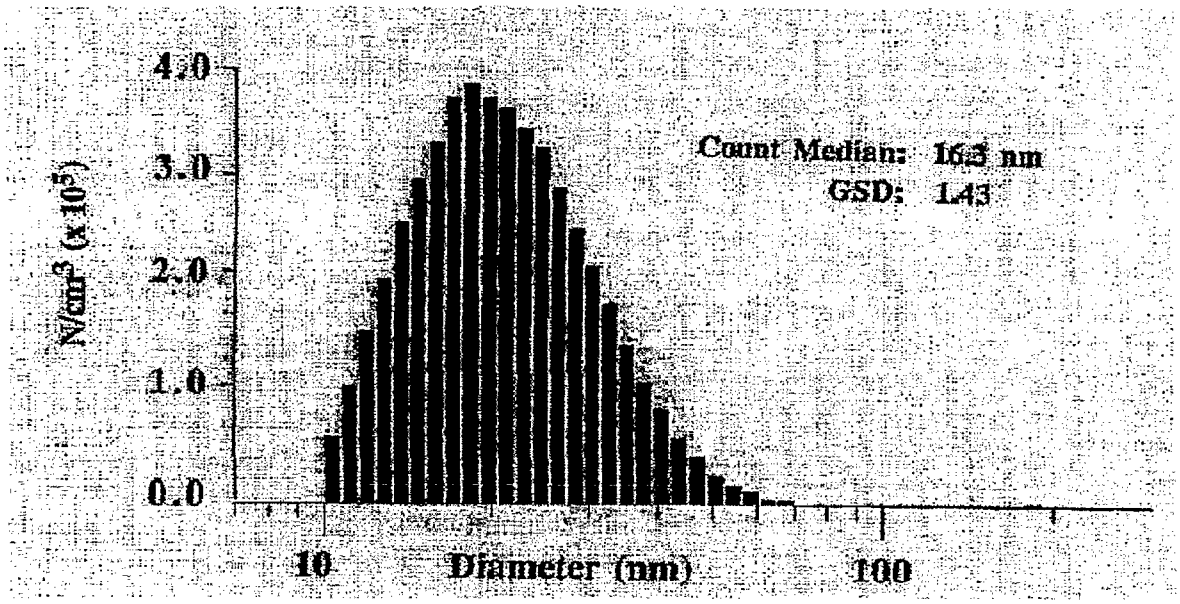

Figure 1. PTFE Fume Particle Size Distribution

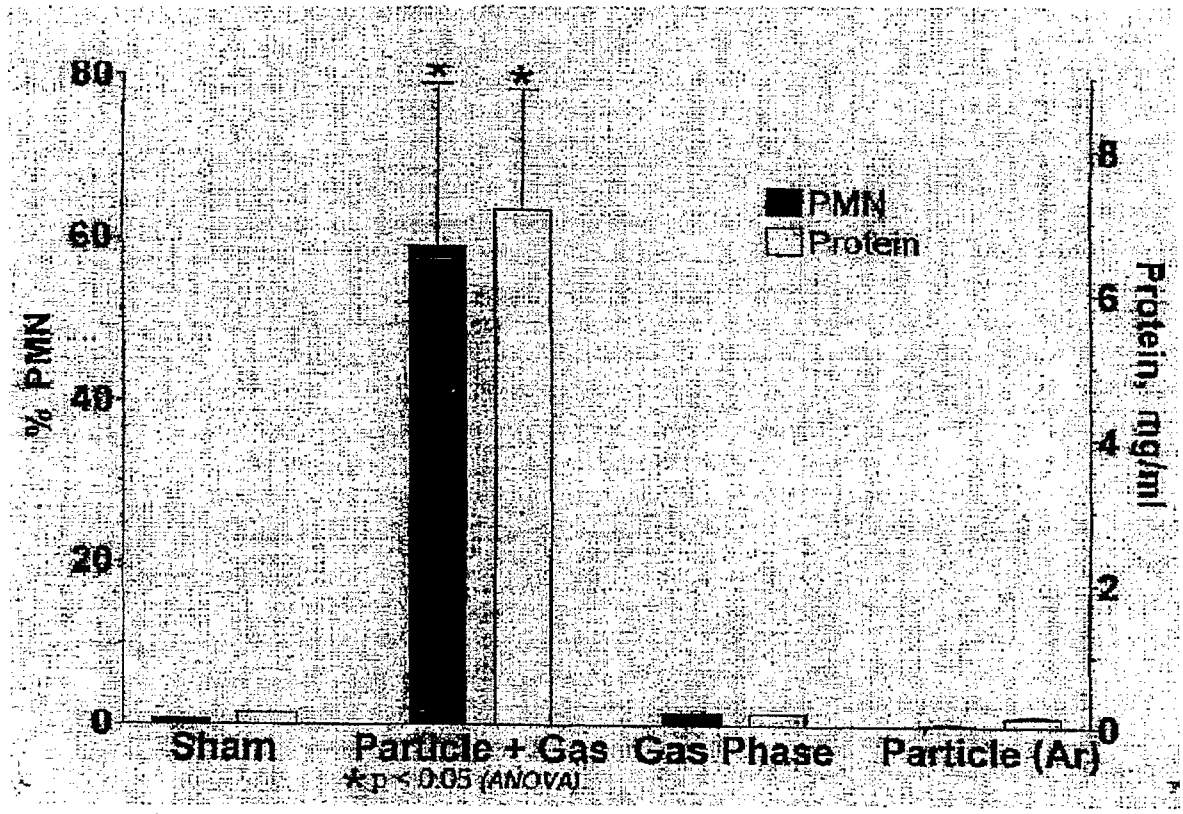

Figure 2. Ultrafine PTFE Fume Exposure: Inflammatory Cells (Neutrophils) and Protein in Lung

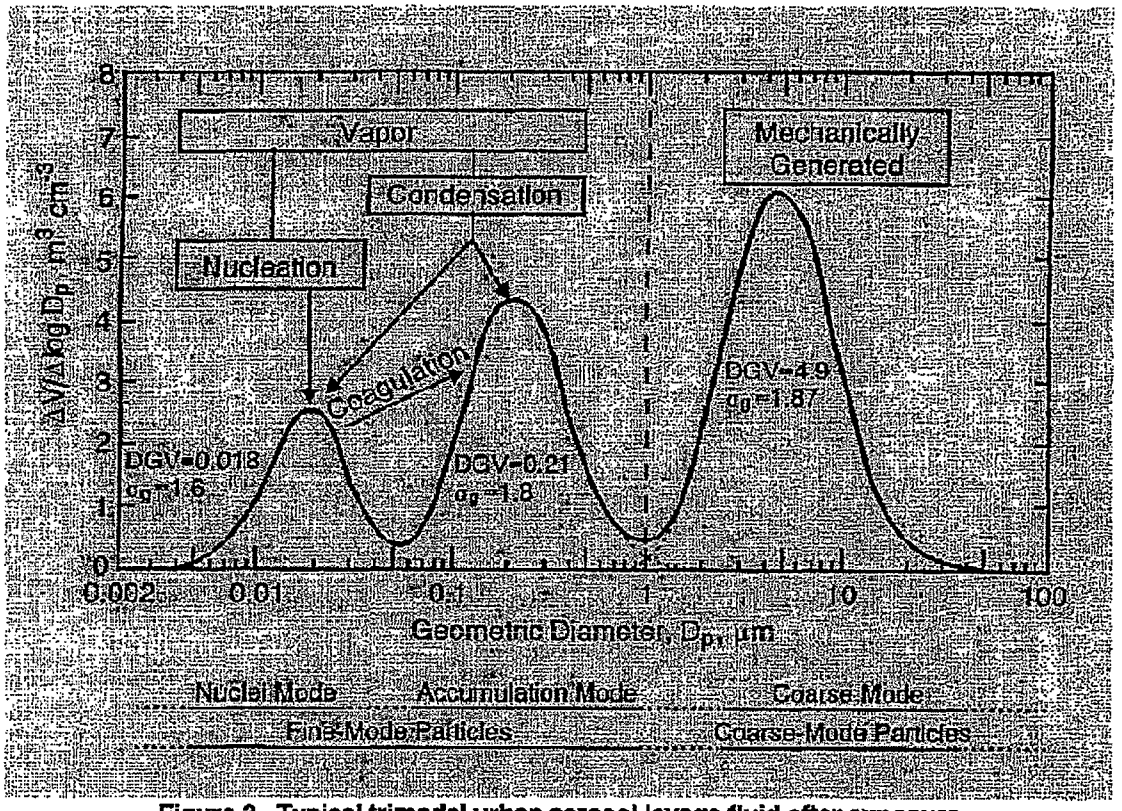

Figure 3. Typical trimodal urban aerosol lavage fluid after exposure of rats to the particle and gas phase (*from EPA, 1996*)

