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Department of the Environment and Heritage

Health Impacts of Ultrafine Particles

Desktop Literature Review and Analysis

A consultancy funded by the Australian Government
Department of the Environment and Heritage

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GLOSSARY

Accumulation Mode Particles	1. Particles with the diameters between about 0.04 and 1 μm. 2. A mode in the atmospheric particle size distribution, formed primarily by coagulation of smaller particles.
Absorption	Penetration of a substance into the body of another substance
Acute	Refers to severe symptoms and a rapid onset and progressive change to an organism leading to a crisis in a relatively short period of time, measured in seconds, minutes, hours, or days, following exposure to a health hazard.
Acute Exposure	Refers to a single, short-term exposure to a toxic substance. Acute exposures are characterised as lasting no longer than 1 day.
Acute Health Effect	An effect that develops either immediately or a short time after exposure.
Adiabatic	A body is said to undergo an adiabatic change when its condition is altered without gain or loss of heat.
Adsorption	The condensation of gases, liquids, or dissolved substances on the surfaces of solids
Adverse Health Effect	Abnormal or harmful effect to an organism (e.g., a person) caused by exposure to a chemical. It includes results such as death, other illnesses, altered body and organ weights, altered enzyme levels, etc.
Aerodynamic Diameter	Refers to the size of particles. It is the diameter of a sphere of unit density that behaves aerodynamically (has the same settling velocity in air) as the particle of the test substance. It is used to compare particles of different size, shape, and density, and to predict where in the respiratory tract such particles may be primarily deposited.

Air Contaminant	Any particle matter, gas, or combination thereof, other than water vapour
Air Pollutant	Any substance in air that could, in high enough concentration, harm humans, animals, vegetation, or material. Pollutants may include almost any natural or artificial composition of airborne matter capable of being airborne. They may be in the form of solid particles, liquid droplets, gases, or in combination thereof. Generally, they fall into two main groups: (1) those emitted directly from identifiable sources and (2) those produced in the air by interaction between two or more primary pollutants, or by reaction with normal atmospheric constituents, with or without photoactivation.
Air Pollution	The presence of contaminants or pollutant substances in the air that interfere with human health or welfare, or produce other harmful environmental effects.
Air Pollution Episode	A period of abnormally high concentration of air pollutants, often due to low winds and temperature inversion that can cause illness and death.
Air Quality Criteria	The levels of pollution and lengths of exposure above, which adverse health and welfare effects may occur.
Air Quality Standards	The level of pollutants prescribed by regulations that are not to be exceeded during a given time in a defined area.
Airborne Particles	Total suspended particulate matter found in the atmosphere as solid particles or liquid droplets. Chemical composition of particles varies widely, depending on location and time of year.
Ambient Air	The external air environment (does not include the air environment inside buildings or structures).
Ambient Measurement	A measurement of the concentration of a substance or pollutant within the immediate environments of an organism; taken to relate it to the amount of possible exposure.

Asthma	A respiratory disease caused by spasmodic contraction of the bronchioles in the lungs. Characterised by attacks of wheezing, shortness of breath and/or coughing and resulting in difficult breathing.
Bias	A systematic error introduced through some aspect of the study design. It cannot be controlled for in the analysis and efforts must therefore be made to prevent it through good study design and data collection.
Black smoke	Surrogate for suspended particles used in UK and is defined according to a special measuring procedure, indicating the density of blackness on a certain filter system.
Carbonaceous particles	Particles consisting mostly of carbon compounds.
Cardiovascular	A medical term that refers to the heart and blood vessel system.
Cardiovascular and Blood Toxicity	The adverse effects on the heart or blood systems, which result from exposure to toxic chemicals.
Chronic	Refers to a change to an organism over a long period of time, measured in weeks, months, or years following repeated exposure to a health hazard.
Chronic Exposure	A long-term exposure to a toxic substance.
Chronic Health Effect	Refers to an adverse health effect that develops slowly over a long period of time or from prolonged exposure to a health hazard without implying a degree of severity.
Coarse particles	Particles with the diameter between 2.5 and 10 μm.
Combustion	A chemical reaction in which a material combines with oxygen with the evolution of heat: "burning". The combustion of fuels containing carbon and hydrogen is said to be complete when these two elements are all oxidised to carbon dioxide and water. Incomplete combustion may lead to (1) appreciable amounts of carbon remaining in the ash;

	(2) emission of some of the carbon as carbon monoxide; and (3) reaction of the fuel molecules to give a range of products of greater complexity than that of the fuel molecules themselves (if these products escape combustion they are emitted as smoke).
Community time series epidemiology	Epidemiological studies that assess the impact of exposures on day-to-day variation in health events within a community.
Confounder	Variable that influences a health effect apart from air pollution. In particular, a confounder is associated with the exposure and the outcome and effect estimates would be biased if the variable would be neglected in the analyses.
COPD (chronic obstructive pulmonary disease)	A disease process that decreases the ability of the lungs to perform ventilation. Diagnostic criteria include a history of persistent dyspnea on exertion, with or without chronic cough, and less than half of normal predicted maximum breathing capacity. Diseases that cause this condition are chronic bronchitis, pulmonary emphysema, chronic asthma, and chronic bronchiolitis.
Diesel exhaust	Diesel exhaust emissions contain hundreds of chemical compounds, which are emitted partly in the gaseous phase and partly in the particulate phase of the exhaust. The major gaseous products are carbon dioxide, oxygen, nitrogen, and water vapour; carbon monoxide, sulfur dioxide, nitrogen oxides, and hydrocarbons and their derivatives are also present. Benzene and toluene are present in the lower range (percentage weight) in the gaseous part of the hydrocarbon fraction. Other gaseous exhaust compounds are low-relative-molecular-mass polycyclic aromatic hydrocarbons. A main characteristic of diesel exhaust is the release of particles at a rate about 20 times greater than that from gasoline-fuelled vehicles. The particles are composed of elemental carbon, organic compounds adsorbed from fuel and lubricating oil, sulfates from fuel-sulfur, and traces of metallic components. Most of the total particulate matter occurs in the submicrometre range, between 0.02 and 0.5 μm.
Dose	The amount of a chemical substance to which a person has been exposed or adsorbed into the body.

Dose response	A relationship in which a change in the amount, intensity, or duration of an exposure is associated with either an increase or decrease in risk of a specified health outcome.
Dose-Response Assessment/ Relationship	The amount of a chemical that an organism (such as a person) is exposed to is called the dose, and the severity of the effect of that exposure is called the response. A dose-response assessment is a scientific study to determine the relationship between dose and response, and how much dose is correlated with how much response.
Emission	Release of pollutants into the air from a source.
Epidemiology	Science concerned with the study of disease in a general population. Determination of the incidence (rate of occurrence) and distribution of a particular disease (as by age, sex, or occupation), which may provide information about the cause of the disease.
Exposure	(1) The time integral of the concentration of a toxicant, which is in the immediate vicinity of various ports of entry (such as lung, gastro-intestinal tract and skin). (2). Qualitatively, contact between a potentially harmful agent and a receptor (e.g., a human or other organism) that could be affected. Exposure may be short term (acute) or long term (chronic).
Exposure Assessment	The process of measuring or estimating the intensity, frequency, and duration of human exposures to an agent currently present in the environment or of estimating hypothetical exposures that might arise from the release of new chemicals into the environment.
Exposure Limits	Established concentrations which, if not exceeded, will not generally cause adverse effects to the exposed population.
Fine Particles	Particles with the diameter smaller than 2.5 μm.
Forced expired volume in one second	The volume expired in the first second (FEV_1) of maximal expiration after a maximal inspiration and is a useful measure of how quickly full lungs can be emptied.

Hazard Evaluation	A component of risk evaluation that involves gathering and evaluating data on the types of health injuries or diseases that may be produced by a chemical and on the conditions of exposure under which such health effects are produced.
Hazard Assessment	Evaluating the effects of a stressor or determining a margin of safety for an organism by comparing the concentration, which causes toxic effects with an estimate of exposure to the organism.
Hazard Identification	Determining if a chemical or a microbe can cause adverse health effects in humans and what those effects might be.
Hazardous air pollutants	(1) Chemicals that cause serious health and environmental effects, (2) According to law, a pollutant to which no ambient air quality standard is applicable and that may cause or contribute to an increase in mortality or in serious illness.
Health hazard	Evidence based on scientific data (human or animal) that acute or chronic effects might occur.
Heterogeneous nucleation	Formation of droplets on condensation nuclei
Homogeneous nucleation	Formation of droplets in the absence of condensation nuclei; also called self-nucleation.
Immunosuppression	Decrease in the immune response.
In vitro	In glass, referring to a study in the laboratory usually involving isolated organ, tissue, cell, or biochemical systems.
In vivo	In the living body, referring to a study performed on a living organism.
Inflammation	The response of the tissues of the body to injury, infection or irritation. Its chief symptoms are redness, heat, swelling, and pain.

Inhalation	Breathing into the lungs of a (contaminated) substance in the form of a gas, vapour, fume, mist, or dust.
Interaction	Modification of the toxic effects of one substance by another. Depending on the substances involved, the effects of interaction can be amplified or mitigated.
Mass concentration	The concentration of particles in air expressed as mass per unit volume.
Mobile sources	Motor vehicles and other moving objects that release pollution; mobile sources include cars, trucks, buses, planes, trains, motorcycles and gasoline-powered lawn mowers. Mobile sources are divided into two groups: road vehicles, which include cars, trucks and buses, and non-road vehicles, which include trains, planes and lawn mowers.
Morbidity	Sickness or illness; departure from a state of physical or mental well-being.
Mortality	Death.
Mortality Rate	The proportion of a population that dies during a specified time period. Also referred to as the death rate.
Mutagen	A chemical substance or physical effect capable of inducing transmissible changes in the genetic material of a living cell that results in physical and functional changes in the descendants. Depending on the type of cells affected, ova or spermazoids, both male and female can be affected. Mutations can lead to birth defects, miscarriage, or cancer.
Nanoparticles	Particles below 50 nm in diameter (0.05 μm).
Nucleaction Mode	Particles with the diameters between approximately 0.003 and 0.03 μm.
Panel study	An epidemiological or toxicological study performed with volunteers.

Particulate Matter (also particles)	Generally refers to all airborne pollutants, which are not gases. Particulate matter can include droplets of liquids or solid matter.
Personal Air Samples	Air samples taken with a pump that is directly attached to the worker with the sampler placed in the worker's breathing zone.
Personal Measurement	A measurement collected from an individual's immediate environment.
Pollutant	Generally, any substance introduced into the environment that adversely affects the usefulness of a resource or the methods for estimating health of humans, animals, or ecosystems.
Pollution	Generally, the presence of a substance in the environment that because of its chemical composition or quantity prevents the functioning of natural processes and produces undesirable environmental and health effects.
Polynuclear Aromatic Hydrocarbons	A class of chemicals typically formed by burning and common in the environment. PAHs are also common to petroleum products and oil. Although most of these compounds are harmless or mildly toxic, some are carcinogenic.
Primary Particles	Particulate matter originated from direct air emissions.
Regression	Statistical term to describe methods for estimating the relationship between a dependent (response) variable Y and one or more independent (explanatory) variables X.
Respirable Particle	Particles able to penetrate and deposit in the lower bronchioles and alveolar region.
Peak expiratory flow	The maximal expiratory flow rate (PEF) achieved and this occurs very early in the forced expiratory manoeuvre.

Respiratory Toxicity	Adverse effects on the structure or function of the respiratory system caused by exposure to a toxic chemical. Respiratory toxicants can produce a variety of acute and chronic pulmonary conditions, including local irritation, bronchitis, emphysema and cancer.
Risk	The probability that damage to life, health, and/or the environment will occur as a result of a given hazard (such as exposure to a toxic chemical). Some risks can be measured or estimated in numerical terms (e.g., one chance in a hundred).
Risk Characterization	An organised process used to evaluate, summarize, and communicate information about the likelihood of adverse health or ecological effects from particular exposures to a toxic chemical in the environment, i.e. how individuals or populations may be affected. It includes discussion of the kind of evidence it uses and how strong that evidence is. Risk characterization is the final step in the process of risk assessment.
Risk Assessment	An organised process used to describe and estimate the amount of risk of adverse human health effects from exposure to a toxic chemical (how likely or unlikely it is that the adverse effect will occur). How reliable and accurate this process is depends on the quantity and quality of the information that goes into the process. The four steps in a risk assessment of a toxic chemical are hazard identification, dose-response assessment, exposure assessment, and risk characterization.
Risk Management	The process of actually trying to reduce risk, e.g., from a toxic chemical, and/or of trying to keep it under control. Risk management involves not just taking action, but also analyzing and selecting among options and then evaluating their effect.
Secondary Particles	Particulate matter formed in the atmosphere by chemical reactions of gases, particularly sulfur dioxide, nitrogen oxides, ammonia and volatile organic compounds.

Soluble Organic Fraction (SOF)	The organic fraction of diesel particles. Includes heavy hydrocarbons derived from the fuel and from the engine lubricating oil. The term "soluble" originates from the analytical method used to measure SOF, which is based on extraction of particulate matter samples using organic solvents.
Total Carbon	The sum of the elemental carbon and organic carbon associated with diesel particles.
Total Particulate Matter	The total particulate matter emissions including all fractions of diesel particles, i.e. the carbonaceous, organic, and sulfate particles.
Toxicant	A substance capable of causing human injury or damage to living body tissue, impairment to the central nervous system, severe illness, and, in severe cases, death. A poison.
Toxicity	The degree of danger posed by a substance to human, animal or plant life.
Toxicology	Scientific discipline involving the study of the actual or potential danger presented by the harmful effects of substances (poisons) on living organisms and ecosystems, of the relationship of such harmful effects to exposure, and of the mechanisms of action, diagnosis, prevention and treatment of intoxications.
Ultrafine Particles	Particles with diameters smaller than 0.1 μm.
Vital capacity	The maximum volume of air which can be exhaled or inspired during either a forced (FVC) or a slow (VC) manoeuvre.
Volatile Organic Compounds	Hydrocarbon-based emissions released through evaporation or combustion.

Volatile Organic Fraction	The organic fraction of diesel particulate matter as determined by vacuum evaporation. It may or may not be equivalent to the SOF fraction. Depending on the exact analytical procedure, the VOF may include the organic material (SOF) as well as some of the sulfate particles which, being composed primarily of hydrated sulfuric acid, are also volatile.
Volatile Organic Substance	Any organic substances, mixture of organic substances, or mixture of organic and inorganic substances which have vapour pressures or sums of partial pressures of substances of 0.02 pounds per square inch (one millimeter of mercury) absolute or greater measured at standard conditions of atmospheric pressure and a temperature of 60 degrees Fahrenheit.

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ACRONYMS AND ABBREVIATIONS

A	Alveolar
AM	Alveolar Macrophages
BALF	Bronchoalveolar Lavage Fluid
BD-AM	Beagle Dogs Macrophages
CAPS	Concentrated Ambient Air Particles
CB	Carbon Black
CFA	Coal Fly Ash
CMD	Count Median Diameter
CNG	Compressed Natural Gas
CO	Carbon Monoxide
COPD	Chronic Obstructive Pulmonary Disease
CP	Coarse Particles
CPC	Condensation Particle Counter
CS	Conventional Sulfur
DECSE	Diesel Emission Control – Sulfur Effects
DEP	Diesel Exhaust Particles
DMA	Differential Mobility Analyser
DMPS	Differential Mobility Particle Sizers
DPF	Diesel Particle Filters
DPM	Diesel Particulate Matter
EAS	Electrical Aerosol Spectrometer
EC	Elemental Carbon
ECG	Electrocardiograph
EELS	Electron Energy Loss Spectroscopy
ELPI	Electrical Low Pressure Impactor
EnTOX	The National Research Centre for Environmental Toxicology
EPA	Environment Protection Authority
ER	Emergency Room
FP	Fine Particles
FVC	Forced Vital Capacity
FEV_1	Forced Expiratory Volume in 1 Second
GAM	Generalised Additive Modelling
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor

HAP	Hazardous Air Pollutants
HBEC	Human Bronchial Epithelial Cells
HC	Hydrocarbons
HEAPSS	Health Effects of Air Pollution on Susceptible Subpopulation
HEI	Health Effect Institute
HRTEM	High Resolution Transmission Electron Microscope
HVCI	High-Volume Cascade Impactor
IC	Ion Chromatography
ICP-AES	Inductively Coupled Plasma-Atomic Emission Spectroscopy
ICP-MS	Inductively Coupled Plasma-Mass Spectrometry
ILAQH	International Laboratory for Air Quality and Health
IPL	Isolated Perfused Rat Lung
LAS-X	Optical Laser Aerosol Spectrometer
LS	Low Sulfur
MARK	Mitogen-Activated Protein Kinase
MAS	Mobile Aerosol Spectrometer
MC	Mass Concentration
MCT	Monocrotaline
MI	Myocardial Infarctions
MIU	Medical Institute of Environmental Hygiene
MMAD	Mass Median Aerodynamic Diameter.
MMD	Mass Median Diameter
µg	Micrograms (10^{-6} grams)
µg/m^3	Microgram per cubic metre
µm	Micrometres (10^{-6} metres)
NAAQS	National Ambient Air Quality Standard
NACN	Acetyl Cysteine
NASA	National (US) Aeronautics And Space Administration
NAG	N-Acetyl Glucosaminidase
Nano-TDMA	Nano-Tandem Differential Mobility Analyser
NC	Number Concentration
NF	Nuclear Factor
Nm	Nanometres (10^{-9} Metres)
NMD	Number Median Diameter
NMHC	Non-Methane Hydrocarbons

NMHC	Non-Methane Hydrocarbons
NO	Nitric Oxide
NO_2	Nitrogen Dioxide
NOPL	Naso-Oro-Pharyngo-Laryngeal
NO_x	Oxides of Nitrogen
NYC	New York City
O_3	Ozone
OECD	Organisation for Economic Cooperation And Development
OFA	Oil Fly Ash
OPC	Optical Particle Counters
PAH	Polycyclic Aromatic Hydrocarbons
Pb	Lead
PDTC	Pyrrolidine Dithiocarbamate
PEF	Peak Expiratory Flow
PH	Pulmonary Hypertension
PIXE	Particle Induced X-Ray Emission Spectroscopy
PM	Particulate Matter
$PM_{0.1}$	Mass Concentration of Particles with an Aerodynamic Diameter Less Than or Equal to a Nominal 0.1 Micrometres (Ultrafine Particle).
PM_1	Mass Concentration of Particles with an Aerodynamic Diameter Less Than or Equal to a Nominal 1 Micrometres (Submicrometre Particles).
$PM_{2.5}$	Mass Concentration of Particles with an Aerodynamic Diameter Less Than or Equal to a Nominal 2.5 Micrometres (Fine Particles)
PM_{10}	Mass Concentration of Particles with an Aerodynamic Diameter Less Than or Equal to a Nominal 10 Micrometres (Coarse Particles).
Ppm	Parts Per Million
PTFE	Polytetrafluoroethylene
QHSS	Queensland Health Scientific Services
QUT	Queensland University of Technology
ROR	Reactive Oxygen Radicals
SAS	Statistical Analysis Software
SEM	Scanning Electron Microscopy
SHR	Spontaneously Hypertensive Rats

SMPS	Scanning Mobility Particle Sizers
SO_2	Sulfur Dioxide
SOF	Soluble Organic Fraction
STEM	Scanning Transmission Electron Microscope
SWCL1	Swedish Class 1 Diesel
TB	Tracheobronchial
TEM	Transmission Electron Microscopy
TEOM	Tapered Element Oscillating Microbalance ®
THC	Total Hydrocarbons
TIMS	Thermal Ionisation Mass Spectrometry
TPM	Total Particulate Matter
TSP	Total Suspended Particles
UFCB	Ultrafine Carbon Black
UFP	Ultrafine Particles
UKULSD	UK Ultra Low Sulfur Diesel
ULTRA	The European Project “Exposure And Risk Assessment For Fine And Ultrafine Particles In Ambient Air”. The Studies were Carried Out in Amsterdam, The Netherlands, Erfurt, Germany, And Helsinki, Finland, During Winter and Spring 1998-1999.
ULS	Ultra Low Sulfur
ULSD	Ultra Low Sulfur Diesel
UN/ECE	United Nations/European Commission on The Environment
Urbd	Urban Dust
US EPA	United States Environmental Protection Agency
VOF	Volatile Organic Fraction
WHO	World Health Organization
XPS	X-Ray Photoelectron Spectroscopy
XRF	X-Ray Fluorescence Spectroscopy

1. EXECUTIVE SUMMARY

1.1 PROJECT

A desktop literature review and analysis of health impacts of ultrafine particles was commissioned by the Australian Department of Environment and Heritage with the following purpose:

- In relation to the health effects of ultrafine particles:
  - establish the state of knowledge; and
  - develop recommendations on the research priorities for Australia to address the information gaps for this issue.

- In relation to the link between the sulfur content of diesel fuels and the number of ultrafine particles in diesel emissions:
  - establish the state of knowledge; and
  - develop recommendations on the research priorities for Australia to address the information gaps for this issue; and
  - develop recommendations on appropriate management responses.