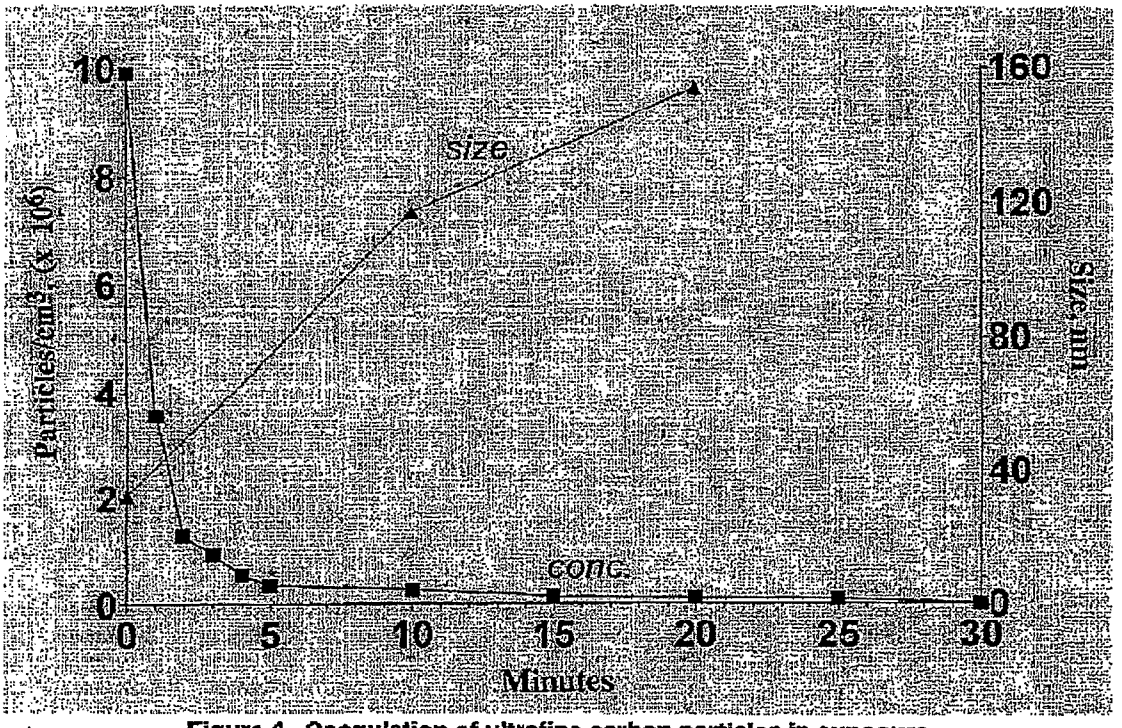

Figure 4. Coagulation of ultrafine carbon particles in exposure chamber under no flow conditions

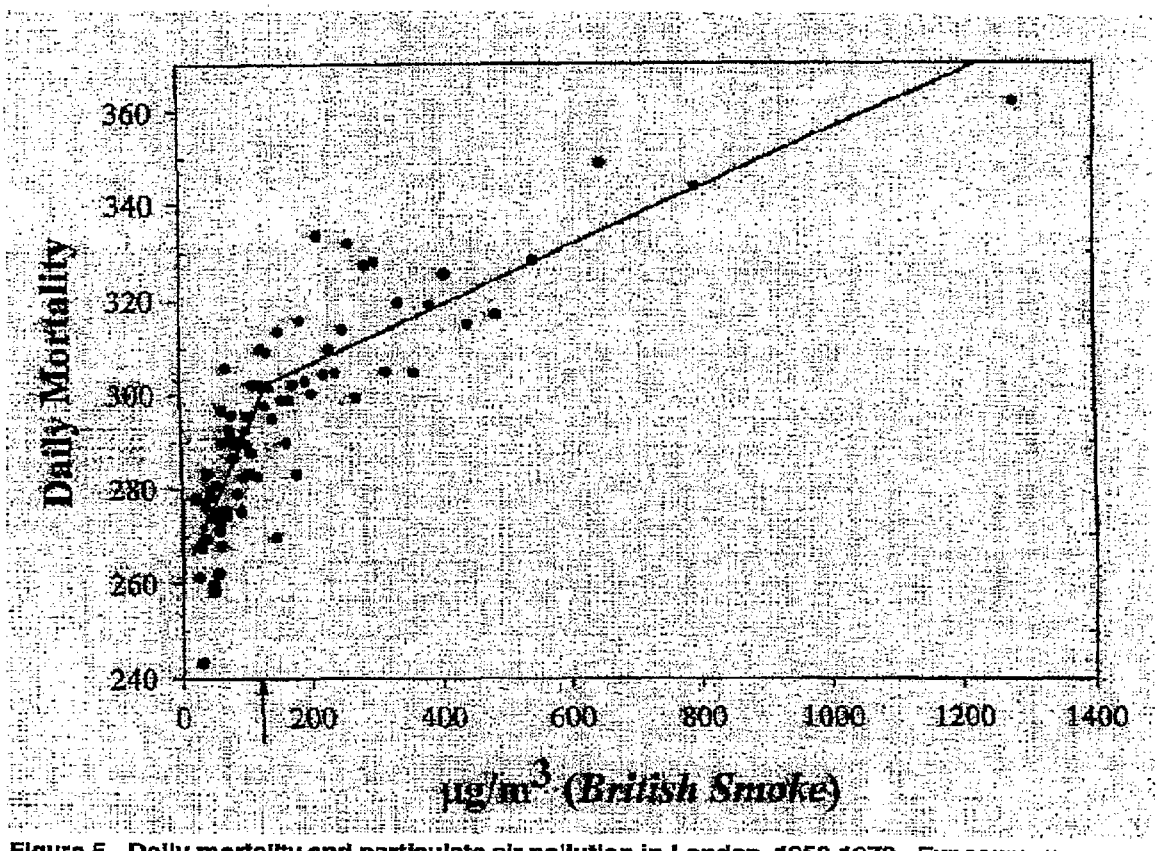

Figure 5. Daily mortality and particulate air pollution in London, 1958-1972. Exposure-response relationship and regression lines; with inflection point at ~130 $\mu g/m^3$.