1.2 ULTRAFINE PARTICLES: SOURCES, PROPERTIES, AND OCCURRENCE

Ultrafine particles have been defined as those, which are smaller than 0.1 micrometre (μm). It should be kept in mind, however, that the divisions between ultrafine and larger particles, similar to the other divisions between different particle size classes, are somewhat arbitrary. On the one hand there are no rigid boundaries created by nature between these size classes, but on the other hand all natural sources (as opposed to laboratory generators) generate particles with a certain range of diameters – polydisperse particles – therefore there is no sharp boundary delineating the contribution of particles from a given particle source.

What is known?

1. Particles in the ultrafine, and more generally, submicrometre ranges are generated mainly from combustion, gas to particle conversion, nucleation processes or photochemical processes, with some of them being primary (emitted directly by the source) and some secondary in nature (formed in the air from the precursors emitted by the sources).

2. In terms of numbers, the vast majority of airborne particles are in the ultrafine range. The total mass of the ultrafine particles is, however, often insignificant in comparison with the mass of a small number of larger particles, with which most of the mass of airborne particles is associated. The biggest contribution to the particle surface area in turn, is from particles somewhat above the ultrafine size range.

3. Chemical composition of particles is multi-factorial and depends on particle source as well as post-formation processes. The most important chemical properties of particles include elemental composition, inorganic ions and carbonaceous compounds (organic

and elemental carbon). Primary particles generated from combustion processes consist mainly of soot, which is formed from hydrocarbons burning under fuel-rich conditions. The main chemical constituents of secondary particulate matter in urban locations commonly include sulfuric acid and ammonium sulfate, ammonium and other nitrates and organic compounds. There is also a whole suite of trace metals associated with ultrafine particles. Chemical composition of particles differs significantly from place to place and depends on the type of the local sources, relative contributions from the sources and in the case of internal combustion sources, on the fuels on which the sources operate.

4. Since ultrafine particles reach high concentrations in terms of their numbers but their mass is often very small, measurements of particles in ultrafine or broader, submicrometre ranges are more commonly based on particle number rather than mass concentration. Particle number concentration and number size distribution are usually measured in real time, while particle mass concentration, mass size distribution and morphology, require that samples are first collected, and then the properties investigated under laboratory conditions, using appropriate instrumentation. In general, the instrumentation used for particle number concentration and size distribution measurements is complicated and expensive, as the particles which they investigate, can range down to molecular sizes. Analysis of particle chemical composition is almost entirely conducted using sophisticated laboratory instrumentation, which again, requires that a representative sample be collected.

5. Since the sources contributing to the formation of particles in the ultrafine (and more generally, the submicrometre range) and coarse particle size ranges are different, correlation between fine and coarse airborne particles is frequently absent. Furthermore, the ultrafine particle size range tends to dominate particle number size distribution whereas the coarse particle size range tends to dominate the particle mass size distribution. The degree of correlation between particle number and mass depends on specific local conditions, of which the degree of contribution from different sources is of key importance. In general, from the measurements of particle mass, only limited information, or no information at all can be obtained about particle number and vice versa.

6. Particle number concentration levels in clean environments are usually of the order of a few hundred particles/cm^3. Clean environments for the purpose of this report are those, which are not influenced by human activities. In urban environments, background particle number concentrations range from a few thousand to about twenty thousand particles/cm^3. Background concentrations mean the concentrations measured at monitoring stations, which are not influenced by a nearby emission source. Near roads and in the tunnels, vehicular traffic constitutes the most significant urban source of particle number, and particle number concentrations can be ten times higher or more than the background, and can reach or exceed levels of 10^5 particles/cm^3. This is in contrast to PM_{10} and $PM_{2.5}$ mass concentrations, which near roads have been shown to be no more than 25 – 30% above background level (calculated as the difference between the maximum at the road and the background levels). Therefore, people living and working in close proximity to an urban arterial road are likely to be exposed to levels of ultrafine particles well above ‘normal’ ambient levels and only to somewhat elevated PM_{10} and $PM_{2.5}$ levels.

7. Particle number concentration, like the concentration of gaseous pollutants and other surrogates for very small particles, decreases significantly with distance from the road. The relationship between concentration and distance is usually approximated by exponential (or power law) decay. The concentration decreases to the urban background levels at a distance usually not greater than about 300 m from the road.

Recommendations for future work

While there is a general understanding of sources generating ultrafine particles, the range of the particle concentration levels encountered in different environments, the general nature of their chemical composition and the dispersion in atmospheric systems, the two main areas, which require further work include:

1. Developing national and local databases and knowledge of ultrafine particles. This includes local and national:

   - Concentration levels of ultrafine particles and time series of the concentrations.
   - Chemistry of ultrafine particles
   - Source contribution and inventory of primary and secondary ultrafine particles
   - Relationships between different particle metrics (for example particle number and $PM_{2.5}$ concentrations). While most commonly there is only a limited relationship or no relationship, in some local environments such relationships may exist.

   Since all the above ultrafine particle characteristics vary from place to place and depend on a myriad of local conditions, time and season, this local and national knowledge is essential to conduct local risk assessment and for identifying local control and management strategies.

2. Standardisation of measurement techniques and study designs. There is no standardisation in relation to the instruments or techniques used for investigation of ultrafine particles, and therefore it is often difficult to compare the results reported by different studies. Much more developmental work is needed, to enable cheaper, reliable and repeatable measurements of particle number concentrations in submicrometre range, down to the size of a few nanometres.

1.3 ULTRAFINE PARTICLES AND HEALTH

Epidemiological studies

A number of recent epidemiological studies have addressed the association between ambient ultrafine particle concentrations and mortality or morbidity of urban populations. The number of these studies is however relatively small (8). Moreover, the vast majority of these studies were conducted in the framework of the European "Exposure and risk assessment for fine and ultrafine particles in ambient air" (ULTRA) program by the same teams of researchers from Finland, Germany, and the Netherlands.

The studies reviewed are limited to the investigations of the acute health effects of short-term exposure, which evaluate the impact of day-to-day variation in ambient pollution on health. These studies have correlated morbidity and mortality with daily pollution levels. The general approach of these studies was to compare the effects of ultrafine particles to those of fine particles. Study outcomes span the range from mortality counts of populations to changes in specific parameters or biomarkers in individuals. The main findings of the reviewed studies can be summarised as follows:

1. A study conducted in Germany on daily mortality showed comparable and independent increases in mortality in association with fine and ultrafine particles.

2. The mortality data suggest that fine particles have immediate health effects whereas ultrafine particles have more delayed effects. Immediate effects seem to be attributable to respiratory disease mortality whereas delayed effects are based on an increase in cardiovascular disease mortality.

3. Panel morbidity studies with asthmatic subjects indicate that both fine and ultrafine particles are associated with the respiratory health of the exposed population. A decrease of respiratory function (e.g., peak expiratory flow) and an increase in symptoms and medication use are associated with elevated concentrations of ultrafine particles, independently from fine particles.

4. There is an indication that the acute effects of the number of ultrafine particles on respiratory health are stronger than those of the mass of the fine particles.

5. The acute effects of ultrafine particles on respiratory health of adult asthmatics are more severe than those found for children with asthma symptoms.

6. Inflammatory events in the lungs develop over a time scale ranging from hours to days. It is likely that a lag time exists between exposure to ultrafine particles and the acute respiratory health effects of the exposed population. Cumulative effects over 5 days seem to be stronger than same-day effects.

7. There is an association between exposure to ultrafine particles and cardiovascular morbidity in the population with chronic heart diseases. A panel study among subjects with coronary heart disease indicated that there are independent associations between both fine and ultrafine particles and the probability of specific electrocardiogram changes used as an indicator of myocardial ischemia (ST segment depression). The study report increased odds ratios for 45 subjects ranging from 1.03 to 3.29 with 95% confidence intervals ranging from 0.54 to 6.32.

In conclusion: Both fine and ultrafine particles appear to affect health outcomes such as mortality and respiratory and cardiovascular morbidity and appear to do so independently of each other. However, the database at present is too limited (both in numbers of studies and numbers of subjects) and geographically restricted, to allow clear conclusions on the mode of action or generalization to other settings. Further studies are currently under way but more studies in other settings need to be initiated to improve our understanding of ultrafine particles and health outcomes.

Clinical studies

The following conclusions are based on a limited review of the literature relating to controlled exposure studies.

1. *Dosimetry:* There are inconsistent findings in respect of insoluble ultrafine particles crossing the air-blood barrier of the lung to enter the systemic circulation. Data suggest that for any concentration of ambient air ultrafine particles, populations with moderate to severe chronic obstructive pulmonary disease (COPD) will receive an increased dose of airway/lung-deposited ultrafine particles relative to persons with normal health.

2. *Lung Function:* A modest but statistically significant increase in Airway Resistance across groups of healthy and asthmatic subjects with no significant change in FVC (Forced Vital Capacity) and FEV_1 (Forced Expiratory Volume) following diesel exhaust inhalation was reported in one study. The other studies summarised found no change in lung function parameters following exposure to ultrafine particles. Results of controlled exposure studies of effects of ultrafine particles on lung function as measured by plethysmography and spirometry have not been consistent.

3. *Inflammation:* Chemical speciation may be important to the promotion of an inflammatory airway response by ultrafine particles in normal healthy individuals. Magnesium oxide fume, sulfuric acid aerosol and sodium chloride aerosol appear not to generate an inflammatory response. Inhalation exposure to concentrated ambient air particles, diesel exhaust particulate matter and zinc oxide fume have consistently produced neutrophil infiltration of the bronchial airway. Neutrophil infiltration is an indicator of inflammation. Apart from neutrophil infiltration of the bronchial airways and related markers of inflammation at this site, the studies have not shown a consistent pattern of inflammatory response to ultrafine particle inhalation. Acute inflammation is the body's response to tissue injury and a defence against invasion by an infectious organism. Inflammation involves highly complex and dynamic cellular processes and interactions involving the activation and subsequent control and termination of the inflammatory response. These control processes include control over the rates of synthesis and release of cytokines and other proteins that govern the translocation, activation and function of leukocytes. Blood, bronchial biopsies and bronchoalveolar lavage samples represent a snapshot of this time-varying biological environment. Apparent inconsistencies across studies may be due to differences in the elapsed time between exposure and sample collection.

4. *Plausible biological mechanism for associations between ultrafine particles and health effects:* Findings of upregulation of adhesion molecules on airway vascular endothelial cells amongst healthy subjects and elevated fibrinogen concentrations in peripheral blood provides some support for cardiovascular mortality and morbidity. The absence of any worsening of asthma or any other indicator of inflammation amongst asthmatics is difficult to reconcile with epidemiological studies.

Toxicology

The general conclusions from these studies are that there are complex interrelationships between ultrafine particles and materials adsorbed onto them. There are very few studies, which examine particles on their own. Studies have shown that cytokines are produced by these particles as mediators of the inflammatory response. The complex interrelationships between these cytokines have not yet been demonstrated in appropriately designed experimental studies.

All of the studies available to us demonstrate that the primary determinant of the effect of ultrafine particles is their number and their surface area and not the weight of particles present. This means that the traditional use of PM weight measures is inappropriate in evaluation of the likely biological effects of ultrafine particles.

Recommendations for priorities for future Australian studies to address gaps in knowledge in the area of health effects of ultrafine particles in general and in the Australian context

Knowledge has been gained from the epidemiological studies reported to date and improved scientific understanding of the characteristics and dynamics of ultrafine particles in atmospheric systems compared to a few years ago. Future exposure/epidemiological studies are likely to provide much clearer answers on the associations between ultrafine particles and health outcomes. The design of such studies can now target the specifics of ultrafine particles, which differ from other size fractions and characteristics of ambient particulate matter. The recently published World Health Organization "Guidelines for concentration and exposure-response measurement of fine and ultrafine particulate matter for use in epidemiological studies" (WHO 2002), is an example of the progress in understanding how ultrafine particle specifics should be dealt with in study design to provide the most useful data relevant to study outcomes. Specific recommendations for future health outcome studies include:

1. Studies should be conducted over longer periods of observation. This relates both to studies of acute and to studies of short-term effects. Such studies with longer periods of observation would enable comparisons to be made between periods likely to have high exposure and periods likely to have low exposure. Longer periods of observation would also allow an evaluation of the lag phase between exposure and effect.

2. Study designs and statistical approaches used should be such that the effects related to particle size of interest (<0.1 micrometres) could be decoupled from other characteristics of the particles or complex pollutant mixtures.

3. Studies should be conducted with larger sample sizes. Larger samples would enable better modelling of the role of age, sex, and other demographic and clinical variables in the effect of ultrafine particles on the outcome of interest. In addition, studies should specifically target potentially susceptible subgroups such as for example children and provide information on the susceptibility on relevant groups of the population.

4. Taking note of the reported differences in ultrafine particle concentrations and other characteristics between different geographical locations (resulting from the differences in the local sources, their strength and characteristics, meteorology, topography, etc), as well as the differences in demographic, socio-economic and urban use factors, etc, it is expected that the type and the magnitude of the responses will differ between different locations. Therefore it is recommended that health outcome studies should be conducted in selected places in Australia to quantify the relationship between exposure to ultrafine particles and health outcomes in an Australian setting. The outcomes of such studies would provide appropriate guidance to the decision makers on the most desirable steps in controlling exposure to ultrafine particles in Australia.

1.4 LINK BETWEEN SULFUR CONTENT OF FUEL AND ULTRAFINE PARTICLES

Nanoparticle formation and emissions in diesel combustion process

1. Size and concentration of nucleaction mode particles

- The nucleaction mode extends through sizes from 3 to 30 nm (0.003-0.03 μm). This range places nucleaction mode particles entirely within the nanoparticle range.
- The maximum concentration of nucleaction mode particles occurs at 10-20 nm.
- The nucleaction mode, depending on the engine technology and particle sampling technique, typically contains only 0.1-10% of the total PM mass, but it often includes more than 90% of the total particle count. Sometimes the nucleaction mode particles represent as much as 99% of the total particle number.

2. Chemical Properties:

- The nature of nucleaction mode particles continues to be the subject of laboratory research.
- Nucleaction mode particles and accumulation mode particles are externally mixed across a wide size range, with the chemical components being distributed between two particle types: (a) “less volatile” particles, probably comprised of an elemental carbon core with a small organic component; and (b) “more volatile” particles.
- The volatility of the diesel nanoparticles was found to resemble that of C24-C32 normal alkanes, which implies a significant contribution of lubricating oil to these particles.
- The organic component of total diesel particles and nucleaction mode particles appears to be comprised predominantly of unburned lubricating oil, whereas the fuel contribution to the total organic component appears to be relatively small, no more than 20 % and probably much less.

3. *What influences the nucleaction mode particles:*

- The nucleaction mode is much more sensitive to engine operation, dilution and sampling conditions than is the accumulation mode.
- Cold temperatures favored nucleaction mode formation.
- The formation of nanoparticles from particle precursors is influenced by the residence time in the dilution tunnel or exhaust system. Short residence time in the exhaust and sampling system prior to dilution favor nanoparticle formation, while short residence time in the dilution system suppresses nanoparticle growth.
- Storage and release of volatile material in the exhaust system, and prior engine operating history influence the formation of nucleaction mode particles.

4. *Control and mitigation:*

- Engine technology effects were observed to be larger than fuel effects for accumulation mode particles, which reflected the observations for particle mass. Fuel effects were observed to be greater than engine technology effects for nucleation mode particles, which reflected the observation for particle numbers.
- Diesel particle filters (DPFs) can effectively remove accumulation mode (solid) particles from the exhaust, but can emit volatile precursors that lead to nanoparticle formation and a large nucleation mode under high load conditions.

Influence of the fuel sulfur level on nanoparticle formation

1. Sulfuric acid nanoparticles form as a result of condensation of hydrated sulfuric acid. They are formed from gaseous precursors as temperature decreases in the exhaust system, and after mixing with cold air, be it in the laboratory dilution tunnel or in the ambient air. The diameter of the original nucleus is believed to be about 1 nm.

2. Fuel sulfur enhances nucleation but is not the major component of the nucleaction mode.

3. Nanoparticles are more easily formed when fuels with high sulfur content are used, but under some engine conditions, such as light load, nucleaction mode formation is independent of fuel sulfur content and heavy hydrocarbons such as those found in lubricating oil could play a major role.

4. Particle number emissions with low sulfur fuels (below 50 ppm) can be up to 100 times lower than with high sulfur fuels (500 ppm), while the particle mass emissions remain almost the same.

5. The reduction of particle number emissions with reduced fuel sulfur content is greater in engines that emit a smaller concentration of accumulation mode particles, smaller mass emissions (new technology vehicles or vehicles with DPFs).

6. The reduction in particle number emission with the reduction of sulfur level will not show any statistically significant change as the vehicles reach a certain age.

Recommendations for future investigations

1. All of the studies except one examined only several vehicles/engines from a limited fleet with most of the engines of a newer design. In order to assess the magnitude of the problem an investigation should be designed to better represent the current and future fleets.

2. New engine designs and after-treatment technologies will present new particle production challenges and solutions. These should be investigated.

3. The reduction of fuel sulfur level is very often accompanied by a significant change in other fuel properties such as aromatic content and volatility. In many of the studies, so far, these parameters were not decoupled. The specific influence of fuel and lubricants should be studied by testing matrices where key parameters of interest, such as sulfur, volatility and aromatic content are decoupled.

4. The effect of not only fuel but also lubricant sulfur content should be studied to determine the influence of this parameter on the formation and emissions of nanoparticles.

5. Further work is required to develop sampling and measurement standards for particle size and number so that comparable datasets can be produced. For this purpose assessment and adaptation of the existing instruments and techniques should be conducted.

Recommendations on Management Response

Since sulfates are just one of several components of the particle mass (PM) emissions, lowering fuel sulfur levels has only limited potential as a means of PM control. The reduction of diesel fuel sulfur levels from 3000 ppm to 500 ppm, as legislated in the U.S. in 1994, yielded relatively large benefits of about 0.04-0.08 g/bhp-hr PM reduction. However, a further reduction of fuel sulfur from the 500 ppm to lower levels has only small incremental PM reduction benefit of about 0.008-0.016 g/bhp-hr. The main benefit in reducing sulfur levels further below 500 ppm towards 50 ppm and lower will be in the reduction in particle number emissions. This reduction will be in the number of particles emitted in the nanoparticle range. Further, to achieve EUROIV and even EUROIII standards of emissions new diesel emission control technologies have to be implemented (aftertreatment devices such as DOC, DPF, etc.). The influence of the sulfur level on the emission of nanoparticles with after-treatment devices is still unknown.

Previous studies have shown that the reduction of nanoparticle emission with the reduction of fuel sulfur level below 500 ppm depends on the age/mileage of the vehicle. In order to assess the scale of the problem for the whole Australian diesel fleet more data are needed on the reduction of nanoparticle emission as a function of age/mileage of the vehicles. The only available scientific data come from a single study, which was conducted on only one type of vehicle present in the diesel fleet (buses).

2. PURPOSE AND SCOPE OF THE REPORT

2.1 THE PURPOSE OF THE REVIEW

A desktop literature review and analysis of health impacts of ultrafine particles was commissioned by Australian Department of Environment and Heritage with the following purpose:

- In relation to the health effects of ultrafine particles:
  - establish the state of knowledge; and
  - develop recommendations for research priorities for Australia to address the information gaps for this issue.

- In relation to the link between the sulfur content of diesel fuels and the number of ultrafine particles in diesel emissions:
  - establish the state of knowledge; and
  - develop recommendations for research priorities for Australia to address the information gaps for this issue; and
  - develop recommendations for appropriate management responses.

2.2 THE SCOPE OF THE REVIEW

As specified by the Australian Department of Environment and Heritage, work to achieve the objectives of this study was summarised as follows:

- Undertake a literature search to identify relevant high quality studies that have been conducted, are underway or are proposed on:
  - the health impacts of ultrafine particles; and
  - the relationship between the sulfur content of diesel fuels and the number of ultrafine particles in diesel emissions.
- Consult widely with stakeholders as needed within Australia and overseas to identify relevant research and obtain views on information gaps and research priorities for both topics.

- Conduct a literature review for both topics, fully referencing all material and using only information from recognised research.

- Recommend priorities for future Australian studies designed to address information gaps on both topics.

- In relation to the link between the sulfur content of diesel fuels and the number of ultrafine particles in diesel emissions:
  - assess the scale of the problem; and

- make recommendations for management responses considered necessary, taking into account the existing framework for managing air quality in Australia.

3. PREPARATION AND ORGANISATION OF THE REPORT

3.1 GENERAL COMMENTS ABOUT THE REPORT

To better understand the material presented in this report and its relation to the overall scope of work, two specific points are emphasised:

Terminology used

The focus of this report is on one specific size fraction of airborne particle matter, which is *ultrafine particles*, defined as those with diameters below 0.1 μm. Particles in this size range are monitored usually in terms of their number concentration (number of particles per unit volume of air). The monitoring instruments available for number concentration monitoring, usually, however, do not have cut-off points of 0.1 μm. This means that they do not measure concentrations of particles that are just within diameters below this value. Most commonly, as explained in section 4.3, they measure concentrations in a much broader window, covering some fraction of the *submicrometre* range (particle sizes below 1 micrometre or even broader than this). Under most circumstances, ultrafine particles constitute the main contribution to the total particle concentration of such windows (see section 4.2). However, it would be technically incorrect to refer to such measurements or investigations as dealing with ultrafine particles only. In most cases, the correct terminology in relation to ambient atmospheric measurements or to exposure and epidemiological studies is particle number concentration within the specified size range investigated. Similarly, in relationship to the investigations of particle formation in the process of diesel combustion and the role of fuel sulfur content on this process, the division most commonly used is not between ultrafine particles and larger, but between particles belonging to nucleaction or accumulation modes. This division is directly related to particle formation mechanisms and, consequently, their chemistry. All nucleaction mode particles are ultrafine particles: however, only some fraction of accumulation mode particles is within the ultrafine range, while the rest is outside this range. It was required in this report that correct terminology was to be used to avoid confusion on one hand; but, on the other hand, it was also required that the role of ultrafine particles, within the overall size windows investigated and in relation to different modes was clearly explained.

Exclusion from the report

- Since the focus of the report is on ultrafine particles, or as explained above, in a more general sense, on particle number concentration, studies on particle mass concentrations, most commonly, PM_{10} or $PM_{2.5}$ and health effects due to exposures to these particle size fractions are not discussed in this report. In a number of places in this report, however, references are made to other particles size ranges for various purposes. For example in chapter 3, a general overview is provided of airborne particle matter and all particle sizes are discussed in this context.

- In the review of the links between health effects and ultrafine particles, studies that did not directly investigate ultrafine particles or their specific, well-defined,

components or fractions or studies that linked health effects to complex mixtures of pollutants are excluded. The authors of this report are of the opinion that even if ultrafine particles were important components of such mixtures, the absence of good characterisation and quantification of the composition of the mixtures means that the effects related to ultrafine particles cannot be decoupled. The effects from ultrafine particles cannot be decoupled from the effects of other components of the mixtures on the one hand, and from possible synergistic (or antagonistic) effects in different components of the mixture on the other hand. These could be different to those caused by ultrafine particles.

4. OVERVIEW OF ULTRAFINE PARTICLES

Airborne particulate matter is a complex mixture of particles ranging in size over five orders of magnitude: from molecular dimensions to the sizes that are distinguishable with the naked eye. The particles may be in liquid or solid state and may differ in other physical properties such as shape, surface area, electrical charge, light scattering properties, etc. In addition, there are substantial differences in particle chemical properties and thus in the toxicological and carcinogenic effects they cause. These effects depend primarily on the origin of the particles, and thus on their sources; but they also depend on post-formation processes.

Before discussing health effects related to human exposures to ultrafine particles and the relationship between the sulfur content of diesel fuels and the number of ultrafine particles in diesel emissions – the foci of this report – it was considered important to provide some background information on ultrafine particles and to discuss these particles in the broader context of all airborne particulate matter.

This chapter provides a brief overview of the key aspects necessary to develop a general understanding of airborne particles. It includes classification into different size fractions, source contribution to various size fractions and source identification. It also includes the physical and chemical properties of the particles and their behaviour in atmospheric systems, particularly during transport from the emission sources. A review of measuring methods for ultrafine particles and more generally in relation to particle number concentration is also provided. Finally, the current state of knowledge on ultrafine particles is summarised.

4.1 PARTICLE CLASSIFICATION BY SIZE

Various classifications and terminologies have been used to define particle size ranges. The division most commonly used is between **fine** and **coarse particles**, with the boundary between these two fractions being widely accepted as 2.5 μm. Fine particles are smaller than this and coarse particles are larger. **Ultrafine particles** have been defined as those smaller than 0.1 μm. Another classification is into **submicrometre** particles, which are smaller than 1 μm, and **supermicrometre** particles, which are larger than 1 μm. The terminology that has been used in the wording of the ambient air quality standards, and also for characterization of indoor and outdoor particle mass concentrations includes the **$PM_{2.5}$** and **PM_{10}** fractions. $PM_{2.5}$ (fine particles) is the mass concentration of particles with aerodynamic diameters smaller than 2.5 μm. PM_{10} is the mass concentration of particles with aerodynamic diameters smaller than 10 μm (more precisely the definitions specify the inlet cut-offs for which 50% efficiency is obtained for these sizes). There have been references made in the literature to PM_1 or $PM_{0.1}$ fractions, which imply mass concentrations of particles smaller than 1 and 0.1 micrometres, respectively. These terms should be used with caution, as particles below 1 micrometre, and, even more importantly, those below 0.1 micrometres, are more commonly measured in terms of their number rather than their mass concentrations (as discussed below). Therefore these terms are misleading.

A classification, which is related to particle formation mechanisms, but which also implies particle size range, is the division of the size distribution into modes, which

correspond to peaks within particle size distributions. The location of such modes is variable, depending on the specific sources and other local atmospheric conditions. Particles can be classified into the following modes:

- **Nucleaction mode**: particles in this mode are formed by nucleation of atmospheric gases in a supersaturated atmosphere and their size is of the order of nanometres.

- **Accumulation mode**: particles in this mode originate from primary emissions as well as through gas to particle conversion, chemical reactions, condensation and coagulation.

- **Coarse mode**: particles generated by mechanical processes.

It should be kept in mind that the divisions between the different particle size classes are somewhat arbitrary. On the one hand there are no natural boundaries between these size classes. On the other hand all natural sources (versus laboratory sources) produce particles within a certain range of diameters (polydisperse particles). Therefore there is no sharp boundary delineating the contribution of particles from a given particle source. This argument also extends to effects produced by the particles. For example it cannot be expected that there will be much difference between 0.09 and 0.11 micrometre particles in terms of their composition and behaviour in atmospheric systems, nor in their penetration into the lung or the health effects they cause, despite the second particle being outside the defined ultrafine range.

4.2 SOURCES AND CHARACTERISTICS OF AMBIENT PARTICLES

Particles in ambient air are mixtures generated by a large number of sources: motor vehicles, power plants, wind blown dust, photochemical processes, cigarette smoking, nearby quarry operation, etc. A primary particle is a particle introduced from the source into the air in solid or liquid form, while a secondary particle is formed in the air by gas to particle conversion. Particles in the ultrafine and, more generally, in the submicrometre ranges are generated mainly from combustion, gas to particle conversion, nucleation processes or photochemical processes. Some of these are primary and some secondary in nature. Particles in supermicrometre size ranges result mainly from mechanical processes and are generated as primary emissions.

Physical properties of particles and the relationships between the properties

The most important physical properties of aerosol particles include: number concentration, number size distribution, mass concentration, mass size distribution, surface area, shape and electrical charge. To a large extent these are the physical properties of the particles that underlie particle behaviour in the air and ultimately removal from atmospheric systems. The efficiency of various forces acting on particles and the processes to which they are subjected in the air depends strongly on the particle physical properties of which size is one of the most important. The health and environmental effect of particles is strongly linked to particle size. It is their size that is a predictor of the region in the lung where the particles will deposit and of the outdoor and indoor locations to which the particles are able to penetrate or be transported. Sampling of particles and choice of

appropriate instrumentation and methodology is primarily based on particle physical properties.

Particles suspended in the air range in size from about 0.001 μm to about 100 μm (Baron and Willeke, 2001): the former is a molecular size and the latter is the size above which particles sediment rapidly due to gravitational force and are thus removed from the air. Almost all of the sources generate particles with a range of sizes, (so called poly-disperse particles) rather than particles of a single size (monodisperse particles). The spread of particle size distribution is characterised by an arithmetic or geometric (logarithmic) standard deviation. The most common methods of characterisation of a particle distribution are in terms of its size: mean size - which is the average of all sizes, median size - which means equal number of particles above and below this size, or modal size - which is the size with the maximum number of particles. The terms used include: count, number or mass median diameter - which are abbreviated as CMD, NMD or MMD, respectively. MMAD is mass median aerodynamic diameter.

Particles generated by most sources have a lognormal size distribution, which means that the particle concentration versus particle size curve is "normal" (bell shaped) when the particles are plotted on a logarithmic scale. Geometric standard deviation characterises the width of the peak in the distribution. When a single pollution source is investigated and when it operates under steady conditions (for example steady parameters of the combustion process), the size distribution obtained is likely to have one distinctive peak and sometimes additional, usually much smaller peaks. Those peaks are called modes of the distribution. Different emission sources are characterised by different size distributions. However, these distributions are not unique to these particle sources alone. For such mixtures of particles of different size distributions, the measured distribution may or may not display individual peaks from the contributing sources, and thus may or may not be used for source identification (source signature). In many cases however the characteristics of size distribution can be a useful tool in source characterisation

Particle distributions are most commonly presented either in terms of number or mass distributions, or sometimes as surface distributions. The relationships between particle number, surface area and mass are presented in Figure 4.1 based on an example of a typical urban air particle size distribution measured in Brisbane (Morawska, 2000). This relationship was derived using measured particle number size distribution and calculating particle surface and mass distribution assuming their sphericity and density being equal to 1 g/cm^3.

The form of presentation of particle size distributions used in the left hand side of Figure 4.1 is quite common, but is somewhat simplistic. It does not properly reflect the logarithmic nature of the distribution. A proper way for presenting particle distributions, and that which is most commonly used in the literature in representing size distributions of aerosols is by plotting in a logarithmic scale $d\mathrm{N}/d\log D_P$, $d\mathrm{A}/d\log D_P$, and $dM/d\log D_P$, which represent particle number, surface and mass respectively, per logarithmic interval of size, as shown in the right hand side of Figure 4.1.

It can be seen from Figure 4.1 that, in terms of number, the vast majority of airborne particles are in the ultrafine range. The total mass of the ultrafine particles is, however,

often insignificant in comparison with the mass of a small number of larger particles with which most of the mass of airborne particles is associated. Therefore the peak in the number distribution spectrum appears in the area where there is almost no mass in the mass distribution spectrum and vice versa. The peak in the mass distribution spectrum is where the particle number is very low. Particle surface area, in turn, is the largest for particles somewhat above the ultrafine size range.

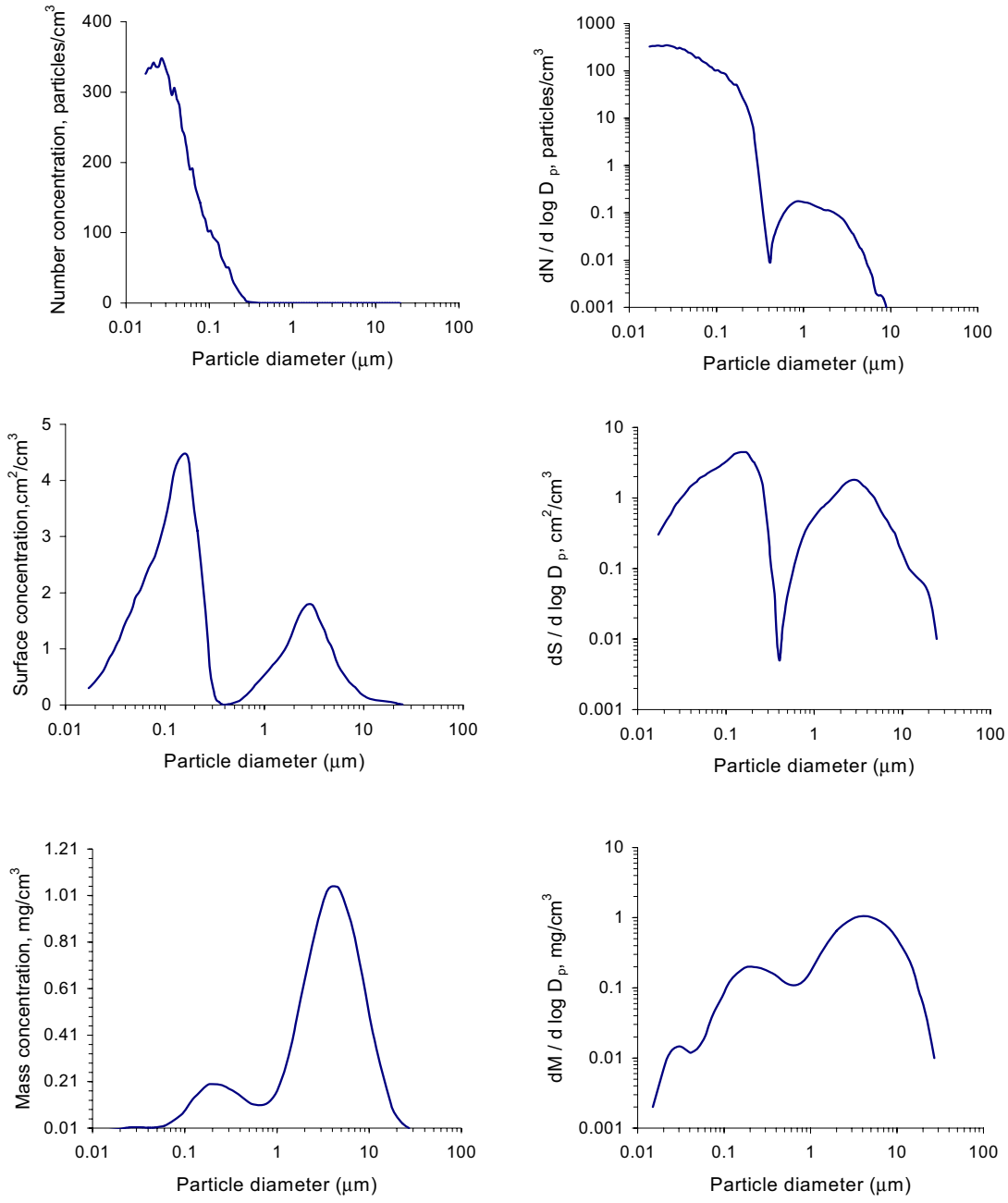

Figure 4.1 Typical - measured in Brisbane - urban air particle number size distribution (top two graphs) - calculated from the number distribution, surface (middle graphs) and mass/ size distributions (bottom graphs), respectively. Two different representations of vertical axis are used for each pair of size distributions.

Particles generated by combustion sources, including vehicle emission, are generally small. A significant proportion of diesel emission particles have diameters smaller than 0.1 μm (Morawska, 1998; Ristovski, 1998). Gasoline particles are mostly agglomerates ranging from 0.01 – 0.08 μm. Particles from CNG emissions are smaller than from diesel or even petrol emissions and range from 0.01-0.07 μm, with majority being between 0.020 and 0.060 μm. (Ristovski, 2000). The majority of particles emitted from biomass burning, which includes controlled burning and uncontrolled fires, are ultrafine, with only a small fraction in the larger size range, and with most of the mass present in particles less than 2.5 μm in aerodynamic diameter (WHO, 1999). Figure 4.2 presents examples of size distributions of particles generated from various combustion sources including vehicles operating on diesel and petrol, forest fire (vegetation burning) and environmental tobacco smoke.

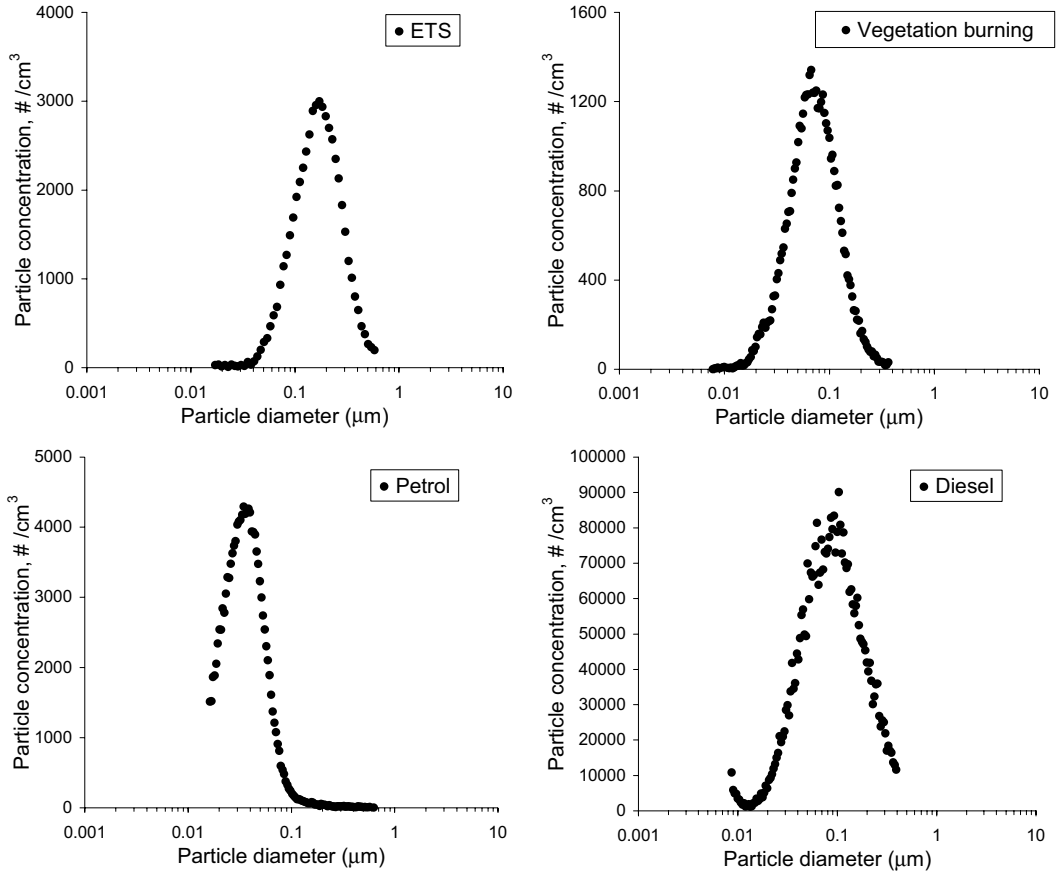

Figure 4.2 Size distribution of environmental tobacco smoke, vegetation burning, petrol smoke and diesel smoke.

As discussed above, particles in different size ranges result usually from different generation processes and only occasionally the same source generates particles with broad size distributions covering both fine and coarse ranges (for example in close proximity to forest fires there are airborne combustion products of the fire as well as large diameter particles that are entrained into the smoke column). Thus different sources contribute to generation of particles in the submicrometre range which is predominant in particle

number, and different sources contribute to larger particles, which predominate in mass. Therefore, it is only occasionally that there is a correlation between fine and coarse airborne particles, or a correlation between particle number and mass. Figure 4.3 presents a scatter-plot of particle data in terms of PM_{10} and particle number in the submicrometre size range collected over a period of two years in downtown Brisbane (Morawska, 1998). As can be seen, there is no correlation between these two particle characteristics which indicates that different sources contribute to generation of particles in the submicrometre range and to the larger particles. An example of a somewhat better correlation is shown in Figure 4.4 where the linear relationship shown in the lower part of the figure presents the number concentration values (right hand side vertical axis) plotted against simultaneously measured PM_1 mass concentrations (Morawska, 1999). While the correlation appears to be better than for PM_{10}, still only less than 25 % of the variance is accounted for by assuming a linear relationship. The correlation is better because both PM_1 and particle number are generated by the same sources which are mainly combustion and gas to particles conversion process, with only very little contribution to PM_1 from the sources generating coarse particles. The correlation is still quite poor because the instruments measuring particle number and mass operate on different physical principles, have different averaging times, have somewhat different cut-off points, have different sensitivity to particles in different size ranges, and the TEOM (measuring particle mass) underestimates the mass due to the evaporation of some fraction of semi volatile particles (particle measurement methods are discussed in more detail below).

Various studies conducted on correlation between different particle characteristics, and in particular on correlation between mass of particles in different size ranges, often point out better correlations than those presented above. The degree of correlation depends on specific local conditions, of which the degree of contribution from different sources is of key importance. Better correlations are achieved for conditions when the majority of particles in the fine and coarse size ranges are related to the same source. In general, only limited information or no information at all can be obtained about particle number from the measurements of particle mass and vice versa.

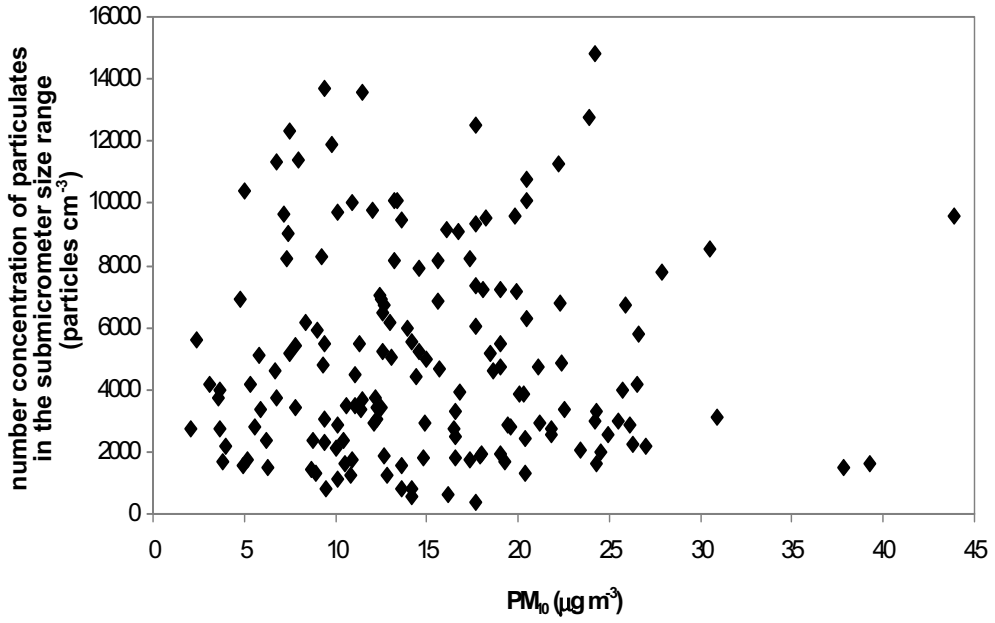

Figure 4.3 Number concentration of particles in the size range 0.016 to 0.626 µm versus PM_{10} mass concentration (Morawska, 1998).