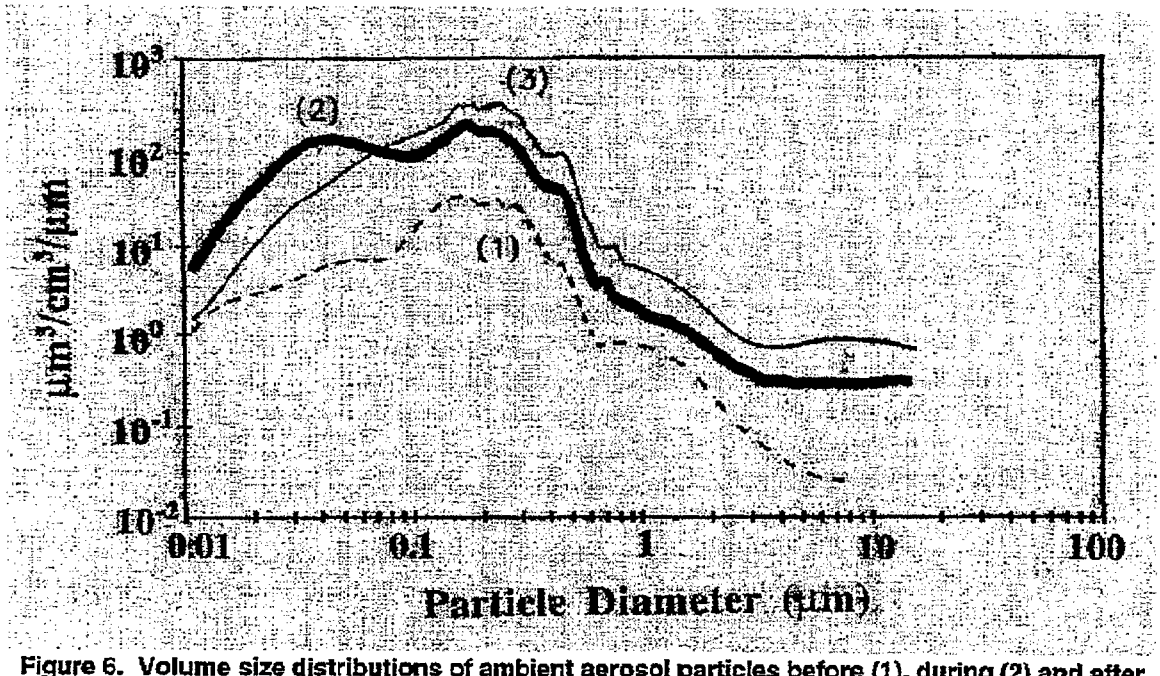

Figure 6. Volume size distributions of ambient aerosol particles before (1), during (2) and after (3) episodic event in Frankfurt, January/February, 1989 (*from Brand et al., 1992*).

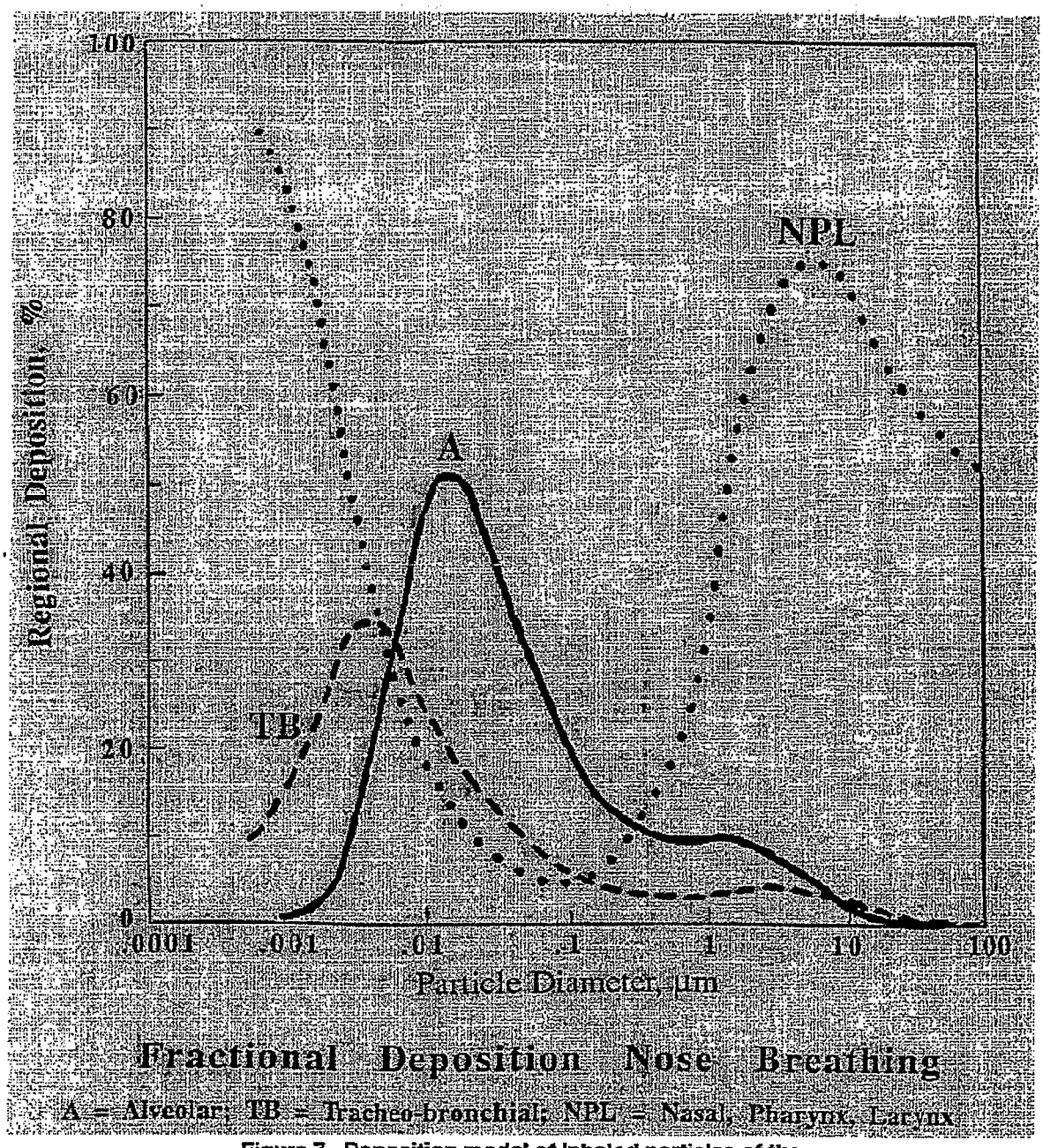

Figure 7. Deposition model of inhaled particles of the International Commission on Radiological Protection (*ICRP, 1995*).

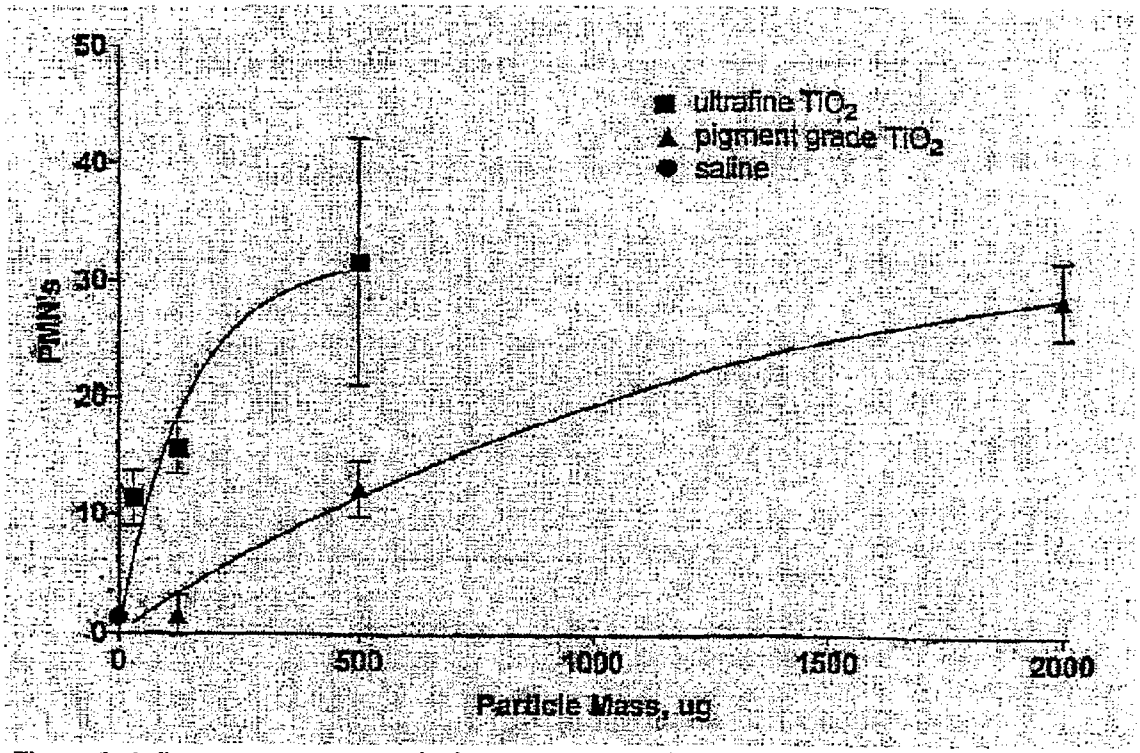

Figure 8. Inflammatory response in the lung as determined by neutrophils in lung lavage fluid following instillation of different doses of ultrafine and fine TiO_2 particles in rats.

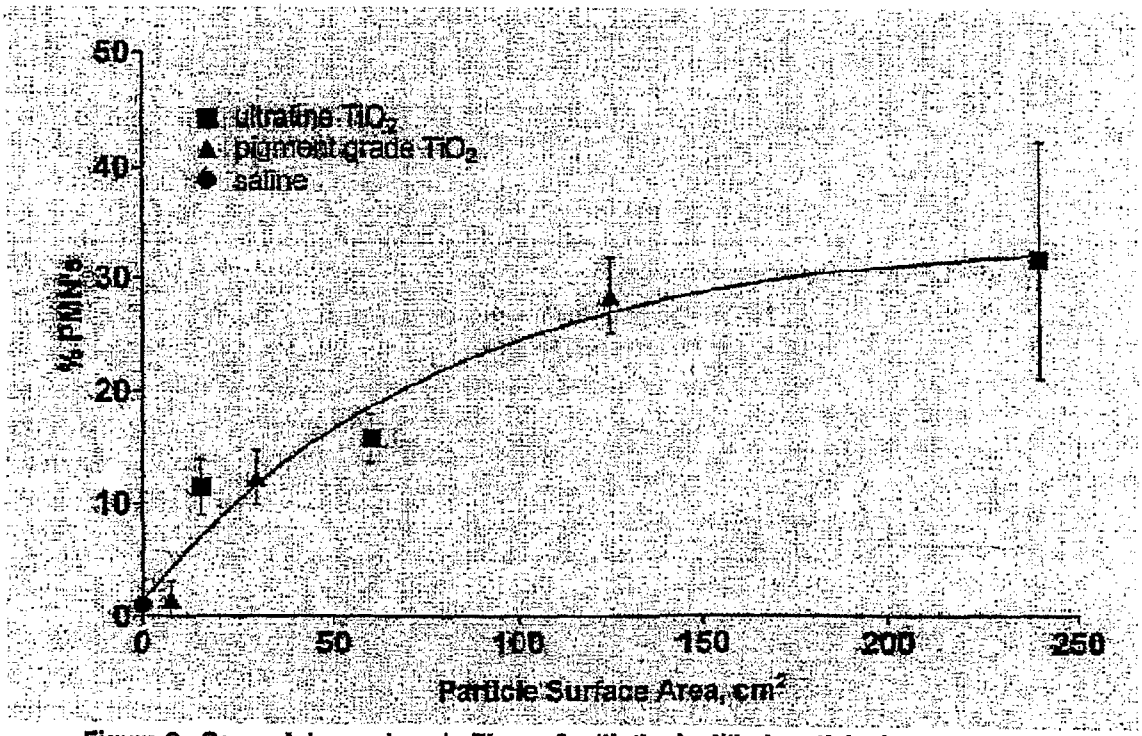

Figure 9. Same data as show in Figure 8 with the instilled particle dose expressed as particle surface area.

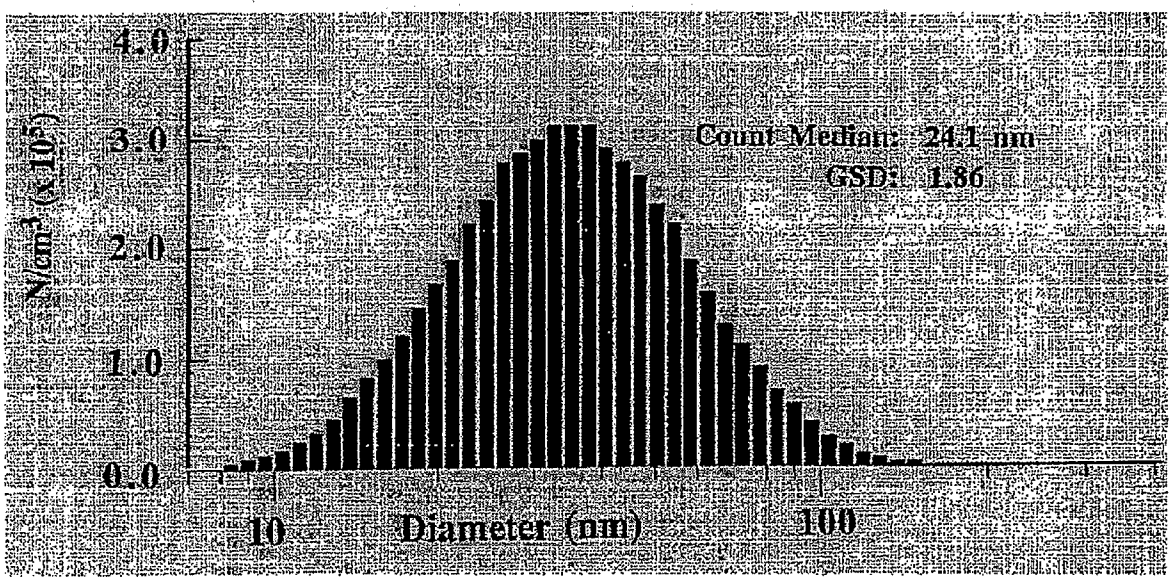

Figure 10. Size distribution of ultrafine carbon particles used in laboratory experiments.

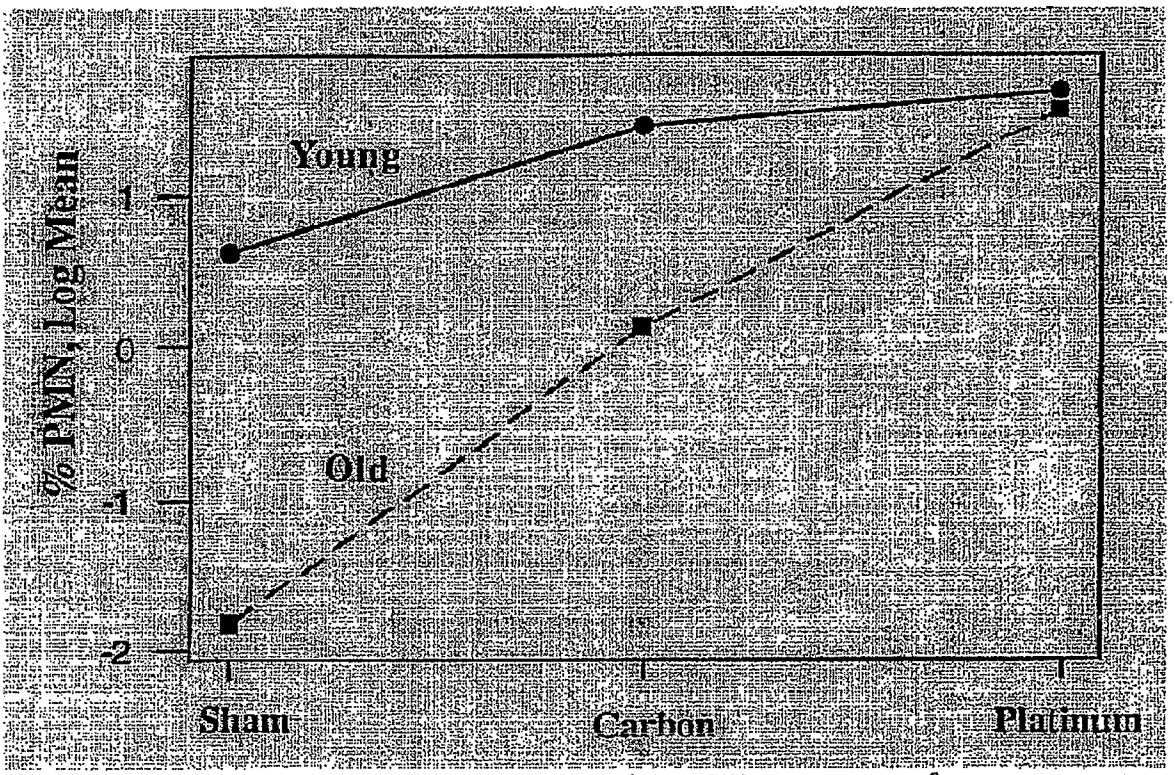

Figure 11. Effects of inhaled ultrafine carbon and platinum particles (~110 μg/m^3, 6 hr.-exposure) on occurrence of inflammatory cells in lung lavage in emphysematous young (8 wks.) and old (18 mos.) mice. Compared to sham-exposed controls, only the old emphysematous mice showed a significant response.