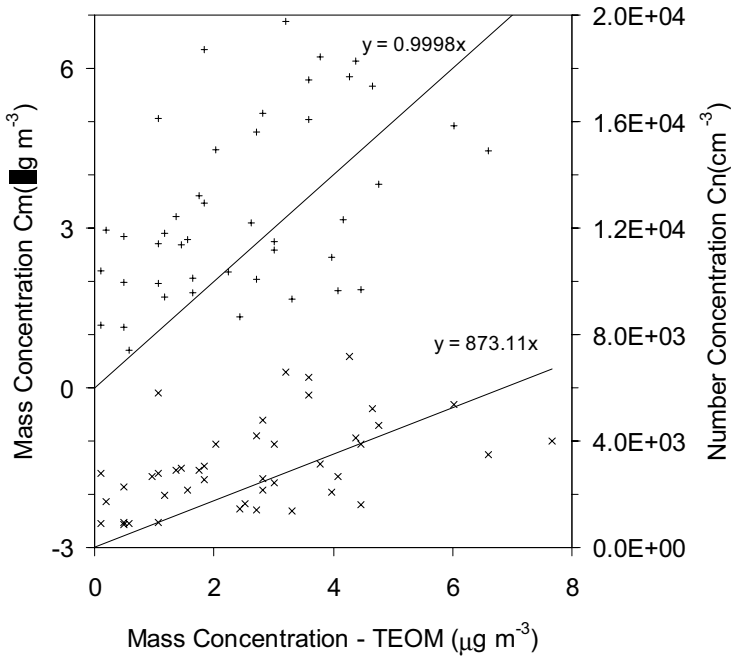

Figure 4.4 Submicrometre mass "+" (left hand side vertical axis) and number concentrations "x" (right hand side vertical axis) derived from SMPS measurement versus PM_1 mass concentration measured by TEOM. (Morawska, 1999)

Summary of reported particle number concentrations

Over the last few years an increasing number of studies have reported particle number concentrations for various places and environments around the world. Again, in most cases for which particle size distributions were reported, it was also seen that ultrafine particles were the dominant fraction in the total number concentration measurement. However, in all the cases reported, the measurements covered broader windows than the ultrafine particles. Table 4.1 summarizes the reported particle number concentration levels in various places in Australia and Figure 4.5 compares background concentrations with the concentrations in the vicinity of the roads measured in Australia and in other countries in a graphical form for easier comparisons.

It can be seen from Table 4.1 and Figure 4.5 that particle concentration levels in clean environments are of the order of a few hundred of particles/cm^3. However, under certain circumstances, particularly as a result of particle generation through natural biogenic activities, the concentrations can be higher, up to several thousand of particles/cm^3 (see also Figure 4.6 below). Clean environments for the purpose of this report mean those which are not influenced by human activities.

Particle number concentrations in urban background environments range from a few thousand to about twenty thousand particles/cm^3. Background concentrations mean the concentrations measured at monitoring stations which are not influenced by local emission sources operating in their immediate proximity.

Table 4.1 Particle number concentration levels in various places in Australia

Location (Reference)	Average particle number concentrations* (particles/cm^3)	Maximum particle number concentrations (particles/cm^3)
Cape Moreton (Johnson, 2003)	~ 0.8 x 10^3	~4 x 10^3
Urban concentrations in six Australian cities (Ayers, 1998)	10^4 – 5 x 10^4	
Immediate vicinity to busy roads (QUT, 2002)**		1.8 x 10^5
Brisbane CBD (away from emission sources) (Morawska, 1998	7.40 x 10^3	4 x 10^4
Brisbane (150 m downwind from busy road) (Hitchins, 2000)	1.8 x 10^4	6 x 10^4

*Particle size ranges differed between studies
**This site could be classified as a road site

In proximity to roads or in tunnels where vehicle traffic constitutes the most significant source of particle numbers, particle concentrations can reach and exceed levels of 10^5 particles/cm^3. The actual levels depend in the first instance on traffic conditions on the road (traffic flow and mode), and on meteorological conditions and the topography of the site. Concentrations are higher in the street canyons for example, and under calm

conditions with low wind speed. Dispersion of particles is lower under such conditions which results in a build up of higher concentrations.

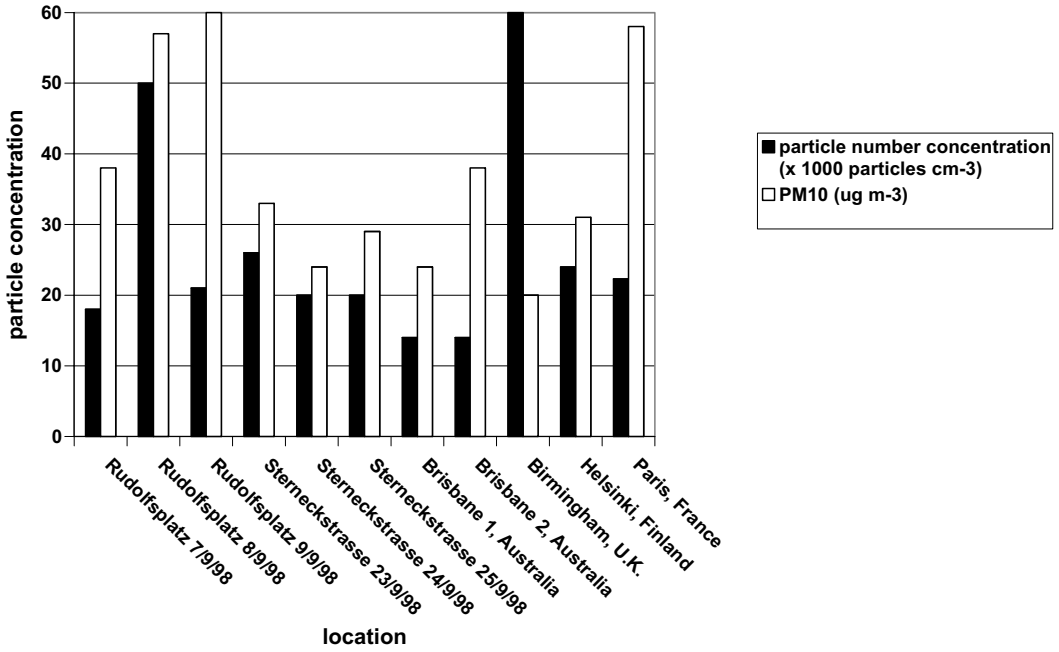

Figure 4.5 Particle number and mass (PM_{10}) concentrations measured at the roadside locations (a) and at the non-roadside locations (b) (Morawska, 2003). In the figure: University, Rudolfsplatz and Sterneckstrasse are sites in the city of Salzburg, Austria and Gaisberg Mountain and Tamsweg are sites in the Alps, outside Salzburg.

Chemical composition and its relation to particle formation mechanisms

The chemical composition of particles is multi-factorial and depends on particle sources as well as post-formation processes. The most important chemical properties of particles include:

- Elemental composition
- Inorganic ions
- Carbonaceous compounds (organic and elemental carbon)

Interest in the elemental composition, in general, derives from the potential health effects of heavy elements like lead, arsenic, mercury and cadmium, and the possibility of using the elements as source tracers. Water-soluble ions such as potassium, sodium, calcium, phosphates, sulfates, ammonium and nitrate associate themselves with water in the indoor environments and can also be used for source apportionment. Carbonaceous compounds are composed of organic and elemental carbon. The former can contain a wide range of compounds such as polycyclic aromatic hydrocarbons, pesticides, phthalates, flame retardants and carboxylic acids some of which are tracers for certain sources while the latter is sometimes termed “soot”, “black carbon” and “graphitic carbon”.

Particles arising through different formation mechanisms display significantly different properties both physical (as discussed above) and chemical. In particular, there are substantial differences in the chemistry of primary and secondary particles.

Primary particles generated from combustion processes constitute mainly of soot, which is formed from hydrocarbons burning under fuel-rich conditions. Soot formed under such conditions appears most commonly as an ensemble of ultrafine particles, but the size of the agglomerates can extend up to a few hundred nanometres (Bockhorn, 2000). The formation of soot, which is the conversion of a hydrocarbon fuel molecule containing few carbon atoms into carbonaceous agglomerate containing some millions of carbon atoms, is an extremely complicated process. It is a gaseous-solid phase transition where the solid phase exhibits no unique chemical and physical structure. Therefore, soot formation encompasses chemically and physically different processes, including formation and growth of large aromatic hydrocarbons and their transition to particles, the coagulation of primary particles to larger aggregates, and the growth of solid particles by picking up growth components from the gas phase. The above-mentioned processes constitute the formation of the bulk of soot. In addition, numerous other processes decide on the "fine structure' of soot. More details on the mechanisms of soot formation are discussed by Bockhorn (2000).

Secondary particles are generated through the following processes (Baron and Willeke, 2001; Bockhorn, 2000): (i) gas-phase chemical reactions occur involving specific precursors - gases produce low-volatility products which are capable of homogenous nucleation to form new particles which are of molecular sizes that can then increase in size by coagulation and are captured by pre-existing ambient particles; (ii) low-volatility gas-phase reaction products condense onto pre-existing ambient particles - the so-called heterogeneous nucleation process. While homogenous nucleation may potentially increase both the number and the mass of aerosol particles per unit volume in the atmosphere, heterogenous nucleation can only increase the mass. Interest in the phenomena of secondary particles and the need to develop a good understanding of the mechanisms leading to the formation of these particles has significantly increased along with the realisation of the effect of sulfur content in diesel fuels on formation of secondary sulfuric particles of nanometre sizes (this process is described in more detail in chapter 6). What follows is a general overview on secondary particle formation.

The main chemical constituents of secondary particulate matter in urban locations commonly include sulfuric acid and ammonium sulfate, ammonium and other nitrates and organic compounds (Derwent, 2000). The sulfur and nitrogen containing secondary particle constituents are largely derived from the processes occurring during combustion of fuel containing sulfur and through photochemical oxidation of man-made SO_2 and NO_x precursors. In contrast, the organic constituents appear to have been derived from natural biogenic precursors. The natural biogenic hydrocarbons play an important role in the formation of tropospheric aerosols. The sunlight driven photo-oxidation of high-molecular weight hydrocarbons has been shown to produce low vapour pressure reaction products that partition between the gaseous and aerosol phases. In the aerosol phase these reactions products are known as secondary organic aerosols. Of the natural biogenic hydrocarbons, terpenes have been shown to be effective sources of SOAs (Hoffmann, 1997), while of the man–made hydrocarbons, aromatics are the most important sources (Odum, 1996). Figure 4.6 presents changes to

ambient particle concentration and size distribution attributed to particle formation from biogenic precursors. The evolution in particle size distribution of ambient particles was measured deep inside the Brisbane Forest Park approximately 7-8 km away from any anthropogenic source on a sunny day with temperature around 20-25^0 C. It can be seen from the figure that there was a significant increase in particle concentration between the first and subsequent measurements, with a clear peak forming of count median diameter around 0.04 μm. This peak was attributed to particles formed from natural biogenic hydrocarbons in the process of sunlight driven photo-oxidation (Morawska, 1999).

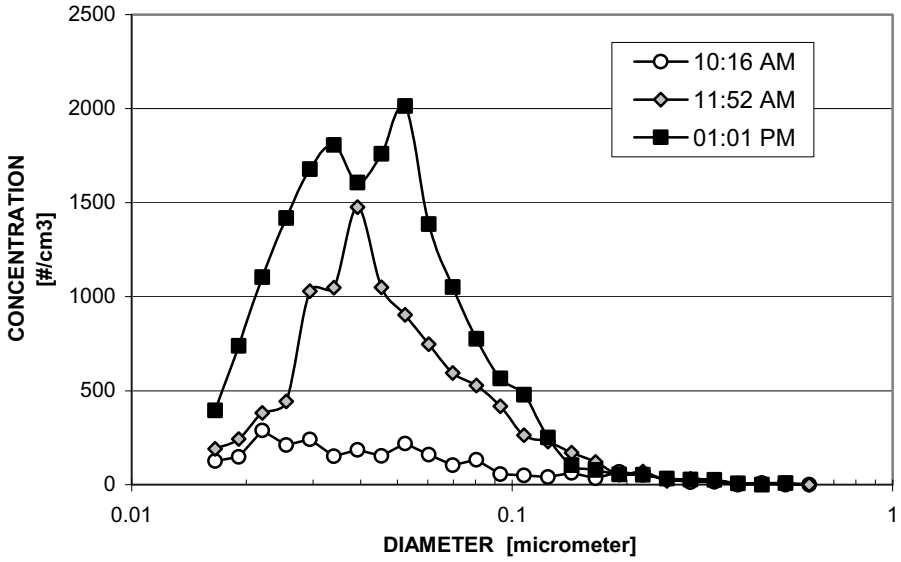

Figure 4.6 Changes to ambient particle concentration and size distribution attributed to particle formation from biogenic precursors measured in the Brisbane Forest Park.

There are still major questions existing in relation to the different mechanisms of the formation of secondary particles, the relative contributions of the different mechanisms and the absolute concentration levels of particles resulting from these processes. For example (Derwent, 2000) summarised the results of modelling of secondary emissions in the UK. A highly sophisticated Lagrangian dispersion model has been used in the UK to describe the formation of particle sulfate by the photochemical oxidation of SO_2. A comparison of model particle sulfate with observations for five rural monitoring sites showed overall good agreement. By contrast, formation of secondary organic aerosols is much less understood and the questions asked are of a much more basic nature. It was concluded that the formation of secondary organic aerosols from the photo oxidation of terpenes is likely to be several times greater in magnitude than that from aromatic hydrocarbon photo-oxidation. It was concluded that the major gaps in knowledge in relation to the ambient secondary particles in the UK include:

- Fundamental lack of knowledge as to how much of the ultrafine secondary particulate matter in the UK atmosphere arises by the homogenous or heterogenous nucleation routes.

- There are so few measurements of ultrafine particulate matter in the UK that it would be difficult to check the model performance against observations in any comprehensive manner.

An example of a comprehensive characterisation of size classified particle chemical composition was presented by (Cass, 2000). Measurements of ultrafine particle mass concentration made in seven Southern Californian cities showed that ultrafine particle concentrations in the size range 0.056-0.1 μm aerodynamic diameter (lower stage of the cascade impactor used in the study) average 0.55-1.16 $\mu g/m^3$. The chemical composition of the particles averages (for all the sites) 50% organic compounds (32 – 67%), 14% trace metal oxides (1-26%), 8.7% elemental carbon (3.5-17.5%), 8.2% sulfate (1-18%), 6.8% nitrate (0-19%), 3.7% ammonium (0-9%) ion, 0.6% sodium (0-2%) and 0.5% chloride (0-2%). The most abundant catalytic metals identified in the ultrafine region include: Fe, Ti, Cr, Zn, with Ce also present. The numbers in brackets present the ranges of concentrations for the individual sites, which can be quite large and depend on the local composition of the emission sources.

Combustion emissions – general summary

Of great concern in relation to health risks is the chemistry of emissions from combustion sources. Under ideal conditions, complete combustion of carbon would result only in generation of carbon dioxide (CO_2) and water. Any products other than CO_2 are often called products of incomplete combustion and include particulate matter and gases. Combustion particles are mainly of anthropogenic origin. The majority of them in terms of number are in ultrafine size range (while in terms of mass, in the submicrometre range), of complex chemistry, carrying most of the trace elements, toxins, carcinogens, etc generated by the combustion process. Therefore the discussion below focuses on the chemistry of combustion emissions. A more specific discussion on vehicle emissions is provided in the section below.

Combustion of different types of fuels results in emissions of various trace elements, which are present in the fuel material. In most cases there is not just one specific element that is related to the combustion of a particular fuel, but an entire source profile of elements. Table 4.2 presents examples of the most common source profiles of trace elements related to specific combustion sources. For comparison, the crustal elements include Mg, Ca, Al, K, Sc, Fe and Mn.

Trace elements are often co-emitted with other pollutants which can be more or less volatile, such as many hydrocarbons. Since most of the trace elements are non-volatile, associated with ultrafine particles and less prone to chemical transformations, they tend to undergo long-range atmospheric transport and remain in the form in which they were emitted. To follow the trace elements back to their potential emission sources, air parcel movements in the form of calculated backward trajectories are combined with the measured concentrations using various methods (Gao, 1996). Whether elemental signatures can provide meaningful qualitative and quantitative source apportionment will depend on how independent the regional signatures are, as well as on the variability and stability during long distance transport. Elemental concentrations could be highly variable, therefore the concentrations alone are not considered to be reliable regional tracers. Use of elemental signatures to apportion aerosols into major regional

sources requires an understanding of the behaviour of the trace element bearing aerosols during transport, and also of the seasonal variations of the signatures.

Table 4.2 Characteristic elements emitted from various combustion sources. (Morawska, 2002).

Emission Source	Characteristic Elements Emitted
Oil fired power plants	V, Ni
Motor vehicle emissions	Br, Ba, Zn, Fe, Pb (in countries where leaded petrol is used)
Refuse incineration	Zn, Sb, Cu, Cd, Hg
Coal combustion	Se, As, Cr, Co, Cu Al
Refineries	V
Nonferrous metal smelters	As, In (Ni smelting), Cu
Use of pesticides	As
Iron and steel mills	Mn
Plant producing Mn metal and Mn chemicals	Mn
Copper refinery	Cu

All of the combustion sources generate large amounts of volatile and semi-volatile organic compounds. Semi-volatile organic compounds can be present in the air either in the vapour or in particle from (solid or liquid). Exposure to many of the organic compounds emitted to the air has been associated with various types of health effects.

Polynuclear Aromatic Hydrocarbons (PAH), some of which are strongly carcinogenic, are one class of compounds contained in the organic fraction of the fine particulate matter. PAH compounds are synthesised from carbon fragments into large molecular structures in low-oxygen environments, such as occurs inside the flame envelope in the fuel-rich region of the flame structure. If the temperature is not adequate to decompose compounds upon exiting from the flame zone, then they are released into the free atmosphere and condense or are adsorbed onto the surface of particles. Many different combustion systems are known to produce PAH compounds. The most studied PAH is benzo[a]pyrene (B[a]P), which is a physiologically active substance that can contribute to the development of cancer in human cells.

A compilation of the health effects of selected non-heterocyclic PAH has been published by (World Health Organization (WHO)). The PAHs considered in the document included: acenaphthene, acenaphthylene, anthracene, benzo[a]pyrene, benzo[a]anthracene, dibenzo[a,h]anthracene, fluoranthene, naphthalene, phenanthrene, and pyrene. The US EPA introduced a list of the 16 priority PAHs that include: (acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo[a]pyrene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, naphthalene, phenanthrene, pyrene (Collier et al 1998).

High concentrations of PAHs have been found in soot generated from wood burning stoves and coal burning stoves (Mumford, 1987). PAHs have also been found in gasoline and diesel soot, and the relative abundance of individual PAH species may be different for different types of soot. This makes it is possible to use PAHs as source signatures of different types of fuels on one hand, but may also result in different health effects due to inhalation of emissions from different fuels.

Semi-volatile aliphatic hydrocarbons (ALIsv) present in ambient air are n-C_{19}, n-C_{20}, n-C_{24}, n-C_{25}, while particle related aliphatic hydrocarbons (ALIp) are n-C_{21}, n-C_{22}, n-C_{29}, n-C_{31}, (Colombo, 1999). The aliphatic signal of diesel and gasoline engines consists of a narrow band of C_{15-27} n-alkanes maximising at C_{20-21}, a very similar pattern to lubricating oils n-C_{13-27}, maximising at C_{19}. The signal of diesel fuel has a broader spectrum extending to n-C_{33}, with a higher proportion of lower molecular weight components (n-C_{10-22}, maximising at C_{19}) (Simoneit, 1985).

Other types of semi-volatile compounds include guaiacol and its derivatives (e.g., 4-methylguaiacol, 4-ethylguaiacol) that result solely from the pyrolysis of wood lignin. Guaiacol and most of its derivatives appear to be relatively stable in the atmosphere. Therefore, these compounds can serve as unique tracers of wood smoke (Hawthorne, 1992). Another important semi-volatile example is that of 3-nitrobenz[a]anthracene, a strongly carcinogenic compound present in diesel emissions. Organic acids, of which the major constituents are monocarboxylic (emitted from combustion of fossil fuels and biomass) and diacarboxylic acids (Limbeck, 1999), can also contribute to source apportionment and have been linked to health effects.

Table 4.3 presents a summary of the most common organic compounds emitted by a number of combustion sources. This table was compiled from the summary presented by (Cass, 1998).

From the point of view of the effect on human health, the specific physical form of the semi-volatile compounds when they are inhaled could be of significance. They could be either in vapour form, or could be associated with particles of specific sizes. There is very little information available on this aspect, which is due not only to the recency of scientific interest, but also mainly to the difficulties in investigating organic composition of small amounts of mass. The mass of particles in the fine and ultrafine range is very small and, in order to collect sufficient mass for standard organic chemistry analyses, long sampling times are required, which are logistically and financially prohibitive for most studies of exposure or health effects.