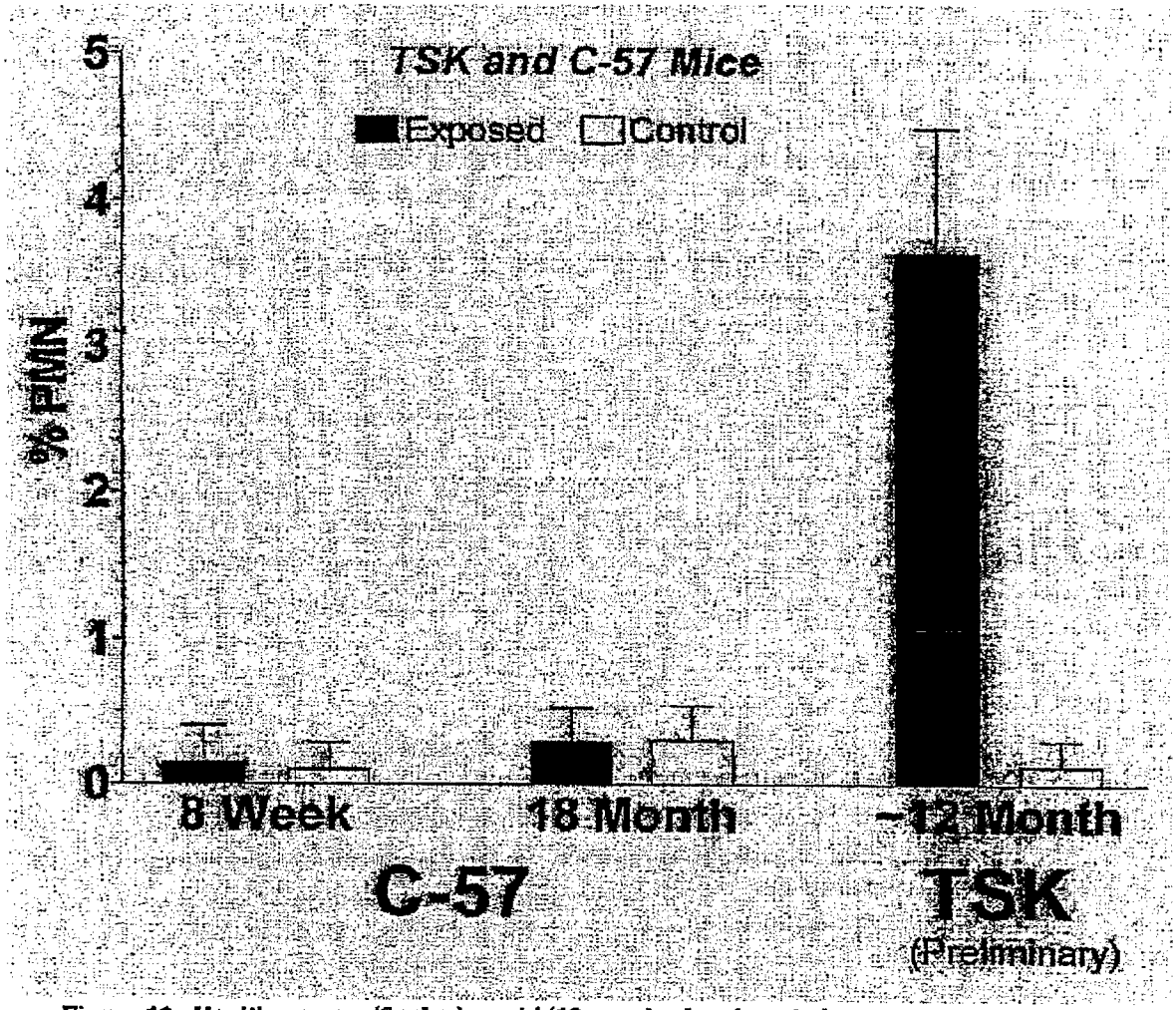

Figure 12. Healthy young (8 wks.) or old (18 mos.) mice do not show response to inhaled ultrafine carbon particles (125 $\mu g/m^3$, 6 hr.). Preliminary data in 12 month-old Tsk mice with genetic lung emphysema indicate some response.

PROGRESS IN EMISSIONS PARTICULATE DOSIMETRY AND TOXICOLOGY

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INTRODUCTION

Under a cooperative agreement with the Office of Heavy Vehicle Technologies, U.S. Department of Energy, the Lovelace Respiratory Research Institute is conducting research and development to provide health-related guidance for heavy vehicle engine, fuel, and exhaust after-treatment technology development. Advances in engine design, fuels, and exhaust after-treatment technologies aimed at reducing diesel particulate mass and nitrogen oxides have changed the nature of emissions. An example is the potential trend toward emission of increased numbers of ultrafine (< 0.1 micron) particles of organic condensate, which may accompany the reduction of elemental carbon particles (Bagley, 1996). Existing epidemiological and laboratory animal data on the toxicity of diesel particulate emissions are derived from exposures to emissions from older-technology engines. It is possible for changes in emissions to result in either greater or lesser toxicity; thus, health information is needed to help guide choices among strategies to increase engine efficiency and reduce emissions.

The current effort at Lovelace has two facets: 1) development of standardized short-term *in vitro* (using cultured cells and tissues) and *in vivo* (using intact animals) toxicity assays for comparing the toxicities of particulate emissions; and 2) evaluation of the disposition (clearance, penetration, and translocation to other organs) of inhaled ultrafine particles deposited in the respiratory tract. Standardized rapid assays of biological activity are needed because it is not practical to conduct long-term animal studies of the influence of each change in diesel technology on the toxicity of the resulting emissions, and epidemiological studies of newer emissions will not be possible for many years. Ultrafine particles are thought to penetrate lung tissues more rapidly, translocate to other organs to a greater degree, and have greater toxicity per unit of mass than larger fine particles, but existing information is largely anecdotal. There are no quantitative data on the distribution of ultrafine particles after deposition in the lung. Thus, improved rapid-response comparative toxicity assays and new information on the disposition of ultrafine particles are needed to estimate the potential health implications of the different diesel technologies and to compare the toxicity of petroleum diesel emissions to those of other engine and fuel technologies.

DEVELOPMENT OF RAPID-RESPONSE TOXICITY ASSAYS

Short-term toxicity assays based on cultured cells and instillation of material into rodent lungs have been used to compare the toxicities of different materials and to explore mechanisms of toxicity, but there has not been a concerted effort to compare the value of these systems for demonstrating subtle differences in toxicity among samples of generally similar toxicity. Cultured slices of other organs have been used for similar purposes, but there has been little exploration of the utility of lung slices as an assay of particle toxicity. A preliminary report by Monteil et al. (1997) suggested that rat lung slices might provide a useful assay for diesel soot toxicity. This project explored the relative values of a cultured cell line of human lung origin (A549), cultured rat lung slices, and instillation of material into intact rat lungs for determining gradations of toxicity among particle samples. The cell line has the advantage of using a single, standardized cell type that is readily available, but is the least representative of the actual complex lung tissue among the three assays. The lung slices have the advantage of consisting of several cell types organized normally into tissues, but are technically complex to manage and have the least background of previous information among the assays. Instillation into rodent lungs requires live animals, and does not exactly duplicate inhalation, but there is considerable experience with the assay, and it is the most realistic biological system among the three assays. There is not a substantial difference in cost among the three assays.

Methods

The variables explored included the best markers of toxicity and the optimum sampling time for each assay, and the ability of the assays to discriminate among different test particles

comprising a range of toxicity. A key measure of toxicity for all the assays was the concentration of several different chemical signals elaborated by cells either during normal metabolism or in response to challenge with foreign material. These agents are thought to include some of the earliest and most sensitive signals of cellular responses to toxic materials. Some of these chemical signals, such as protein synthesis or adenosine triphosphate content, indicated the general health of the cells. Others, commonly termed Acytokinese constitute the chemical signaling among cells for activation of defense or repair mechanisms, such as recruitment of inflammatory cells from the blood, or stimulation of cell division and growth. These cytokines included interleukin-8, growth-regulated oncogene alpha, macrophage inflammatory protein-2, and tumor necrosis factor alpha. Measurements also included the early gene transcription factor NF-kB and the cytoplasmic enzyme, lactate dehydrogenase. These agents were measured in the culture medium for cell and lung tissue cultures, and in the bronchoalveolar lavage (lung washing) fluid of instilled lungs. For instilled lungs, the number and type of inflammatory cells entering the lungs and the histopathology of the lungs were also evaluated.

A range of particle types were used for these studies, with the goal of comparing the responses of the assays to particles of differing type and toxicity. Diesel soot particles (DEP) were scraped from filters collected from General Motors LH6 6.2 L V-8 engines burning EPA certification fuel and operated on the FTP light-duty cycle at the Institute. The carbon black (CB) was Elftex-12 furnace black obtained from Cabot. Both the DEP and CB would have been very similar, if not identical, to the materials used at Lovelace in a 2-year inhalation carcinogenesis bioassay of rats (Nikula et al., 1995). Residual oil fly ash (ROFA) was obtained from the heat exchange section of the Boston Edison Mystic River power plant, and was provided by Dr. John Godleski of Harvard University. Nickel subsulfide (Ni_3S_2) was provided by Midwest Research Institute, and was identical to material used at Lovelace in a 2-year inhalation carcinogenesis bioassay (U.S. NTP, 1994). Ambient particulate (Ottawa) collected in Ottawa, Canada (dust EHC-93) was provided by the Health Ministry of Canada. Silica (S) (5 Fm Min-U-Sil) was obtained from U.S. Silica. Titanium dioxide (TiO_2) was obtained from Fischer Scientific.

Results

A549 Cell Culture: The methods and results from the A549 cell culture studies are presented in detail by Westhouse et al. (1998a). Overall, although the cultured cells detected large differences in particle toxicity, there was a disappointing degree of variability among individual cultures, and they did not display a very fine or consistent gradation of toxicity among the different particle types. Two examples serve to illustrate this response. Figure 1 illustrates differences in protein synthesis, a measure of cell health, in cultures treated with 40 Fg particles/cm^2 of surface and analyzed 20 hours later. The data are the means and standard deviations of three cultures for each particle. As seen in the figure, only ROFA and Ni_3S_2 reduced protein synthesis markedly. These particles have high chemical toxicity related to soluble metals. The responses to the other particles varied somewhat, but were not distinguished by the assay. Thus, silica, which is known to have high short-term toxicity in the lungs of rats (Benson et al., 1985), was not distinguished from Ottawa, TiO_2, CB, or DEP.

Figure 2 illustrates differences in the secretion of IL-8, a cytokine which recruits inflammatory cells, by A549 cell cultures treated with 40 Fg particles/cm^2 of surface and analyzed 20 hours later. The data are the means and standard deviations of four cultures for each particle. The figure shows that ROFA caused a striking response and Ottawa caused a slight response, but the assay did not distinguish among the toxicities of the other particles. In this case, the responses to ROFA and Ni_3S_2 were very different, but the responses to Ni_3S_2 and silica were nearly identical.

Cultured Rat Lung Slices: Results of the lung slice studies are presented in detail by Westhouse et al. (1998b). After considerable effort to optimize and standardize the culture and particle treatment conditions for the assay, its responses to DEP, CB, and ROFA were compared. The assay indicated a graded toxicity from CB with the least toxicity to ROFA with the greatest. An example is given in Figure 3, which illustrates differences in secretion of macrophage inflammatory protein2 (MIP-2). MIP-2 recruits inflammatory cells, by slices cultured for 11 days after instillation with 1 mg particles/ml incubation medium. The data are means and standard deviations of three cultures for each particle, expressed as percentages of the mean control, or untreated, response.

Although the lung slice assay appeared to provide a greater distinction between CB and DEP than the A549 cell culture, there was only slightly greater distinction between DEP and ROFA, which has been shown by many investigators to be highly toxic. In addition, there was considerable variability in the control data, and

the assay was more complex, time-consuming, and costly to perform than either cell culture or animal instillation.

Intratracheal Instillation in Intact Animals: Nine week old male F344 rats were anesthetized with halothane, a cannula was placed in the trachea via the mouth, and 3 mg of particles suspended in 0.5 ml saline was instilled into the lower trachea via a smaller tube. The rats recovered, then groups of five were killed at either 4, 6, 12, or 24 hours or 1 week after instillation. The lungs were removed and lavaged using a common and well-standardized technique (Henderson, 1991) via the trachea with saline, which was then analyzed for cytokines, lactate dehydrogenase, and inflammatory cells.

Although the ability of the rat instillation assay to distinguish among particles having slightly different, but generally low toxicity is still being explored, preliminary results suggest that the integrated response of intact animals may give better results than the two *in vitro* assays. Figure 4 illustrates the levels of the enzyme, lactate dehydrogenase, in lavage fluid at 6 and 24 hours and 1 week after instillation of CB or DEP. This enzyme is present in the cytoplasm of lung cells, and its presence in the lung lining fluid is a reflection of cell toxicity and death (Henderson, 1991). The data represent the means and standard errors from 5 rats/group. The enzyme was slightly elevated by both particles at 6 hours. The levels at both 24 hours and 1 week indicated that CB was more toxic to lung cells than DEP. This finding is consistent with the results of an earlier long-term inhalation study (Nikula et al., 1995), which also indicated that this CB was slightly more toxic than DEP from the same type of engine per unit of material retained in the lung.

Figure 5 illustrates the influx of neutrophils, inflammatory cells from the blood, into the lung lining fluid of rats at different times after instillation of DEP or CB. Inflammation is a common early response to toxic materials, is important in eliciting asthmatic responses, and is also a common feature of the early stages of several other lung diseases. Although it is assumed that cells secrete cytokines before neutrophils are called into the lung, the influx of neutrophils is an indication that the body has recognized a significant intrusion by foreign material. The data represent the means and standard errors of 5 rats/group. Both DEP and CB attracted neutrophils into the lung, but the time course and magnitude of the responses to the two particles differed. The neutrophil response to CB was apparent by 4 hours, peaked at 6 hours, and was still evident at 1 week. The neutrophil response to DEP was not significant until 12 hours and was not significant at 1 week. The CB elicited a greater response than DEP at all times.

Summary

The results of this development work suggested that overall, intratracheal instillation into the lungs of intact rats followed by bronchoalveolar lavage and histopathology was likely to give a more realistic and cost-effective assay of relative particle toxicity than either the cell culture or lung slice culture assays. The comparison between cell cultures and lung instillation is still being completed, and it remains possible that cell cultures may still prove to be a useful tool for rapid toxicity comparisons. At this time, it does not appear that the lung slice assay offers any advantage over lung instillation. Responses of lung slices may resemble those of intact lungs more closely than do responses in cell cultures, but the assay is just as costly as lung instillation and is more technically demanding and more difficult to interpret. The lung instillation assay has the great advantage over the other assays of allowing the full interplay among the various biological responses present in intact individuals. Because the assay does not involve inhalation, the exposure route of concern, it is not likely to produce data useful for quantitative estimates of risk to humans. It appears that it can, however, meet the need for a rapid, low-cost assay of relative toxicity of particles in the lung, and especially if positive and negative control particles are included for comparison with the test particles.

DISPOSITION OF INHALED ULTRAFINE PARTICLES

The work on ultrafine particle disposition is continuing, and few definitive results are yet available. This effort required considerable technical development to enable the detection and quantitation of very small amounts of tiny particles dispersed in various tissues and fluids. The lack of detection ability has largely been responsible for the lack of previous work in this area. Another major difficulty due to the high surface area to mass ratio of ultrafine particles is ensuring that the material does not dissolve before it is measured in the body. Our research approach was to: 1) produce radiolabeled particles that could be aerosolized in uniform sizes; 2) determine the time course of the dissolution of the particles; and 3) measure the time course of the distribution of particles in rats and monkeys. Work to date has focused on the use of radiolabeled silver particles (^{110m}Ag), because of our previous experience in generating

this material in the appropriate size range and because silver particles in small quantities should be inert in the lung.

The ^{110m}Ag particles were generated by heating radiolabeled foil and allowing the vapor to condense into particles under controlled cooling and dilution conditions. After trials defining the conditions necessary to reproducibly generate ^{110m}Ag in fine and ultrafine sizes, the solubility of the particles in simulated body fluid was tested using a dialysis bag system. These results indicated that a sufficient portion of the material would remain in particulate form to evaluate disposition in the body during times up to a few days after inhalation.