Table 4.3 Summary of the most common organic compounds emitted by a number of combustion sources (Cass, 1998).

EMISSION SOURCE	EMITTED COMPONENTS
Environmental tobacco smoke	Nicotine, iso-alkanes, anteiso-alkanes (anteiso-triacontane, anteiso-hentriacontane, anteiso-dotriacontane, iso-tritriacontane),
Road transport Motor vehicle emissions	Hopanes and steranes (present in lubricating oil for diesel and gasoline vehicles, and in diesel) black elemental carbon (present in a higher fraction in diesel emissions) high molecular PAHs: coronene and benzo[g,h,i]perylene (these are less specific)
Tyre dust	styrene/butadiene copolymer, very high molecular weight even number n-alkanes, benzothiazole
Natural gas powered motor vehicles	some PAHs and oxyPAHs, aldehydes
Vegetation burning Wood combustion	Retene, phytosterols, ligmens, phenolic compounds from lignins, diterpenoids from resins.
Small combustion Meat charbroiling Natural gas fired home appliances	Cholesterol, supplemented by fatty acids benzo[a]anthracene
Vegetative detritus	High molecular weight n-alkanes ranging from C_{27} – C_{34} (high concentration of odd number n-alkanes)

Combustion emissions – vehicles

Vehicle emissions, like other combustion products, are comprised of pollutants in gaseous and particle forms, which are complex in chemistry, and contain many compounds, which have been shown to affect human health. The main gaseous emissions include hydrocarbons (HC), CO, NO_x, CO_2, SO_2 and water vapour. The chemistry of particles originating from vehicle emissions varies and depends on the type of fuel on which the vehicle operates, on its specific composition and on other characteristics, as well as lubricating oil used and its composition. There are thus differences between particles originating from diesel or spark ignition vehicles, the latter including petrol, compressed natural gas (CNG), liquid petroleum gas (LPG) and ethanol fuelled vehicles.

Diesel emission particles are primarily elemental carbon, but they also contain adsorbed or condensed hydrocarbons, hydrocarbon derivatives, sulfur compounds and other materials (Kittelson, 1998). Solvent extractable organic components of diesel aerosols represent 5 – 40% of the particle mass. In general, composition of emitted particulate matter varies greatly and depends on engine technology, test conditions, fuel composition, etc. (Kittelson, 1998). Associated with particles (especially fine and

ultrafine) are many toxins, trace elements and carcinogenic compounds. An example of these is 3-nitrobenzanthrene, a nitrated polycyclic aromatic hydrocarbon (nitro-PAH), which has been shown to have high cancer-causing potential.

Particles emitted from spark ignition vehicles are mostly carbonaceous spherical submicrometre agglomerates consisting of a carbon core with various associated organic compounds. The main components of the particle phase include soot and ash which consist of trace elements such as lead, iron, chlorine and bromine, organic compounds and a low-to-medium boiling fraction of engine oil (Zinbo, 1995). Lubricating oil and other fuel hydrocarbons are the main contributors to emissions of particles of nanometre size (Kittelson, 2002). The sulfate particles present in gasoline engine emissions are mainly from catalyst-equipped vehicles utilising unleaded gasoline (Brodowicz, 1993). Common organic compounds are polycyclic aromatic hydrocarbons (PAHs) such as pyrene, chrysene, benzo[a]pyrene. The semi-volatile fraction of the emissions can be associated either with vapour or with particle phases.

Combustion emissions – wood heaters/woodstoves

Small combustion devices, such as household cooking stoves and space heaters, are counted in billions throughout the world, providing for the very basic household need for heat (see for example: Environment Australia Technical Report No 5, Emissions from domestic solid fuel burning appliances:
http://www.ea.gov.au/atmosphere/airtoxics/publications.html).

The types of fuels and stoves used, however, are very unevenly distributed between developed and developing countries and between rural and urban households. A number of studies characterised the emissions from residential wood burning stoves in developed countries, mainly in the USA. The studies reported that: (1) the particle mass distribution from wood (pine, oak, eucalyptus) combustion have a single mode at approximately 0.1 – 0.2 μm (Kleeman et al., 1999). This means that particle number distribution has its mode below 0.1 μm, thus in the ultrafine range; (2) The particles are compact structures with fractal-like dimensions and contain low mass fractions of volatile compounds (Hueglin et al., 1997); and (3) that operating conditions, such as the amount of air supply, have a strong impact on the particle size distribution and the emission of particle-bound PAHs (Hueglin et al., 1997).

The emissions from wood burning stoves were found to be acidic (pH = 2.8-4.2) (Burnet et al., 1986). Organic compounds were the dominant components of both wood smoke and meat charbroiling smoke. Noticeable elemental carbon was found in wood smoke as well as measurable quantity of Na, K, Fe, Br, Cl, nitrate, sulfate and ammonium. Statistically significant amounts of Na, Al, K, Sr, Ba, Cl, nitrate, sulfate were found in meat charbroiling emissions (Kleeman et al., 1999).

High concentrations of PAHs have been found in soot generated from wood burning stoves and coal burning stoves (Mumford et al., 1987). Although PAHs have also been found in other combustion emissions (gasoline and diesel soot), the relative abundance of individual PAH species may be different for different types of soot. This relative abundance makes it possible to use PAHs as source signatures in receptor modelling for residential wood and coal combustion (Li and Kamens, 1993). Guaiacol and its derivatives (e.g., 4-methylguaiacol, and 4-ethylguaiacol), however, result solely from

the pyrolysis of wood lignin. Guaiacol and most of its derivatives appear to be relatively stable in the atmosphere and therefore these compounds can serve as unique tracers of wood (Hawthorne et al., 1992).

Source inventory

Quantification of emissions from individual pollution sources and generation of emission inventories at local, regional and national levels is important for developing appropriate management and control strategies in relation to air quality. One aim of pollution concentration and emission measurements has been to provide relevant information that would enable a complete inventory of time series to be compiled for all particle size ranges. Estimates of emission inventories and vehicle contribution to the total ambient particle concentration levels have been conducted in many countries and for various urban environments, mainly in relation to TSP or PM_{10}, and less so for $PM_{2.5}$. To date, not enough measurements have been conducted and there are very few data available to compile inventories of vehicle emissions for other particle mass size ranges or for particle number emissions.

One example of a completed inventory is the estimation conducted for source emission contribution to different particle mass size ranges in Europe in 1993 (excluding the former Soviet Union) (Holman, 1999). The following sources were identified as the main contributors to $PM_{0.1}$: road transport (41%), production processes (24%) and power generation (17%). By contrast, the contributions of the first two sources to PM_{10} were only 17 and 14 %, respectively.

Another example is the assessment conducted by the Airborne Particles Expert Group on behalf of the Department of the Environment, Transport and the Regions, the Welsh Office, the Scottish Office and the Department of the Environment (Northern Ireland) (Airborne Particles Expert Group, 1999) An approach was taken such that inventories for $PM_{2.5}$, PM_1 and $PM_{0.1}$ were estimated based on the UK PM_{10} monitoring data and from the mass fractions in these size ranges available for different emission sources and fuel types. While only 33 particle spectra were investigated, the report provides a comprehensive analysis of emission trends for the years 1970 to 1996. After analyzing the contributions of individual combustion sources to particle emission inventories, it was evident that in all size fractions motor vehicle emissions were the major contributor out of all other combustion and non-combustion sources in urban areas. With decreasing particle size, the contribution of road transport to the total emissions increased and for $PM_{0.1}$ it reached 60%. Contributions from other combustion sources tended to decrease with decreasing particle size. One of the conclusions from the data presented in the report is, that there has been a significant decrease in emissions in the PM_{10} and $PM_{2.5}$ ranges during the period of time from 1970 to 1996, less in the PM_1 range and very little in the $PM_{0.1}$ range. This could be related to the increase in the number of vehicles used, as well as to the lack of strategies for decreasing emissions of the ultrafine fraction of particles.

A source emission inventory constructed for the South Coast Air Basin that surrounds Los Angeles (Cass *et al.,* 2000) estimated the primary ultrafine particle emission rate to be 13 tonnes per day. These emissions were attributed primarily to mobile and stationary fuel combustion sources and were estimated to consist of 65% organic compounds, 7% elemental carbon, 7% sulfate, 4% trace elements, with very small

quantities of sodium, chloride and nitrate. Sources contribution to primary ultrafine particles was also estimated and is presented in Figure 4.7. It can be seen from this Figure that the major contributors to the primary particles are on-road vehicles (43.1%) and stationary fuel use (32.2%).

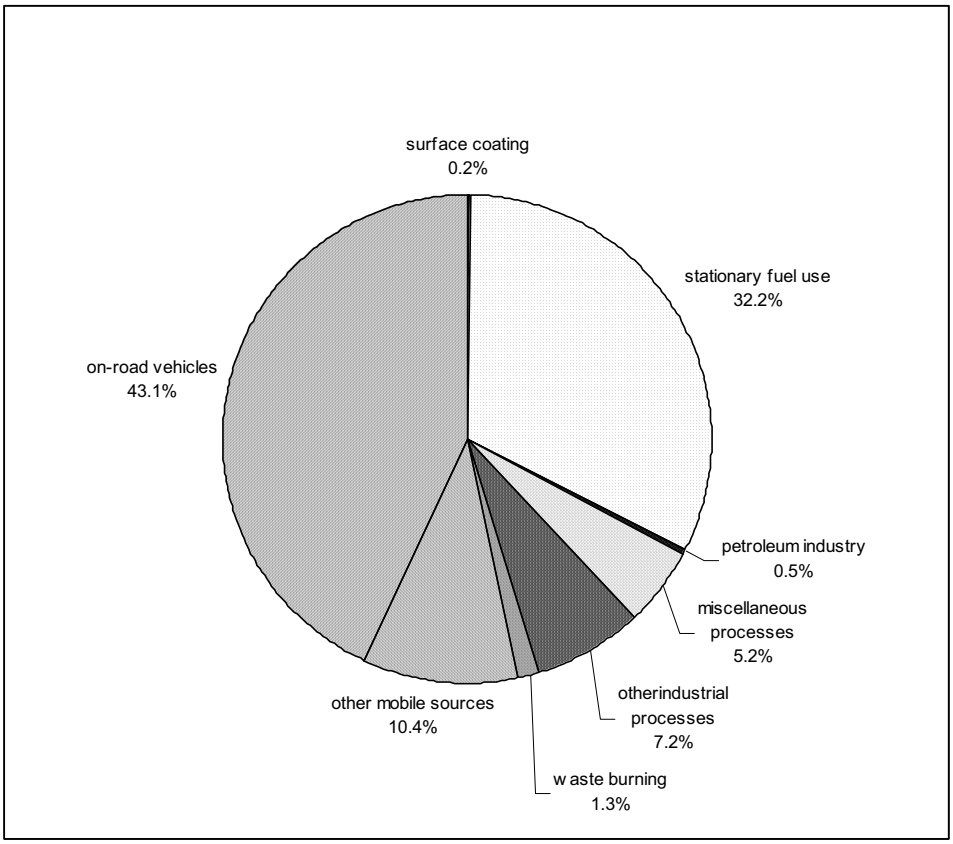

Figure 4.7 Source contributions to primary ultrafine particle emissions in California's South Coast Air Basin (1996) (Cass, 2000).

The authors concluded that the mass emission rate was sufficient to explain the 0.8 $\mu g/m^3$ ambient ultrafine particle concentration measured in the Los Angeles area and that the chemical composition distribution in the emissions was generally similar to measured ambient ultrafine particle concentrations once the trace metals in the emissions were converted to the mass of their oxides. It was further concluded that the ambient ultrafine particles in the Southern California atmosphere in the investigated fraction of the ultrafine range may be explained by primary particle emissions plus secondary aerosols nitrate formation at some places and times.

Residence time in the air and dispersion of particles from the emission sources

Following emission, pollutants, including particles, undergo dilution with ambient air, and then undergo various types of changes and transformations during the transport process. Larger particles are gravitationally deposited on the ground soon after emission, while smaller particles can travel larger distances and remain suspended in the air for hours and days after emission. The residence time of larger particles in the air is short and ranges from minutes to hours. The residence time of submicrometre

particles is much longer, and in the lower troposphere is approximately 10 days – due to lack of efficient removal mechanisms (Raes, 2000). Processes such as coagulation, diffusion, and convection transport, govern the behaviour and fate of these particles in the air.

Of significant interest in recent years has been the dispersion of particles from roads, as vehicle emissions constitute the most important source of pollution in most urban environments. A number of investigations have been reported on dispersion of particle mass (PM_{10} and $PM_{2.5}$), and only a few report on particle numbers. Analysis of the reported studies has shown that, despite significant differences in the designs of the individual studies, clear general trends in relation to particle mass and number concentrations as a function of a distance from the road can be identified (Morawska, 2003):

- There is very little or no gradient in TSP, PM_{10} and $PM_{2.5}$ concentrations within the distance from the road. All the reported studies showed that the decrease in mass concentration between these at the minimum distance from the road, and the background levels ranged from 0 to about 25 – 30%. The absence of a gradient means that the road does not contribute in any measurable way to the concentrations of these mass fractions.

- Particle number concentration, like the concentration of gaseous pollutants, decreases significantly with the distance from the road. Decay in particle concentration was approximated by exponential curves in a number of studies, and it was shown that the impact of the road on particle number concentration, while significant in the immediate vicinity of the road, is not distinguishable past about 300 m from the road. It was shown that dispersion is the main factor responsible for the decrease in particle concentration with distance from the road. Modelling of particle concentration using the same approach as that used for the modelling of dispersion of gaseous pollutants (CALINE4), showed that in some cases an even better approximation of the decay could be by power law (Gramotnev, 2003). While this approach does not include coagulation, the effect of coagulation on a relatively broad particle number window was not significant, as shown by excellent agreement between the theory and the experiment.

A practical implication from these findings is that the exposure to the number concentration of airborne particles is significantly increased within the first 100 or so metres of the road, compared to average urban exposure levels, and is reduced to the urban background level at distances greater than about 300 m from the road. On this basis, it is reasonable to assume that people living and working in close proximity to an urban arterial road will be likely to be exposed to levels of ultrafine and submicrometre particles beyond what could be considered ‘normal’ ambient levels.

4.3 MEASUREMENT METHODS

Characterisation of ultrafine particles includes identification of their physical and chemical properties (not discussed in this report are particle biological properties). Physical properties include mass, number, surface area, size distribution and morphology. Some of these properties can be measured in real time, including number and number size distribution, less frequently mass. Other properties, such as mass and

mass size distribution or morphology, require that samples are collected first, and then the properties are investigated under laboratory conditions using appropriate instrumentation. Particle mass of collected samples is determined using microbalances (this method also requires stringent pre- and post-sampling conditions of the filter). Information about particle size, morphology and surface properties is readily acquired using the scanning transmission electron microscope (STEM), while high resolution transmission electron microscopy (HRTEM) allows acquisition of structural information on particles and atomic clusters to sub-0.2 nm resolution.

Particle chemical composition studies are almost entirely conducted using sophisticated laboratory instrumentation which, again, requires that first a representative sample is collected eg (Maynard, 2000; Baron and Willeke, 2001). Following collection and sample preparation, chemical composition of particles is analysed. Particles down to nanometre size diameters can be analysed using electron energy loss spectroscopy (EELS) and X-ray emission in the STEM. Scanning probe microscopy offers the possibility of analysing nanometre diameter particles under ambient conditions, thus removing some of the constraints imposed by electron microscopy. Imaging methods such as atomic force microscopy and near field scanning optical microscopy enable characterisation of specific aerosols. All the above methods are very costly, complicated and not suitable for field application.

The focus of the brief review in this chapter is on methods for characterisation of ultrafine particle physical characteristics. As explained above, particles in the ultrafine size range reach high concentrations in terms of their numbers; however, mass of these particles is often very small. Therefore measurements of particles in ultrafine or broader, submicrometre ranges are most commonly based on particle number rather than mass concentrations.

Due to different physical properties, different methods need to be used for measuring very small particles which are mostly affected by diffusion, and for large particles which are mostly affected by inertia. The techniques used for characterisation of particle number concentrations in the size range up to 1 micrometre can be broadly divided into two groups:

- Measurements of total number concentrations: These include in the first instance optical particle counters and condensation particle counters.

- Measurements of number size distribution: These include a combination of techniques: in most cases the particles are first classified according to size. This classification is based on particle electrical mobility or diffusive properties, and then the size-classified particles are counted by condensation particle counters.

In general, the instrumentation used for particle number concentration and size distribution measurements are complicated and expensive, as the particles, which they investigate are very small, down to molecular sizes (in other words, they are nanoparticles). The instruments used have different properties and in particular differ in the lower size range of particles they can detect, and in general sensitivity. In addition, while there are absolute methods for calibration of the instruments in relation to the size of the measured particles (by using test calibration particles of known sizes), there are no methods for absolute calibration of the instruments for the concentration levels

measured. It is assumed that when all the operating parameters of the instruments are set according to the specifications, particularly the flow rates, the instruments read the correct concentrations. However, it is known that even the same types of properly operating instruments can yield readings differing up to an order of 20% (Rickeard, 1996). The differences between different types of instruments can be much higher due to the variation in the size ranges covered, sensitivity, etc. There is no standardisation in relation to such instruments or techniques used, and therefore it is often difficult to compare the results reported by different studies. Much more developmental work is needed to enable cheaper, reliable and repeatable measurements of particle number concentrations in the submicrometre range down to the size of a few nanometres (WHO 2002).

Particle number concentration

Particle number concentration is measured in real time by optical particle counters (OPCs) based on the principle of light scattered by single particles. This is achieved by directing the aerosol flow across a light beam and by collecting a portion of the scattered light into a detector. Each particle traversing the light beam results in a detector signal at the output, and the number n of signals during a certain time t_p is proportional to the particle concentration N according to the formula:

$$N = \frac{n}{Q \cdot t_p}$$

Where Q is the air volume flow rate. This technique enables detection of only the particles large enough to deliver a measurable scattering signal. If more than two particles cross the light beam at the same time they will produce one pulse and be counted as a single particle, which results in the so called coincidence error. Occurrence of the coincidence error limits the application of the OPC's to the environments of relatively low number concentrations, usually below 10^4 particles/cm^3. The cut-off size for most OPCs is above 0.1 μm. In order to detect particles smaller than this, condensation particle counters (CPCs) are used. A CPC acts as a particle magnifier in which a liquid (usually an alcohol vapour) condenses on the particles, resulting in their growth to the sizes detectable by an OPC, which follows in a continuous flow arrangement (Agawal, 1980). CPCs usually detect particles down to diameters of 0.01 μm, but frequently down to 0.003 μm.

There is presently no alternative to CPCs for real time particle number concentration measurement. All other existing methods, such as those measuring collective extinction or scattering from an aerosol probe, require information on particle size, shape and composition to derive the true number concentration.

Particle size distribution

Particle size distribution measurements are conducted in relation to particle mass or particle number. Mass distribution measurements involve either sample collection on a multistage impactor followed by gravimetric analyses of the masses collected at different impactor stages, or real time assessment if a quartz microbalance is used. Particle number distribution measurements are conducted using real time methods

based on time of flight measurements (for larger particles), electrical mobility measurements (for smaller particles) and light scattering (both smaller and larger particles).

Multistage impactors

Multistage impactors are the most common instruments used for sizing of airborne particles according to their aerodynamic diameters. Particle mass size distribution is then usually obtained by gravimetrical analyses of masses deposited on each impactor stage. There are also multistage impactors available, which use quartz crystal microbalances to provide real time approximation of particle mass distribution (Chuan, 1976). Each impactor stage consists of an orifice and an impaction plate. Between orifice and plate, the flow performs a 90° change of direction. Due to their larger inertia, particles above a certain size impinge on the plate, where they stick, while smaller particles (of smaller inertia) follow the flow to the next stage. The nozzle-plate geometry and the gas pressure at each particular stage define the cut-off diameter. The smallest diameters measured with commercial impactors are of the order of tens of nanometres.

A somewhat different concept is used in the Electrical Low Pressure Impactor (ELPI) (Marjamaki, 2000), in which the aerosol is charged in a diffusion charger and the electric current of the charged particles is measured at each impactor stage. This method is free from the problems associated with erroneous mass measurements due to bad contact between the particles and the surface of the quartz crystal, and has also good time resolution. Consideration, however, has to be given to a few aspects which limit the instrument application under certain conditions or for certain types of aerosols. In particular, the impactor has to be cleaned periodically to avoid modification of the impactor characteristics due to particle pile-up. This constitutes a limitation in the instrument application for strongly agglomerated particles, such as those resulting from diesel emissions, when the operation time between cleaning events is severely reduced (van Gulijk, 2001). Models suppressing this pile-up effect by using sintered impaction plates soaked with oil, have become available. However, this modification has a somewhat negative effect on the sharpness of the impactor cut-offs. Another limitation of the ELPI arises from dependence of the charging and impaction processes on different equivalent diameters. This makes the interpretation of the measured signal difficult in the case of strongly non-spherical particles. Finally, these instruments require larger than standard vacuum pumps which are noisier, and therefore it may be necessary for indoor applications to place the pump outdoors and connected to the impactor by sufficiently long tubing.