An initial inhalation study in rats has been done, and the results are being analyzed. Twelve week old male F344 rats were exposed by inhalation to ^{110m}Ag particles having a count median diameter of 25 nanometers. A 35-minute exposure produced a lung burden of approximately 130 kiloBequerels of radioactivity representing a deposited particle mass of less than 1 nanogram. Rats were killed at 2, 6, 24, and 72 hours after the end of exposure, and the radioactivity in various tissues was analyzed. The ^{110m}Ag cleared rapidly from the lung, with only 53% and 25% remaining at 24 and 72 hours, respectively. Most of the ^{110m}Ag excreted from the body was excreted in the feces. A tissue digestion and ultracentrifugation procedure was used to determine if the remaining ^{110m}Ag was in particulate or dissolved form. Preliminary data indicate that the material was primarily in particulate form in the lung and liver, and in solubilized form in the blood. Tissue sections will be evaluated by autoradiography to visualize the particles and their location.

The initial results confirm the fact that a significant portion of poorly soluble ultrafine particles leaves the lung rapidly after deposition. Pending completion of the rat study and conclusion that the ^{110m}Ag inhalation technique is deemed acceptable, a similar study will be performed using monkeys. Current information suggests that the majority of the ultrafine particulate material in emissions from new diesel engines consists of organic condensate. Therefore, the next step will be to evaluate the behavior of ultrafine organic particles after inhalation. Together, these results should provide a much improved understanding of the behavior of ultrafine particles after deposition in the lung by inhalation.

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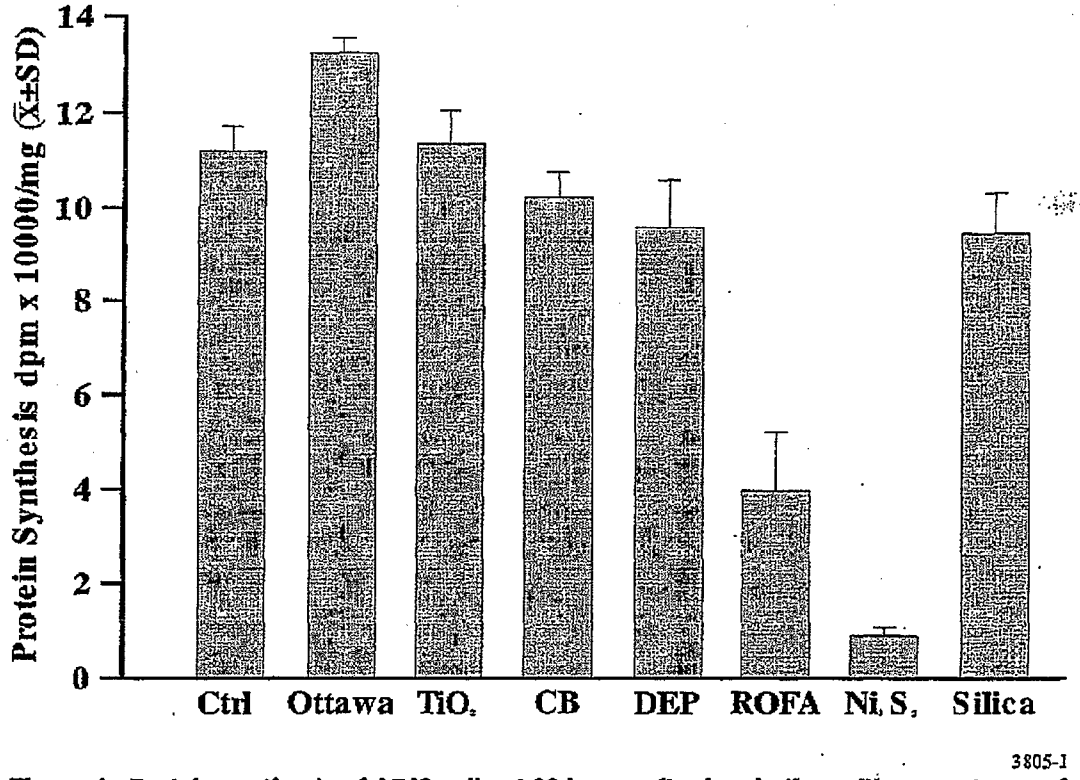

Figure 1. Protein synthesis of A549 cells at 20 hours after incubation with seven types of particles, or no particles (Ctrl).

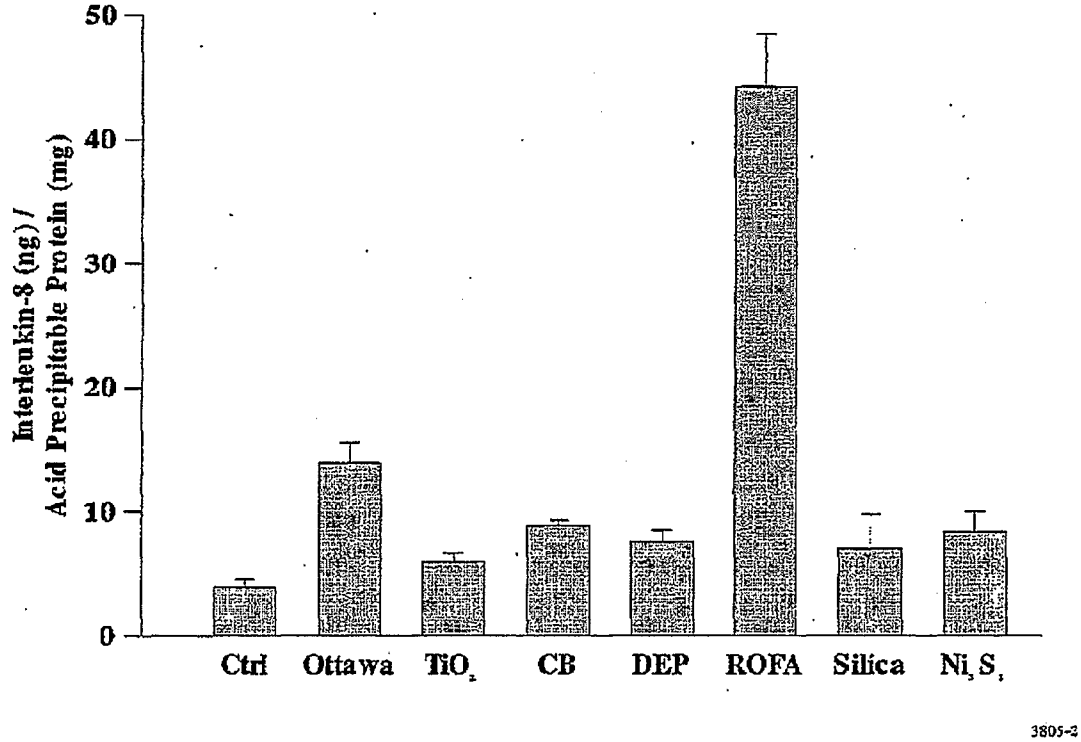

Figure 2. Secretion of the pro-inflammatory cytokine, IL-8 by A549 cells at 20 hours after incubation with seven types of particles, or no particles (Ctrl).