Recently, variable pressure impactors have been used as single-stage size spectrometers (Fernández de la Mora, 1996). Impactor models for laboratory applications are capable of size classification of particles with diameters of the order of a few nanometres. Such instruments, however, require very large pumps, which make them rather unsuitable for most indoor applications. Commercial variable pressure impactors are not available yet.

Electrical mobility measurement

The distribution of electrical mobility equivalent diameter is measured by a differential mobility analyser (DMA) (Fissan, 1983). The aerosol enters a cylindrical chamber through an annular slit and is carried downward in laminar flow parallel to the cylinder's axis. High voltage is applied between the walls of the cylinder and a central rod and charged particles are deflected towards the centre rod by a radial electric field. At a particular applied voltage, particles of a specific mobility exit through the slit in the lower part of the centre rod. More precisely, the extracted particles have a narrow mobility distribution, the mean of which is defined by the deflecting voltage applied between the centre rod and the outer cylinder. To obtain the size distribution, the number concentration of the exiting particles is measured as a function of the applied voltage. If the charge distribution of the particles is known, the size distribution can be calculated from this function. A variety of DMA models are available on the market. The most common application of this technology is in the Differential Mobility Particle Sizers (DMPS) or Scanning Mobility Particle Sizers (SMPS), which consist of a diffusion charger, a DMA and a condensation particle counter (CPC). The deflecting voltage is automatically scanned in a programmed manner, and the CPC response is measured as a function of the voltage. This function is automatically converted to the particle size distribution by a software algorithm. The main difference between the DMPS and SMPS systems is in the manner in which the deflection voltage is scanned. The SMPS system continuously ramps the voltage while the DMPS system scans the voltage in a series of steps. To record mobility distribution takes less than a minute with an SMPS system and several minutes with a DMPS system which provides more precise size distribution data. The inlets of commercially available systems are usually equipped with impactors with a cut-off smaller than 1 μm. This guarantees a well-defined maximum size which is required for data reduction.

Different data reduction algorithms have been applied in these systems, leading to significantly different results. The size distributions obtained with them must therefore be regarded as approximations.

4.4 SUMMARY OF STATE OF KNOWLEDGE OF MEASUREMENT AND TECHNOLOGY IN RELATION TO ULTRAFINE PARTICLES

Ultrafine particles have been defined as those which are smaller than 0.1 micrometres. It should be kept in mind, however, that the divisions between ultrafine and larger particles, similar to the other divisions between different particle size classes, are somewhat arbitrary. On the one hand there are no naturally - occurring boundaries between these size classes, and, on the other hand, all natural sources (versus laboratory generators) generate particles with a certain range of diameters - polidisperse particles - therefore there is no sharp boundary delineating contribution of particles from particle source.

What is known?

1. Particles in the ultrafine, and more generally submicrometre ranges, are generated mainly from combustion, gas to particle conversion, nucleation processes or photochemical processes, with some being primary (emitted

directly by the source) and some secondary in nature (formed in the air from the precursors emitted by the sources).

2. In terms of number, the vast majority of airborne particles are in the ultrafine range. The total mass of the ultrafine particles is, however, often insignificant in comparison with the mass of a small number of larger particles, with which most of the mass of airborne particles is associated. Particle surface area, in turn, is largest for particles somewhat above the ultrafine size range.

3. Chemical composition of particles is multi-factorial and depends on particle source as well as post-formation processes. The most important chemical properties of particles include elemental composition, inorganic ions and carbonaceous compounds (organic and elemental carbon). Primary particles generated from combustion processes constitute mainly of soot, which is formed from hydrocarbons burning under fuel-rich conditions. The main chemical constituents of secondary particulate matter in urban locations commonly include sulfuric acid and ammonium sulfate, ammonium and other nitrates and organic compounds. There is also a whole suite of trace metals associated with ultrafine particles. Chemical composition of particles differs significantly from place to place and depends on the type of the local sources, relative contributions from the sources and the fuels on which the sources operate (in relation to combustion sources).

4. Since ultrafine particles reach high concentrations in terms of their numbers although their mass is often very small, measurements of particles in ultrafine or broader, submicrometre ranges are more commonly based on particle number rather than mass concentrations. Ultrafine particle number and number size distribution are usually measured in real time, while mass, mass size distribution or morphology require that samples are first collected and then the properties investigated under laboratory conditions using appropriate instrumentation. In general, the instrumentation used for particle number concentration and size distribution measurements are complicated and expensive, as the particles which they investigate are very small, down to molecular sizes. Particle chemical composition is almost entirely conducted using sophisticated laboratory instrumentation which, again, requires that a representative sample is collected first.

5. Since different sources contribute to the generation of particles in the ultrafine range (more generally, submicrometre range) which predominate particle number, and different sources contribute to larger particles which predominate mass, it is only occasionally that there is a correlation between fine and coarse airborne particles, or a correlation between particle number and mass. The degree of correlation depends on specific local conditions, of which the relative contribution from different sources is of key importance. In general, only limited information, or no information at all can be obtained about particle number from the measurements of particle mass and vice versa.

6. Particle concentration levels in clean environments are usually of the order of a few hundred particles/cm^3. Clean environments for the purpose of this report mean those which are not influenced by human activities. In urban

environments background particle number concentrations range from a few thousand to about 2 x 10^4 particles/cm^3. Background concentrations mean the concentrations measured at monitoring stations which are not influenced by a local emission source operating in their immediate proximity. In proximity to roads or in tunnels, where vehicle traffic constitutes the most significant urban sources of particle numbers, particle concentrations can be ten times higher or more, and can reach and exceed levels of 10^5 particles/cm^3. This is in contrast to PM_{10} and $PM_{2.5}$ concentrations, which have been shown to be no more than 25 – 30% above background level at roads (calculated as the difference between the maximum at the road and the background levels). Therefore people living and working in close proximity to an urban arterial road are likely to be exposed to levels of ultrafine particles well above 'normal' ambient levels and only to somewhat elevated PM_{10} and $PM_{2.5}$ levels.

7. Particle number concentration, like the concentration of gaseous pollutants and other surrogates for very small particles, decreases significantly with the distance from the road, and this decrease is usually approximated by exponential (or power law) decay. The concentration decreases to urban background levels at a distance usually not greater than about 300 m from the road.

Recommendations for future work

While there is a general understanding of sources generating ultrafine particles, the ranges of the particle concentration levels encountered in different environments, the general nature of their chemical composition and the dispersion in atmospheric systems, the two main areas, which require further work include:

Developing national and local databases and knowledge of ultrafine particles. This encompasses local and national:

- Concentration levels of ultrafine particles and time series of the concentrations;
- Chemistry of ultrafine particles;
- Source contribution and inventory of primary and secondary ultrafine particles; and
- Relationships between different particle metrics (for example particle number and $PM_{2.5}$ concentrations). While most commonly there is only limited relationship or no relationship, in some local environments such relationships may exist and could be utilised.

Since all the above ultrafine particle characteristics vary from place to place and depend on the myriad of local conditions, this local and national knowledge is essential to conduct local risk assessment and for identifying local control and management strategies.

Standardisation of measurement techniques and study designs. There is no standardisation in relationship to the instruments or techniques used for investigations of ultrafine particles, and therefore it is often difficult to compare the results reported by different studies. Much more developmental work is needed, to enable cheaper,

reliable and repeatable measurements of particle number concentrations in submicrometre range, down to the size of a few nanometres.

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5. HEALTH IMPACTS OF ULTRAFINE PARTICLES

5.1 GENERAL OVERVIEW ON HEALTH IMPACTS OF TSP, PM_{10} AND $PM_{2.5}$

Over the past decade, overwhelming evidence has accumulated indicating that airborne particles characterised as Total Suspended Particles (TSP), PM_{10} and $PM_{2.5}$ exert a range of adverse health effects. The identified health effects are diverse in scope, severity, duration, and clinical significance. This diversity reflects the multiple pathways of injury caused by air pollution and the nature of the research evidence, which comes from epidemiological studies, human clinical exposures, animal toxicological studies and *in vitro* experiments.

The evidence on the health effects of air pollution has been summarised in a number of the state-of-the-art reviews (ATS, 1996a; ATS, 1996b; Holgate and Maynard, 1999) as well as in two recent U.S. EPA criteria documents (USEPA, 1996; USEPA, 1999). This section outlines key information on known and potential health effects associated with airborne PM, alone and in combination with other pollutants that are routinely present in the ambient air. The information highlighted here summarizes:

- Nature of the effects that have been reported to be associated with ambient PM;
- Sensitive subpopulations that appear to be at greater risk to such effects; and
- Integral evaluation of the health effects evidence.

Nature of the effects

The key health effects categories associated with PM include:

- Premature mortality;
- Aggravation of respiratory and cardiovascular disease (as indicated by increased hospital admissions and emergency room visits, school absences, work loss days, and restricted activity days);
- Changes in autonomic nervous system function and cardiovascular risk factors such as blood pressure, C-reactive protein or endothelial dysfunction
- Changes in systemic blood markers
- Changes in lung function and increased respiratory symptoms;
- Changes to lung tissues and structure; and
- Altered respiratory defence mechanisms.

Most of these effects have been consistently associated with ambient PM concentrations, which have been used as a measure of population exposure in a number of community epidemiological studies. Additional information and insight into these effects is provided by animal studies, *in vitro* toxicology, and controlled-human exposures to various constituents of PM conducted at higher-than-ambient concentrations. Although mechanisms by which particles cause effects have not been elucidated, there is general agreement that the cardio-respiratory system is the major target of PM effects.

Sensitive subpopulations

The epidemiological studies provide evidence that several subpopulations are more susceptible to the effects of air pollution containing PM. The observed effects in these subpopulations range from the decreases in pulmonary function reported in children to increased mortality reported in the elderly and in individuals with cardiopulmonary disease. Such subpopulations may experience effects at lower levels of PM than the general population, and the severity of effects may be greater.

The subpopulations that appear to be at greatest risk due to exposure to ambient PM include:

- Individuals with respiratory disease (e.g., COPD, acute bronchitis) and cardiovascular disease (e.g., ischemic heart disease) are at greater risk of premature mortality and hospitalisation.

- Individuals with infectious respiratory disease (e.g., pneumonia) are at greater risk of premature mortality and morbidity (e.g., hospitalisation, aggravation of respiratory symptoms). Also, exposure to PM may increase individual susceptibility to respiratory infections.

- Elderly individuals are also at greater risk of premature mortality and hospitalisation for cardiopulmonary causes.

- Children are at greater risk of increased respiratory symptoms and decreased lung function.

- Asthmatic children and adults are at risk of exacerbation of symptoms and increased need for medical attention.

Integral evaluation of health effects evidence

Community epidemiological studies provide evidence that serious health effects are associated with exposures to ambient levels of PM characterised as TSP, PM_{10} and $PM_{2.5}$ and found in contemporary urban airsheds at concentrations below current PM standards (USEPA, 1996). Although a variety of responses to constituents of ambient PM have been hypothesised to contribute to the reported health effects, the relevant toxicological and controlled human studies published to date have not identified an accepted mechanism that would explain how such relatively low concentrations of ambient PM might cause the health effects reported in the epidemiological literature. However, the toxicological studies tend to show that particles become more toxic per unit mass as their size decreases. Thus, attention is focused upon surface area or particle number per unit mass, rather than mass fraction.

Studies on particle mass concentration (PM_{10} and $PM_{2.5}$) indicate that for particle mass there is no threshold in particle concentrations below which health would not be jeopardised. This is presented in the World Health Organization Guidelines for Air Quality (WHO 1999), which shows a linear relationship between PM_{10} and $PM_{2.5}$ and various health indictors (including mortality, hospital admissions, bronchodilator use,

symptom exacerbation, cough, peak expiratory and flow) for concentration levels from 0 to up to 200 $\mu g/m^3$.

5.2 DEPOSITION OF PARTICLES IN THE HUMAN RESPIRATORY TRACT

Whether the inhaled particle will be deposited in human respiratory tract or exhaled, and the actual location of deposition in the tract depends on a number of factors, which can be classified into three main groups:

- The physico-chemical properties of aerosols
- The anatomy of the respiratory tract
- The airflow patterns in the lung airways

Factors grouped under the physico-chemistry of aerosols, including size or size distribution, density, shape, hygroscopic or hydrophobic character and chemical reactions of the particle, will all affect the deposition. Size of particles plays a particularly important role in the fate of the inhaled particles. Large-size particles deposit mainly in the upper part of the respiratory tract due to impaction, interception, gravitational sedimentation, and dispersion. Ultrafine particles, such as generated through combustion processes, have a high probability of deposition in deeper parts of the respiratory tract due to their high diffusivities.

As summarised in the review presented by WHO (2002), over the last three decades or so, a large number of studies have been conducted to investigate particle deposition in the human respiratory tract. A somewhat larger number focused on theoretical modelling than on the experimental determination of deposition. The review by Morawska et al. (1999), showed that the relatively small number of experimental studies of lung deposition for human subjects differ in the area of deposition investigated (total or various fractional deposition), type of aerosol inhaled, characteristics of the aerosol (age, size distribution, concentration, humidity, etc), type of inhalation (natural, artificial, controlled) and finally the experimental techniques and instrumentation used. In terms of theoretical predictions, a comparison between different modelling approaches shows that, with general agreement as to the total deposition levels, there are often significant discrepancies in the values for fractional deposition (i.e., regional in different parts of respiratory tract and generation-by-generation) (Hofmann 1996; Hofmann et al. 2001). The numerical models of particle deposition in the human lung that have been proposed are based on different morphometric lung models, utilise different deposition equations, and employ different computational techniques, it is thus not surprising that the resulting deposition fractions exhibit significant variations at the single airway generation level.

A comprehensive review of the experimental and theoretical modelling studies on particle deposition in the respiratory tract is outside the scope of this report. Further details on this topic can be found in (Morawska et al., 1999), (WHO,2002) and, (Hofmann et al.,2001).

To illustrate the general trends identified in particle deposition in the human respiratory tract, the two commonly used lung deposition models, those of Yeh and Schum (1980) and Yu and Diu (1982) are briefly discussed below. These models represent the

respiratory tract as a branching network of airways, with each generation characterised by the number of airways and their length and diameter. Particle deposition in each generation is computed by deterministic formulae, accounting for gravitational deposition, impaction, and diffusion. Interindividual variation in airway structure leading to variability in the model has been taken into account by including two random scaling factors (one for the tracheo-bronchial region and one for the alveolar region). It was shown that airway size is the single most important factor in the consideration of inter-subject variability of total and regional deposition under normal steady breathing conditions.

Figure 5.1 presents calculated total and regional mass deposition of polydisperse aerosols (Yeh et al. 1993). It can be seen from this figure that both total and fractional deposition of ultrafine particles is very high, and occurs predominantly in the deeper parts of the respiratory tract. All the existing deposition models show this trend, despite the differences between the models in the exact shapes of the deposition curves. The very high probability of deposition of the inhaled ultrafine particles in the deepest part of the respiratory tract is of high significance when considering health outcomes caused by the particles.

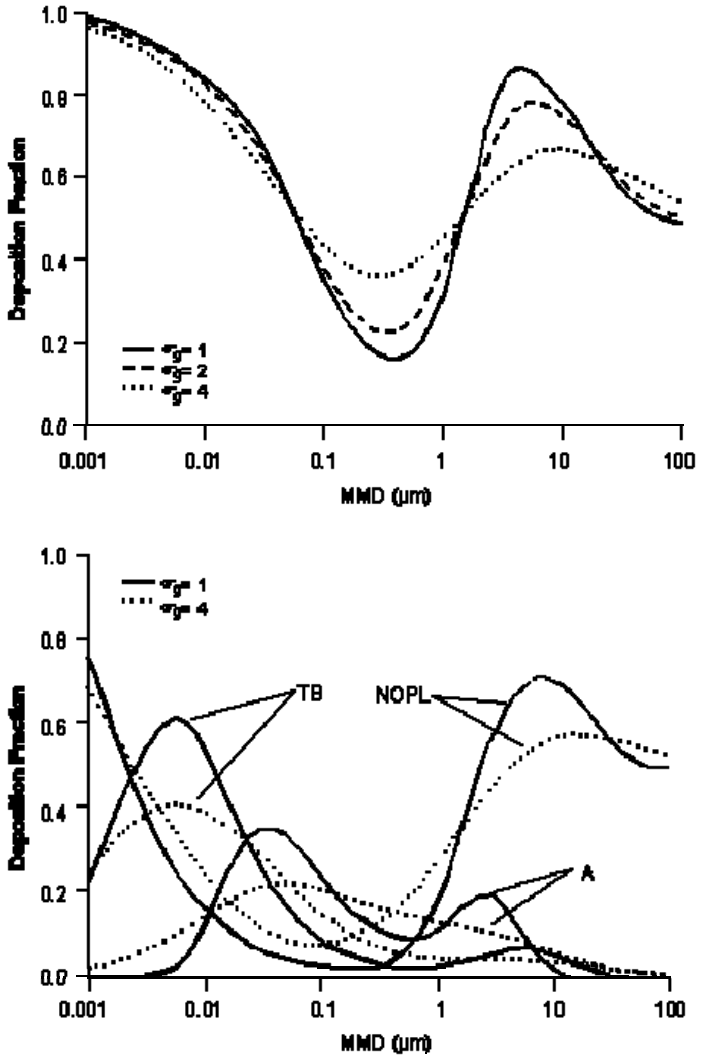

Figure 5.1 Calculated mass deposition of polydisperse aerosols of unit density with various geometric standard deviations (σ_g) as a function of mass median diameter (MMD) for quiet breathing (tidal volume = 750 mL, breathing frequency = 15 min^{-1}). The upper panel is total deposition and the lower panel is regional deposition (NOPL = Naso-oro-pharyngo-laryngeal, TB = Tracheobronchial, A = Alveolar). The range of σ_g values demonstrates the extremes of monodisperse to extremely polydisperse. Source: Yeh et al. (1993).

5.3 LITERATURE REVIEW OF HEALTH EFFECT STUDIES

5.3.1 EPIDEMIOLOGICAL STUDIES

In general, epidemiological studies attempting to link exposure to ambient particulate matter and health examine the following:

1. Characteristics (*e.g.*, size, concentration and composition) of particulate matter that might be responsible for its effect on morbidity and mortality;

2. Social and medical factors that might increase the health risk when particle pollution increases;
3. Possible patho-physiological mechanisms that might lead to death in people exposed to particle air pollution.

The relationship between airborne particles and health outcomes has been extensively investigated through epidemiological research (eg Pope, 2000; Samet et al., 2000; Dominici et al., 2002; Katsouyanni et al., 2001). Very few of the studies, however, have examined the role of ultrafine particles in health outcomes. One of the main reasons for this deficiency in epidemiological research linking health outcomes with exposure to ultrafine particles is that data on ambient concentrations of ultrafine particles is largely unavailable. Within most established networks, ambient particulate matter is typically measured in terms of its mass characteristics, either total particle mass, TSP, PM_{10} or $PM_{2.5}$. Most of the epidemiological research therefore has focused on the links between these mass characteristics of ambient particles and health. As explained in chapter 4, ultrafine particles are best measured in terms of their number concentration, because their mass concentration is very small and insignificant compared with the mass of larger particles and difficult to measure using the available methods. Only rarely, however, is there a correlation between particle number and mass characteristics. Therefore on the basis of existing mass concentration data, it is normally not possible to evaluate the effects of ultrafine particles in terms of their number concentrations on human health.

In the absence of adequate measurement and monitoring data in relation to ultrafine particles, many indirect approaches have been pursued. Indirect approaches have meant that epidemiological studies have been conducted without actually measuring ultrafine particle concentrations or number. These studies have not directly assessed human exposure to ultrafine particles. Indirect attempts to link exposure to ultrafine particles and health effects falls into three groups. The first comes from studies that have directly compared the coarse ($PM_{2.5-10}$) with the fine ($PM_{2.5}$) fractions, with the latter predominantly originating from sources known to produce ultrafine particles (e.g., traffic-related particles monitored as $PM_{2.5}$); the findings of these few studies have not been consistent. The second comes from studies of chemical species or measures of particles (sulfates, acid aerosol and black smoke) that reside mainly in the fine fraction (including ultrafine). Many of these have found associations with adverse health effects. The third group are those few studies that have compared the effects of size/number concentrations with size/mass concentrations. The findings from these have either been inconclusive or have suggested that numbers of particle are more important than mass. All the indirect studies were excluded from this review as the findings they generated do not directly link exposure to ultrafine particles with health effects and therefore do not enable unambiguous interpretations of the reported results in relation to the effects of ultrafine particles on human health.

In addition, there have been numerous epidemiological studies focused on establishing associations between diesel exhaust and health effect of exposed populations[1].

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[1] Jörgensen and Svensson (1970); Cuddihy et al. (1982) Boffetta et al. (1990); Gustavsson et al. (1990); Iyer et al. 1990; Hobbs and Mauderly 1991; Lindquist et al. 1991; Scheepers and Bos 1992; Steenland et al. 1992; Crane 1993; Emmelin et al. 1993; Pepelko and Chen 1993; Muscat and Wynder 1995a; Muscat and Wynder 1995b; Thomas et al. 1995; Muscat 1996; Stober and Abel 1996; Cox 1997; Morgan et al.

Although particle emissions from diesel engines consist predominantly of ultrafine particles (this issue is discussed in detail in Chapters 4 and 6 of this report), these studies were excluded from this review as they did not control (monitor) human exposure to the particles in the size range of particular concern in the present review, i.e. particles in the ultrafine size, range and could not control for the likely effects on health of adsorbed compounds on the particles.