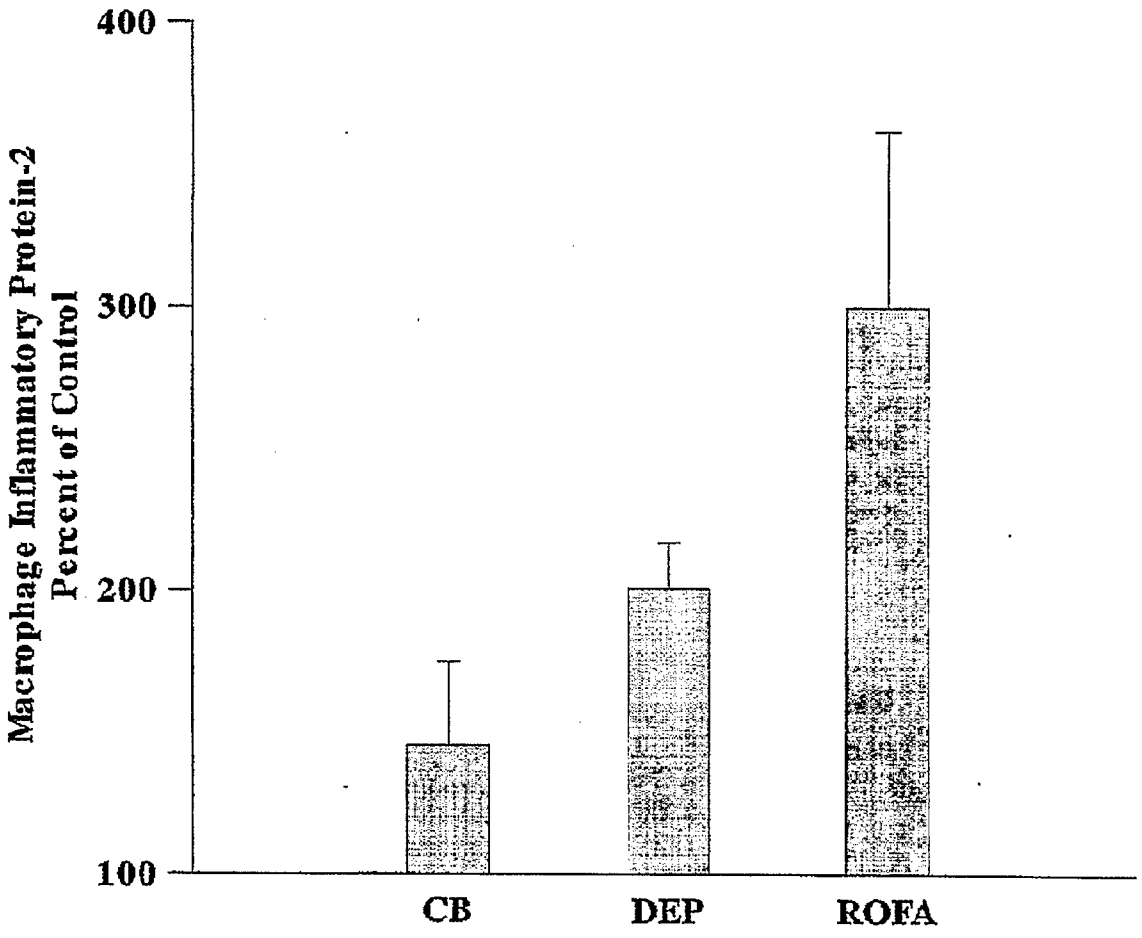

Figure 3. Secretion of the pro-inflammatory cytokine, MIP-2 by rat lung slices at 11 days after incubation with three types of particles.

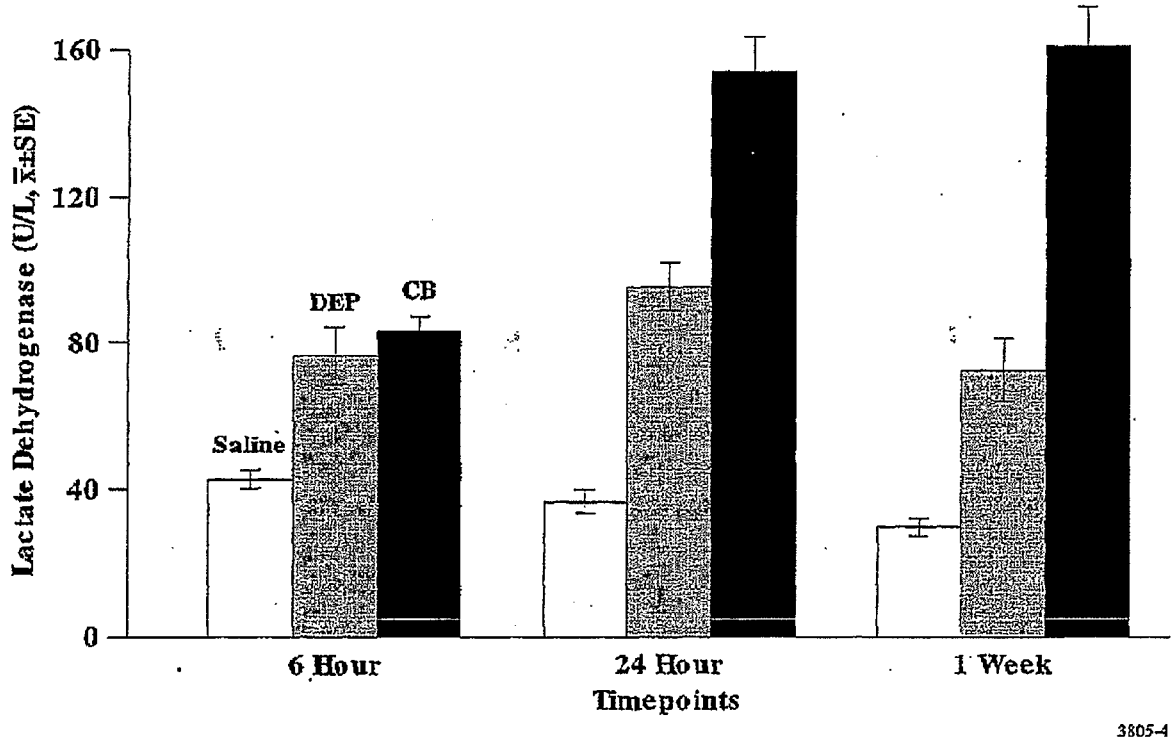

Figure 4. Levels of lactate dehydrogenase in lung lavage fluid of rats at different times after intratracheal instillation of saline or 3 mg of DEP, or CB.

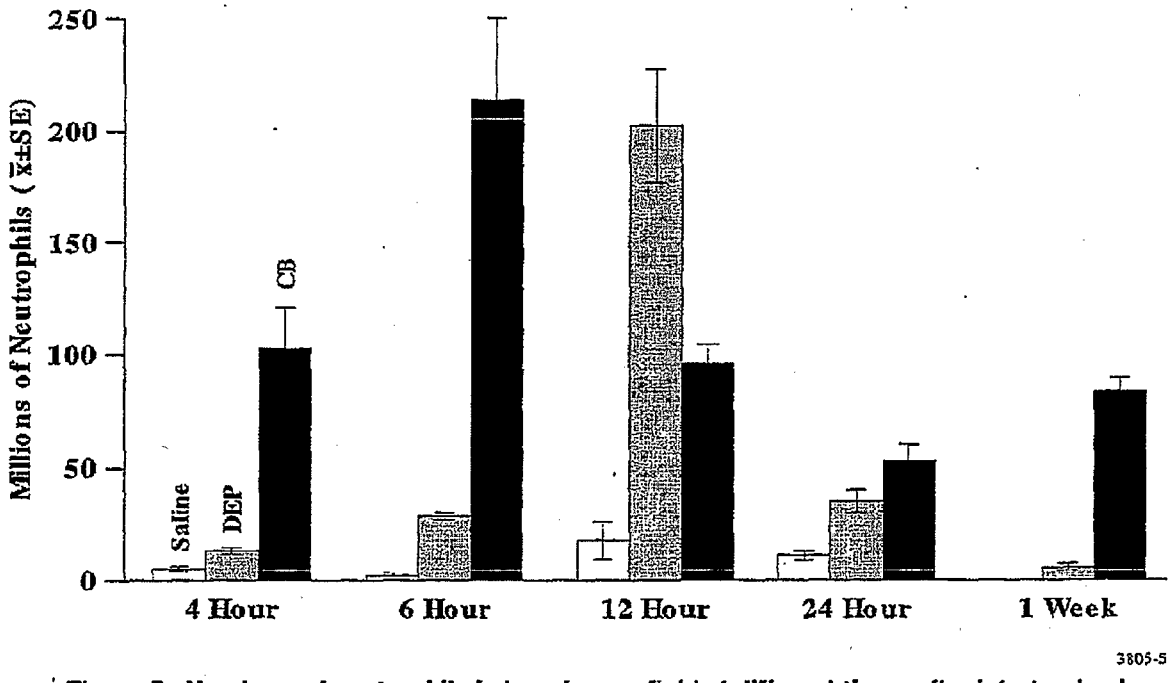

Figure 5. Numbers of neutrophils in lung lavage fluid at different times after intratracheal instillation of 3 mg of DEP or CB.