In contrast to other areas of air quality research, it is only relatively recently, in the early nineties, that measurements of particle size distributions and number concentrations of ultrafine particles have been undertaken in exposure assessment and epidemiological studies as technological and instrumentation advances have made such measurements possible. This section examines the studies which include direct measurement of ultrafine particles and which were available (had been published) by the end of 2002. A summary of the reported epidemiological studies linking health effects with ultrafine particles is presented in Table 5.3.1.1. As can be seen, the number of theses studies is relatively small (8). Moreover, the vast majority of these studies were conducted in the framework of the European ULTRA program by the same teams of researchers from Finland, Germany, and the Netherlands

For the purpose of this review the studies were divided into three groups:

- Mortality
- Respiratory morbidity
- Cardiovascular morbidity

Each of these groups of studies is first reviewed and discussed separately in the relevant sections of this chapter. Then a general summary of the outcomes of all these epidemiological studies is provided at the end of this chapter, followed by discussion of the uncertainties introduced by various aspects of the study designs and data analysis and emerging recommendations for future studies in this area. It has to be noted that, in accordance with the objectives of this report, the summary of the studies outcomes is based on the conclusions made by the researchers conducting the investigations.

One important point need to be made about presentation of the epidemiological papers reviewed in this report. In many of the papers the authors stressed that even if there were some trends identified the results were not statistically significant. Therefore, such results or outcomes could serve as pointers for future investigations or formulations of hypotheses, but not as scientifically defensible conclusions. This is reflected in the conclusions of this report, which are formulated in the form of general hypotheses rather than scientifically defensible statements.

The biological significance of the findings was not discussed in any of the epidemiological studies and thus was not included in the review of the individual papers.

1997; Pott and Roller 1997; van Netten et al. 1997; Bhatia et al. 1998; Comstock 1998; Seidler et al. 1998; Stayner et al. 1998; Steenland et al. 1998; Brueske-Hohlfeld et al. 1999; Lipsett and Campleman 1999; Northridge et al. 1999; Saeverin et al. 1999; Säverin et al. 1999; Larkin et al. 2000; Szadkowska-Stanczyk and Ruszkowska 2000; Boffetta et al. 2001; Boffetta and Silverman 2001; Crump 2001; Dawson and Alexeeff (2001)

The summary and critical analyses of the results presented in the papers on epidemiological studies do not include meta-analysis of the data, as this was outside the scope of this review. In addition, the datasets were considered by statisticians involved with this report and their advice has been that datasets were generally unsuitable for meta-analysis as they are extremely disparate. Further analysis of this data is not justifiable in the context of this report.

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Table 5.3.1.1 Summary of epidemiological studies on the health effects of ultrafine particles.

Ref (Location of study)	Particle aspects	Groups studied	Effects studied	Findings/ Conclusions
Osunsanya et al, 2001 (UK)	PM_{10}, UFP	44 adults (aged > 50) with chronic pulmonary disease	Respiratory morbidity	No association was found between UFP and respiratory symptoms and peak expiratory flow (PEF). The consistent associations between symptoms and PM_{10} suggest that a contribution of the coarser fraction should not be dismissed.
Pekkanen et al, 1997 (Finland)	Size (CP, FP, UFP), PM_{10}, Black smoke	39 children (age 7-12) with asthmatic symptoms	Respiratory morbidity	Number concentrations of UFP were more strongly associated with variations in peak expiratory flow (PEF) than PM_{10} or BS. Different particle size fractions were, however, highly intercorrelated
Pekkanen et al, 2002 (Finland)	Size (FP, UFP), Mass (PM_1, $PM_{2.5}$, PM_{10})	45 adults with coronary heart disease	Cardiovascular morbidity	Observed independent associations of both FP and UFP with the risk of ST-segment depression during repeated exercise tests among subjects. The association was most consistent for measures of particles reflecting accumulation mode particles, but ultrafine particles also had an effect (odds ratio 3.14; 95% CI, 1.56 to 6.32), which was independent of $PM_{2.5}$. Also, gaseous pollutants NO_2 and CO were associated with an increased risk for ST-segment depressions. No consistent association was observed for coarse particles. The associations tended to be stronger among subjects who did not use beta-blockers. Conclusions: The present results suggest that the effect of particulate air pollution on cardiovascular morbidity is at least partly mediated through increased susceptibility to myocardial ischaemia.

Table 5.3.1.1 Summary of epidemiological studies on the health effects of ultrafine particles (Continued).

Ref (Location of study)	Particle aspects	Groups studied	Effects studied	Findings/ Conclusions
Penttinen, 2001 (Finland)	Size (UFP, FP), Mass (PM_{10}, $PM_{2.5}$, PM_1)	54 non-smoking adult asthmatics	Respiratory morbidity	Daily mean number concentration of particles, but not particle mass was negatively associated with daily PEF deviations. The strongest effects were seen for particles in the ultrafine range. However, the effect of ultrafine particles could not definitely be separated from other traffic-generated pollutants, namely nitric oxide, nitrogen dioxide and carbon monoxide. No associations were observed with respiratory symptoms or medication use. Particle mass measurements can be strongly influenced by mechanically produced, soil-derived particles, which may not be associated with adverse health effects. Therefore, air quality monitoring should include particle number concentrations, which mainly reflect ultrafine particles.
Peters et al, 1997 (Germany)	Size (FP, UFP)	27 non-smoking adult asthmatics	Respiratory morbidity	Most of the particles (73%) were in the ultrafine fraction whereas most of the mass (82%) was attributable to particles in the size range of 0.1 to 0.5 μm. Both fractions were associated with a decrease of peak expiratory flow (PEF) and an increase in cough and feeling ill during the day. Health effects of the 5-d mean of the number of UFPs were larger than those of the mass of the FP. In addition, the effects of the number of the ultrafine particles on PEF were stronger than those of PM_{10}. Conclusions: the present study suggests that the size distribution of ambient particles helps to elucidate the properties of ambient aerosols responsible for health effects.
Tiittanen et al, 1999 (Finland)	Size (UFP, FP, CP), Mass ($PM_{2.5}$, PM_{10})	49 children with chronic respiratory symptoms	Respiratory morbidity	No consistent effect of particles was found as the associations varied by lag. Of lags examined, only 1-day lagged $PM_{2.5}$ was statistically significantly associated with decreased morning peak expiratory flow (PEF). Evening PEF was significantly associated with the 1-day lagged number of particles in the size range of 0.1-1.0 μm. One-day lagged PM_{10}, $PM_{2.5}$, and $PM_{2.5-10}$, and the 4-day average of $PM_{2.5}$ were significantly associated with increased risk of cough. Given the short duration of the study, separating the effects of different types of particles was difficult.

Table 5.3.1.1 Summary of epidemiological studies on the health effects of ultrafine particles (Continued).

Ref (Location of study)	Particle aspects	Groups studied	Effects studied	Findings/ Conclusions
von Klot et al, 2002 (Germany)	Size (UFP, FP), Mass (PM_{10}, $PM_{2.5}$)	53 non-smoking adult asthmatics	Respiratory morbidity	Corticosteroid use and bronchodilator use both increased in association with cumulative exposure over 14 days of ultrafine particles and fine particles. A comparable effect was found for cumulative exposure over 5 days. The data suggest that asthma medication use increases with particle air pollution. The effect might be more delayed but stronger on anti-inflammatory medication than on bronchodilators.
Wichmann et al, 2000 (Germany)	Size (FP, UFP), Mass[2] ($PM_{2.5}$, PM_{10})	General population	Cardiovascular and respiratory mortality	Found that both fine and ultrafine particles were associated with increased mortality. However, FPs showed more immediate effects while UFPs showed more delayed effects with a lag of four days between particle concentrations and mortality. Furthermore, immediate effects were clearer in respiratory cases, whereas delayed effects were clearer in cardiovascular cases. Conclusions: FPs cannot be used as indicator for UFPs: the time trends for FPs decreased, while UFPs was stable and the smallest size fraction of UFPs has continually increased since 1991/92.

[2] Calculated from particle numbers.

MORTALITY STUDIES

i. Daily mortality and fine and ultrafine particles in Erfurt, Germany: role of particle number and particle mass - (Wichmann et al, 2000b)

This study examined the relation between components of particle pollution and daily mortality using separate analyses by age and causes of death. The overall *aims* of this study were to characterize ambient air pollution based on measurements of ultrafine particles, fine particles, and gaseous pollutants and to assess the association of ultrafine particles and fine particles with mortality. The study had three specific *objectives*: (1) to identify which size-fractionated ultrafine particles or fine particles were associated with general and cause-specific mortality, (2) to examine whether mortality was more strongly associated with particle mass or number concentrations, and (3) to explore which groups within the population were at greatest risk of death related to air pollution.

- *Location and period* – The study was conducted in Erfurt, Germany, a geographically self-contained community located in a valley surrounded on three sides by mountains, with a population of roughly 200,000 people and about 5 to 6 deaths per day. Data were collected prospectively over a 3.5 year period from August 1995 to December 1998.

- *Mortality data* - Death certificates were obtained from the local health authorities. Anonymous copies of the death certificates included data on immediate, underlying, and contributing causes of death. Based on this information, the investigators identified two main classifications: 1) underlying cause of death, or the disease or condition identified by the physician signing the death certificate as being the underlying cause of death; and 2) prevalent conditions, or any mention of cardiovascular or respiratory disease on the death certificate. In the report, the authors stratified cause-specific mortality based on prevalent conditions.

- *Exposure measurement* – Detailed air pollution data were collected at a single sampling site located in a mixed-use area (residences, offices, a school, and a hospital) 2 km from the centre of the city and approximately 50 m from a major road. The air pollutant mixture at this location was primarily influenced by traffic emissions and domestic heating.

  The mass and number concentration of ultrafine particles, fine particles, and coarse particles, as well as concentrations of gaseous pollutants and temperature, were continuously recorded each day for 40 months.

  To cover all particle sizes, the measurements were taken by three different instruments; each measured particle size by a different method. The three instruments were combined into a measurement system the investigators called the mobile aerosol spectrometer (MAS). Differential Mobility Analyser was used to monitor particles with a diameter of 0.01–0.5 μm followed by a condensation particle counter (a combination of instruments termed a differential mobility particle sizer (DMPS). Optical Laser Aerosol Spectrometer (LAS-X) was used to monitor particles with a diameter of 0.1–2.5 μm.

Condensation Particle Counter (CPC) was used for particles ranging from 0.003 to 3.0 μm.

Particle mass ($PM_{2.5}$ and PM_{10}), sulfate, and acidity were measured using the traditional filter-based Harvard impactors without a denuder.

Mass and number were measured for three size fractions of ultrafine particles (0.01–0.03, 0.03–0.05, and 0.05–0.1 μm in diameter) and three size fractions of fine particles (0.1–0.5, 0.5–1.0, and 1.0–2.5 μm in diameter). More traditional particulate matter measurements included larger particles of 2.5–10 μm and 10–40 μm in diameter, based on total suspended particles, PM_{10}, and $PM_{2.5}$.

Meteorological data (temperature, relative humidity, wind speed, wind direction) were collected at the same sampling site. Gaseous pollutants (SO_2, NO_2, CO) were measured at the central and two state-run sites. In addition to the daily mortality counts, the influenza data were obtained from a commercial source.

- *Statistical methods* – The investigators used a time-series approach to look at short-term changes in particle concentration and mortality over a 3.5-year period. The association with daily mortality was analysed using Poisson regression techniques with generalised additive modelling (GAM) to allow non-parametric adjustment for the confounders. The pollutants were included either untransformed or log transformed, depending on goodness of fit. Models were developed systematically for each pollutant and allowing for interactions, with relative risk estimates based on the interquartile range of the data. Sensitivity analyses were performed for subgroups (age and cause of death) as a test of the stability of the models.

- *Air pollution data* – The average concentrations were: 15773 ± 10321 particles/cm^3 for $NC_{0.01–0.1}$, 17966 ± 11373 particles/cm^3 for $NC_{0.01–2.5}$, 0.64 ± 0.52 μg/m^3 for $MC_{0.01–0.1}$, 25.8 ± 21.4 μg/m^3 for $MC_{0.01–2.5}$, 26.3 ± 20.8 μg/m^3 for $PM_{2.5,}$ 38.2 ± 26.4 μg/m^3 for PM_{10}, 48.9 ± 28.1 μg/m^3 for TSP, 16.8 ± 18.7 μg/m^3 for SO_2, 36.4 ± 15.3 μg/m^3 for NO_2, and 600 ± 500 μg/m^3 for CO. The greatest proportion (88%) of the total daily average number concentration represented particles below 0.1 μm. The greatest proportion (97%) of the total daily average mass was contributed by the larger particles (0.1–2.5 μm in diameter). It has to be noted that the authors have discussed the results for fine particles in terms of $MC_{0.01–2.5}$, which is analogous to $PM_{2.5}$, but $MC_{0.01–2.5}$ includes the size range covered by ultrafine particles (although very little of the mass was contributed by the smaller particles (0.01–0.1 μm).

***Main findings*:**

- All particles had a strong seasonality with maximal concentrations in winter. Over the years, however, the mass of fine particles in each winter decreased while the number remained constant. The investigators interpreted this finding to mean that the number of fine particles had diminished while the number of ultrafine particles had increased.

- The ultrafine particles concentrations showed a strong day of the week effect with concentrations during the weekend 40% lower than during the week. This and a clear increase of the ultrafine particles concentrations during the rush hours suggest that the main source for ultrafine particles was automobile traffic.

- Associations between health effects and particle number and particle mass concentrations have been observed in different size classes, and both immediate effects (lags 0 or 1 days) and delayed effects (lags 4 or 5 days) were found (Table 1 - Wichmann et al 2000b).

- There was a tendency for more immediate effects of the mass concentrations (i.e. in the larger size ranges) and for more delayed effects of the number concentrations (i.e. in the smaller size ranges; Table 2 - Wichmann et al 2000b). However, this pattern could not be separated clearly, and distributed lag models comprising the days 0 to 5 showed similar results.

- The effects could be found for total mortality but also for respiratory and cardiovascular causes (Table 4 - Wichmann et al 2000b). There was a tendency for more immediate effects on respiratory causes and more delayed effects for cardiovascular causes (Table 5 - Wichmann et al 2000b).

- Mortality increased in association with ambient particles after adjustment for season, influenza epidemics, day of week and meteorology, and sensitivity analyses showed the results to be stable.

Table 1 - Wichmann et al 2000b. Regression results for particle number concentration, particle mass concentrations, and gaseous pollutants.

	Interquartile Range (IQR)	Lag (Days)	TR	Relative Risk/IQR	CI	*P*
Particle Number Concentration (particles/cm^3): Best Single-Day Lag						
$NC_{0.01–0.03}$	5,177–14,065	4	log	1.048	1.000–1.099	0.05
$NC_{0.03–0.05}$	1,603–4,127	4	id[a]	1.031	0.998–1.066	0.07
$NC_{0.05–0.1}$	993–2,518	1	log	1.043	0.999–1.089	0.06
$NC_{0.01–0.1}$	8,042–20,732	4	log	1.046	0.997–1.097	0.07
$NC_{0.01–2.5}$	9,659–22,928	4	log	1.041	0.991–1.093	0.11
Particle Number Concentration (particles/cm^3): Polynomial Distributed Lag[d]						
$NC_{0.01–0.03}$		0–5	id	1.030	0.997–1.065	0.06
$NC_{0.03–0.05}$		0–4	id	1.038	1.000–1.077	0.05
$NC_{0.05–0.1}$		0–5	id	1.040	0.997–1.085	0.07
$NC_{0.01–0.1}$		0–4	id	1.041	1.001–1.082	0.04
$NC_{0.01–2.5}$		0–4	id	1.036	1.003–1.069	0.03
Particle Mass Concentration (μg/m^3): Best Single-Day Lag						
$MC_{0.1–0.5}$	9.8–25.2	0	id	1.026	0.995–1.058	0.10
$MC_{0.5–1.0}$	0.81–4.03	0	id	1.015	0.996–1.034	0.13
$MC_{1.0–2.5}$	0.56–1.55	3[b]	id	0.977	0.954–1.001	0.06
$MC_{0.01–1.0}$	11.3–31.0	0	id	1.028	0.996–1.060	0.09
$MC_{0.01–2.5}$	12.0–31.9	0	id	1.031	1.000–1.063	0.05
Particle Mass Concentration (μg/m^3): Polynomial Distributed Lag[d]						
$MC_{0.1–0.5}$		0–5	id	1.035	0.999–1.071	0.05
$MC_{0.5–1.0}$		0–5	id	1.028	1.004–1.052	0.02
$MC_{1.0–2.5}$		0–5	log	1.048	1.011–1.087	0.01
$MC_{0.01–1.0}$		0–5	id	1.040	1.002–1.080	0.04
$MC_{0.01–2.5}$		0–5	id	1.049	1.011–1.088	0.01
Other Particle Mass (μg/m^3): Best Single-Day Lag						
$PM_{2.5}$	13.0–31.5	3[c]	id	0.970	0.941–1.000	0.05
PM_{10}	19.9–47.6	0	id	1.035	1.001–1.069	0.04
TSP	28.8–61.9	1	log	1.023	0.981–1.067	0.28
Other Particle Mass (μg/m^3): Polynomial Distributed Lag[d]						
$PM_{2.5}$		0–1	id	1.022	0.988–1.058	0.20
PM_{10}		0–4	id	1.036	1.004–1.069	0.03
TSP		0–1	log	1.022	0.965–1.083	0.46
Gaseous Pollutants: Best Single-Day Lag						
SO_2 (μg/m^3)	5.5–19.8	0	log	1.060	1.011–1.112	0.02
NO_2 (μg/m^3)	26.0–46.0	4	id	1.029	0.992–1.067	0.12
CO (mg/m^3)	0.3–0.8	4	log	1.055	1.003–1.110	0.04
Gaseous Pollutants: Polynomial Distributed Lag[d]						
SO_2 (μg/m^3)		0–3	log	1.074	1.022–1.129	0.01
NO_2 (μg/m^3)		1–4	id	1.035	0.995–1.077	0.09
CO (mg/m^3)		1–4	log	1.076	1.017–1.138	0.02

With log transformation fit was only slightly less well: lag = 4, TR = log, RR = 1.040, CI = 0.994–1.089, *P* = 0.09.

[b] The second best fit was lag = 0, TR = id, RR = 1.019, CI = 0.997–1.042, *P* = 0.1.

[c] The second best fit was lag = 0, TR = id, RR = 1.019, CI = 0.991–1.049, *P* = 0.19.

[d] The weights are given in Table 3 - Wichmann et al 2000b.

Table 2 - Wichmann et al 2000b. Regression results for total mass and number concentrations and for gaseous pollutants: total mortality, all single-day lags and transformations[a].

Lag (Day)	TR	RR/IQR	CI	*P*
$NC_{0.01\text{-}0.1}$				
0	id	1.022	0.982–1.065	0.22
0	log	1.019	0.969–1.072	0.30
1	id	1.003	0.966–1.042	0.39
1	log	1.026	0.979–1.075	0.22
2	id	0.984	0.946–1.022	0.28
2	log	0.994	0.948–1.042	0.39
3	id	1.009	0.970–1.050	0.36
3	log	1.021	0.973–1.071	0.28
4	id	1.035	0.995–1.077	0.10
4	log	1.046	0.997–1.097	0.07
5	id	1.005	0.967–1.044	0.39
5	log	1.012	0.967–1.059	0.35
$MC_{0.01\text{-}2.5}$				
0	id	1.031	1.000–1.063	0.06
0	log	1.040	0.992–1.089	0.11
1	id	1.013	0.982–1.045	0.29
1	log	1.016	0.969–1.064	0.32
2	id	1.000	0.969–1.032	0.40
2	log	0.998	0.951–1.047	0.40
3	id	0.978	0.947–1.009	0.15
3	log	0.985	0.939–1.032	0.32
4	id	1.004	0.974–1.035	0.39
4	log	1.006	0.962–1.053	0.38
5	id	1.006	0.977–1.037	0.37
5	log	1.023	0.977–1.071	0.25
PM_{10}				
0	id	1.035	1.001–1.069	0.05
0	log	1.030	0.987–1.075	0.16
1	id	1.016	0.984–1.050	0.25
1	log	1.020	0.978–1.064	0.26
2	id	0.997	0.964–1.030	0.39
2	log	0.993	0.951–1.036	0.38
3	id	0.976	0.944–1.010	0.15
3	log	0.988	0.947–1.031	0.34
4	id	1.003	0.971–1.035	0.39
4	log	1.002	0.962–1.044	0.40
5	id	0.995	0.964–1.028	0.38
5	log	1.007	0.966–1.049	0.38

For interquartile ranges see Table 1 - Wichmann et al 2000b.

Table 3 - Wichmann et al 2000b. Weights for distributed polynomial lag models in Table 1 - Wichmann et al 2000b[a].

Pollutants	Lag 0	Lag 1	Lag 2	Lag 3	Lag 4	Lag 5
Particle Number Concentration						
$NC_{0.01–0.03}$	1.35	-0.78	-0.90	-0.03	0.80	0.56
$NC_{0.03–0.05}$	0.14	0.42	-0.13	-0.35	0.92	
$NC_{0.05–0.1}$	0.39	0.16	0.05	0.03	0.11	0.26
$NC_{0.01–0.1}$	0.44	0.07	-0.33	-0.19	1.02	
$NC_{0.01–2.5}$	2.56	-0.69	-2.08	-1.19	2.50	
Particle Mass Concentration						
$MC_{0.1–0.5}$	0.56	0.37	-0.02	-0.30	-0.20	0.59
$MC_{0.5–1.0}$	0.57	0.39	-0.10	-0.48	-0.29	0.91
$MC_{1.0–2.5}$	0.45	2.29	0.12	-2.67	-2.68	3.49
$MC_{0.01–1.0}$	0.72	0.24	-0.13	-0.29	-0.10	0.55
$MC_{0.01–2.5}$	0.72	0.20	-0.17	-0.30	-0.07	0.62
Other Particle Mass						
$PM_{2.5}$	0.16	0.84				
PM_{10}	3.66	1.53	-1.60		-2.93	0.12
TSP	-0.08	1.08				
Gaseous Pollutants						
SO_2	0.82	-0.02	-0.17	0.37		
NO_2		0.48	-0.19	-0.09	0.80	
CO		0.19	-0.10	0.10	0.81	

Table 4 - Wichmann et al 2000b. Regression results by prevalent disease.

Disease Group	Lag	TR	RR/IQR[a]	CI	*P*
$NC_{0.01–0.1}$ (particles/cm^3): Best Single-Day Lag					
Cardiovascular or Respiratory[b]	4	log	1.055	1.001–1.111	0.04
Cardiovascular but Not Respiratory			1.051	0.990–1.115	0.10
Respiratory			1.048	0.956–1.149	0.31
Other Natural			1.029	0.932–1.136	0.57
$NC_{0.01–0.1}$ (particles/cm^3): Polynomial Distributed Lag[c]					
Cardiovascular or Respiratory[b]	0–4	log	1.063	1.013–1.116	0.02
Cardiovascular but Not Respiratory	0–4	log	1.058	1.001–1.119	0.05
Respiratory	0–4	log	1.083	0.994–1.180	0.07
Other Natural	0–4	log	1.030	0.938–1.130	0.33
$MC_{0.01–2.5}$ (μg/m^3): Best Single-Day Lag					
Cardiovascular or Respiratory[b]	0	id	1.033	0.999–1.068	0.05
Cardiovascular but Not Respiratory			1.007	0.969–1.047	0.72
Respiratory			1.098	1.040–1.160	0.00
Other Natural			1.019	0.955–1.087	0.57
$MC_{0.01–2.5}$ (μg/m^3): Polynomial Distributed Lag[c]					
Cardiovascular or Respiratory[b]	0–4	id	1.047	1.014–1.081	0.01
Cardiovascular but Not Respiratory	0–5	id	1.051	1.004–1.099	0.04
Respiratory	0–5	id	1.098	1.029–1.172	0.01
Other Natural	0–5	id	1.029	0.957–1.106	0.30
MC_{10} (μg/m^3): Best Single-Day Lag					
Cardiovascular or Respiratory[b]	0	id	1.036	1.001–1.074	0.05
Cardiovascular but Not Respiratory			1.022	0.981–1.065	0.29
Respiratory			1.083	1.017–1.152	0.01
Other Natural			1.016	0.946–1.090	0.67
MC_{10} (μg/m^3): Polynomial Distributed Lag					
Cardiovascular or Respiratory[b]	0–4	id	1.042	1.007–1.079	0.03
Cardiovascular but Not Respiratory	0–4	id	1.038	0.998–1.081	0.07
Respiratory	0–4	id	1.062	0.997–1.133	0.07
Other Natural	0–4	id	1.016	0.947–1.090	0.36

[a] For interquartile ranges (IQR), see Table 1 - Wichmann et al 2000b.
[b] Results were obtained on the basis of a slightly different confounder model.
[c] The weights are approximately the same as in Table 3 - Wichmann et al 2000b.

Table 5 - Wichmann et al 2000b. Regression results by cardiovascular and respiratory diseases under respective best immediate (0 or 1 day) or delayed (4 or 5 days) model per disease group[a].

	Lag	TR	RR/IQR	CI	*P*
Cardiovascular (But Not Respiratory)[b]					
$NC_{0.01–0.1}$ (particles/cm^3)	0	id	1.004	0.955–1.056	0.43
	4	log	1.051	0.990–1.115	0.10
	0–4	log	1.058	1.001–1.119	0.05
$MC_{0.01–2.5}$ (µg/m^3)	0	id	1.007	0.969–1.047	0.72
	5	log	1.037	0.978–1.099	0.22
	0–5	id	1.051	1.004–1.099	0.04
PM_{10} (µg/m_3)	0	id	1.022	0.981–1.065	0.29
	5	log	0.992	0.941–1.046	0.76
	0–4	id	1.038	0.998–1.081	0.07
Respiratory[b]					
$NC_{0.01–0.1}$ (particles/cm^3)	1	log	1.155	1.055–1.264	0.00
	4	log	1.048c	0.956–1.149	0.31
	0–4	log	1.083	0.994–1.180	0.07
$MC_{0.01–2.5}$ (µg/m^3)	0	id	1.098	1.040–1.160	0.00
	5	log	1.030	0.940–1.130	0.52
	0–5	id	1.098	1.029–1.172	0.01
PM_{10} (µg/m^3)	0	id	1.083	1.017–1.152	0.01
	5	log	1.041	0.956–1.134	0.35
	0–4	id	1.062	0.997–1.133	0.07

[a] The effect sizes are based on models in which all subgroups were forced to have the same delay.
[b] For interquartile ranges (IQR), see Table 1 - Wichmann et al 2000b.
[c] This effect went away when confounder models were different per subgroup.

Summary, critique and conclusions on the mortality study

This study is a major contribution to the state of knowledge on actual particulate matter levels, and the results provided the first evidence that ultrafine particles are associated with mortality. The investigators found comparable effects for ultrafine particles and fine particles and suggest a delayed effect for ultrafine particles versus an immediate effect of fine particles.

Extensive modelling approaches were used by the investigators, which addressed several issues of global concern in regards to epidemiological studies, illustrate the complexities of assessing relationships amongst the variables. For example, instead of evaluating fine particles by measuring $PM_{2.5}$, which contains both ultrafine particles and accumulation-mode particles, the investigators used the size-fractionated metric $MC_{0.01–2.5}$, which represented the mass calculated from particle number. Due to this approach, the artifactual formation of SO_4^{2-} in traditional filter-based PM samplers was avoided. Although earlier work in Erfurt suggests that measurement error for $PM_{2.5}$, PM_{10}, and other air quality parameters may not be a concern for fine particle air pollution. It may still be an issue for the ultrafine fraction. The use of a more precise

particle metric is a start toward disentangling the contribution of relevant particle fractions in complex mixtures of air pollutants, although the issue of dealing with other highly correlated air pollutants remains.

The investigators also adjusted for the occurrence of a major influenza outbreak, which occurred in two waves during the study period

Despite the exhaustive modelling approach used by the investigators, important limitations to the results remain (for example, interpretations regarding timing of effect). Although the ultrafine particle fraction has been shown to be associated with human mortality, no clear pattern emerged of differences between ultrafine particles and fine particles. The information required to characterise human exposure to ultrafine particles in Erfurt is not available (the spatial distribution of ultrafine particles in Erfurt and the time course of a potential effect of ultrafine particles on human health). However, what is known about ultrafine particles in Erfurt raises questions about the relationship between ultrafine particles and mortality.

The dynamics of ultrafine particles raises the question of whether a single monitoring station measuring particle levels can adequately represent the levels for the entire geographical area in which the population resides. The area of Erfurt is approximately 150 square kilometres. Under conditions of low wind velocity or still weather conditions conducive to inversions, a gradient of ultrafine particle levels may occur across the study area if the mobile source density is not uniform where the people are located. Thus as distance increases between mobile sources (such as traffic) and individuals (at work or at home), the exposure to ultrafine particles may decrease, raising uncertainty as to how well a single monitoring station represents the population's exposure to ultrafine particles. Cyrys and associates (1998) reported that concentrations of $PM_{2.5}$, PM_{10}, and other air quality parameters measured at the central site and other sites in Erfurt did not differ appreciably from each other. If so, a single monitoring station might represent exposure adequately for those pollutants measured. However, identifying whether this homogeneity is true for ultrafine particles would require exposure comparisons specific to ultrafine particles, information that is currently not available.

STUDIES ON RESPIRATORY MORBIDITY

Results from a total of six panel studies with asthma and/or COPD patients have been published.

i. Association of respiratory effects with the number of ultrafine particles - (Peters et al., 1997)

This study assessed the short-term effects of ambient air on the respiratory morbidity of adults with lung disease.

- *Location and period* – The study was conducted between October 1991 and March 1992 in Erfurt, a German city with 200, 000 residents. The main sources of air

pollution are a power plants, traffic, and domestic heating (using coal and natural gas).

- *Population* – Twenty-seven non-smoking adults (44-80 years old) were recruited from an outpatient clinic specializing in lung diseases where they had been identified as having history of asthma. Asthma medications were used by 23 participants; 87 % used these medications > 95 of 100 person-days. Thirteen subjects reported allergies to house dust, pollen, animal hair, or fungal spores.

- *Health effect measurement* – The subjects reported daily symptoms and performed peak expiratory flow measurements 3 times a day before and after medication use.

- *Exposure measurement* – ultrafine particles were measured with an electrical mobility analyser (Model 3071; TSI), which counts particles within the range 0.01- 0.3 μm. LAS-X (counts particles within the range 0.1- 2.5 μm) was used for monitoring of fine particles. PM_{10} were measured with Harvard impactor. All measurements were performed at a single sampling site located 1 km south of the city centre, 40 m away from a major road.

- *Air pollution data* – Most of the particles were in the ultrafine fraction, whereas most of the mass was attributable to particles in the size range 0.1-0.5 μm. Since ultrafine particles and accumulation-mode particles did not have similar time patterns, comparison of their health effects was possible (correlation coefficient, r = 0.51).

- *Confounding factors* – 24hr mean temperature, quadratic temperature, relative humidity, quadratic relative humidity, linear trend, quadratic trend, weekend as indicator variable (weekend vs. not weekend), presence of viral infections coincident with air pollution episodes. Particle size data were categorised as 0.01μm to < 0.1μm; 0.1μm to < 0.5μm; and 0.5μm to < 2.5μm.

- *Statistical methods* – Regression models were used to estimate the association between averaged time series of health outcomes and particle air pollution while controlling for time-varying factors. Polynomial distributed lag structures used 5 day means to summarize cumulative impact. Analyses performed using SAS statistical software

Main findings:

Associations Between Particles and Peak Expiratory Flow

- Both fine and ultrafine particle pollution were associated with small, but consistent, decreases in PEF (Table 1- Peters 1997). Analyses were adjusted for a linear trend, mean daily temperature, and weekend.

- Decreases in PEF in association with particulate air pollution on the same day were observed for the ultrafine and fine particles, but only the estimates for $MC_{0.01\text{-}0.1}$ and

$MC_{0.01-0.25}$ achieved statistical significance (Table 1 - Peters 1997). Concurrent values of PM_{10} were not associated with decreases in lung function.

- Evidence for a cumulative effect of the exposure to ultrafine particles and PM_{10} on PEF in the evening was found in polynomial distributed lag structures. For $NC_{0.01-0.1}$ and PM_{10}, a quadratic polynomial fitted the data best, with a maximum of 2 to 3 days prior to the decreases in PEF. Five-day means of the exposure quantified the association between health outcomes and cumulative exposure, including the last 4 days. Twofold to threefold larger effect estimates for the 5-day mean than for the same-day values of $NC_{0.01-0.1}$, $MC_{0.01-0.1}$, and $MC_{0.01-0.25}$ were calculated and therefore supported the temporal association determined by polynomial distributed lag structures. In addition, statistically significant effect estimates were observed for the 5-day means of $MC_{0.01-0.25}$, $MC_{0.1-0.5}$, and PM_{10}.

- Estimates for the association between measures of particles on the same day and the lung function measurement on the next morning were slightly smaller than in the evening before (Table 1 - Peters 1997). Statistically significant associations between particulate air pollution on the previous day and PEF in the morning were observed for the ultrafine particles ($NC_{0.01-0.1}$, $MC_{0.01-0.1}$, and $NC_{0.01-2.5}$), and the fine particles ($MC_{0.1-0.5,}$ $MC_{0.1-0.5,}$ and PM_{10}. Only particles larger than 0.5 µm ($MC_{0.5-2.5}$ and $NC_{0.5-2.5}$) were not strongly associated with decreases in lung function. Some evidence for a cumulative impact of air pollution on the PEF in the morning was observed for ultrafine particles; nearly two-fold larger estimates for 5-day means of $NC_{0.01-0.1}$, $MC_{0.01-0.1}$, and $NC_{0.01-2.5}$ were calculated compared with the estimates for the same day.

- Strong evidence was found for an association between the 5-day mean of $NC_{0.01-0.1}$ and PEF in the evening and also in the morning. This association was stronger than the associations observed for 5-day means of $MC_{0.1-0.5}$ and PM_{10} (Table 3 - Peters 1997).

Associations Between Particles and Respiratory Symptoms

- A linear increase in the prevalence of feeling ill during the day was detected during the winter. The prevalence of feeling ill during the day increased in association with all measures of particulate air pollution on the same day, and the odds ratios had similar sizes (Table 2 - Peters 1997). Odds ratios for feeling ill during the day were larger for the 5-day means of $NC_{0.01-0.1}$ and PM_{10} than the odds ratios for $NC_{0.01-0.1}$ and PM_{10} on the same day. The cumulative association for PM_{10} was supported by a second order polynomial with a maximum of 2-day prior, which fitted best for PM_{10}. However, none of the lag structures for $NC_{0.01-0.1}$ achieved statistical significance. Two pollutant models were not able to distinguish between the contribution of prolonged exposure to PM_{10} and $NC_{0.01-0.1}$ (Table 3 - Peters 1997) and attributed the increases in prevalence of feeling ill during the day to both measures of particulate matter.

- A linear increase in the prevalence of cough was detected during the winter. Weak associations between particulate air pollution on the same day and the revalence of cough were observed for the number of ultrafine particles ($NC_{0.01-0.1,}$ $NC_{0.01-2.5}$), but

the estimate for $MC_{0.01-0.1}$ achieved statistical significance. Fine particles ($MC_{0.1-0.5}$ and $MC_{0.01-2.5}$) were associated with increases in cough, of which $MC_{0.01-2.5}$ was the best predictor. The symptom cough showed the strongest associations with particles larger than 0.5 μm ($MC_{0.5-2.5}$ and $NC_{0.5-2.5}$) and PM_{10}. The increases of the prevalence of cough were best predicted by concurrent exposure to $PM_{10.}$ However, an association between a 5-day mean of $NC_{0.01-0.1}$ and cough was confirmed by two pollutant models being stronger than the associations between cough and 5-day means of PM_{10} or $MC_{0.1-0.5}$.

Conclusions:
Exposure to ultrafine particles and fine particles were associated with a decrease of peak expiratory flow and an increase in cough and feeling of illness during the day. Health effects were associated with the number of ultrafine particles rather than the mass of the fine particles. The effects were most strongly associated with the mean of the particle number over the previous 5 days.

Table 1 - Peters 1997. Associations between particulate air pollution and peak expiratory flow*.

	Evening Peak Expiratory Flow (L/min)				Morning Peak Expiratory Flow (L/min)			
	Same-day		Five-day Mean		Previous day		Five-day Mean	
Particle Size	β	CI	β	CI	β	CI	β	CI
$NC_{0.01-2.5}$	-1.49	-3.32 to 0.33	-3.58	-5.28 to -1.89	-1.42	-2.61 to -0.23	-2.38	-3.61 to -1.14
$NC_{0.01-0.1}$	-1.37	-3.13 to 0.39	-4.04	-6.06 to -2.01	-1.20	2.39 to -0.01	-2.55	-3.95 to -1.14
$NC_{0.1-0.5}$	-1.49	-3.11 to 0.13	-2.24	-3.93 to -0.55	-1.14	-2.23 to -0.05	-1.57	-2.76 to -0.38
$NC_{0.5-2.5}$	-1.03	-2.42 to 0.35	-1.35	-3.10 to 0.41	-0.65	-1.56 to 0.25	-0.73	-1.97 to 0.51
$MC_{0.01-2.5}$	-1.38	-2.78 to 0.01	-2.18	-3.80 to -0.57	-1.01	-1.92 to -0.11	-1.42	-2.57 to -0.28
$MC_{0.01-0.1}$	-1.42	-2.73 to -0.11	-3.90	-5.60 to -2.21	-1.21	-2.13 to -0.30	-2.29	-3.45 to -1.12
$MC_{0.1-0.5}$	-1.40	-2.80 to 0.00	-2.13	-3.67 to -0.59	-1.05	-1.96 to -0.14	-1.44	-2.53 to -0.36
$MC_{0.5-2.5}$	-1.17	-2.57 to 0.24	-2.02	-3.89 to -0.14	-0.78	-1.69 to 0.13	-1.02	-2.36 to 0.32
PM_{10}	-0.37	-1.83 to 1.08	-2.31	-4.54 to -0.08	-1.30	-2.36 to -0.24	-1.51	-3.20 to -0.19

* The mean effect (β) and 95% confidence intervals (CI) refer to increase in particle concentrations by one interquartile range.

Table 2 - Peters 1997. Associations between particulate air pollution and respiratory symptoms*.

	Feeling ill during the Day				Cough			
	Same-day		Five-day Mean		Same day		Five-day Mean	
Particle Size	OR	CI	OR	CI	OR	CI	OR	CI
$NC_{0.01-2.5}$	1.29	1.05 to 1.58	1.39	1.15 to 1.68	1.16	0.97 to 1.38	1.17	1.01 to 1.37
$NC_{0.01-0.1}$	1.21	0.98 to 1.50	1.44	1.15 to 1.81	1.12	0.95 to 1.33	1.26	1.06 to 1.50
$NC_{0.1-0.5}$	1.27	1.08 to 1.50	1.23	1.07 to 1.42	1.13	0.98 to 1.30	1.03	0.91 to 1.16
$NC_{0.5-2.5}$	1.23	1.07 to 1.14	1.20	1.04 to 1.39	1.24	1.11 to 1.38	1.06	0.93 to 1.20
$MC_{0.01-2.5}$	1.24	1.09 to 1.41	1.21	1.06 to 1.38	1.19	1.07 to 1.33	1.02	0.91 to 1.15
$MC_{0.01-0.1}$	1.22	1.05 to 1.41	1.33	1.12 to 1.58	1.13	1.00 to 1.28	1.13	0.98 to 1.30
$MC_{0.1-0.5}$	1.23	1.09 to 1.40	1.19	1.05 to 1.35	1.18	1.06 to 1.31	1.02	0.91 to 1.14
$MC_{0.5-2.5}$	1.27	1.11 to 1.46	1.25	1.06 to 1.46	1.24	1.10 to 1.39	1.05	0.92 to 1.21
PM_{10}	1.20	1.01 to 1.44	1.47	1.16 to 1.86	1.32	1.16 to 1.50	1.30	1.09 to 1.55

* The odds ratio (OR) and 95% confidence intervals (CI) refer to increase in particle concentrations by one interquartile range.

Table 3 - Peters 1997. Two pollutant models*.

	Evening Peak Expiratory Flow (L/min)				Morning Peak Expiratory Flow (L/min)			
	Same-day		Five-day Mean		Previous day		Five-day Mean	
Particle Size^	β	CI	β	CI	β	CI	β	CI
$NC_{0.01-0.1}$	-0.40	-1.89 to 1.09	-4.25	-7.14 to -1.36	-0.60	-1.59 to 0.39	-2.74	-4.85 to -0.63
$MC_{0.1-0.5}$	-1.27	-2.78 to 0.25	-0.91	-2.55 to 0.73	-0.81	-1.80 to 0.17	-0.65	-1.84 to 0.55
$NC_{0.01-0.1}$	-0.86	-2.87 to 1.14	-3.70	-6.12 to -1.27	-1.05	-2.24 to 0.15	-2.56	-4.34 to -0.78
PM_{10}	-0.53	-2.38 to 1.32	-0.36	-3.35 to 2.62	-0.62	-1.96 to 0.72	0.02	-2.17 to 2.21

	Feeling ill during the Day				Cough			
	Same-day		Five-day Mean		Previous day		Five-day Mean	
Particle Size^	OR	CI	OR	CI	OR	CI	OR	CI
$NC_{0.01-0.1}$	1.06	0.83 to 1.35	1.29	0.97 to 1.72	1.05	0.87 to 1.27	1.36	1.10 to 1.67
$MC_{0.1-0.5}$	1.24	1.03 to 1.50	1.12	0.94 to 1.34	1.11	0.95 to 1.30	0.93	0.80 to 1.07
$NC_{0.01-0.1}$	1.17	0.91 to 1.50	1.25	0.93 to 1.68	0.98	0.80 to 1.19	1.22	0.97 to 1.52
PM_{10}	1.13	0.92 to 1.39	1.27	0.93 to 1.73	1.20	1.07 to 1.34	1.06	0.83 to 1.35

* The regression coefficients (β) and 95% confidence intervals (CI) refer to increase in particle concentrations by one interquartile range (see Table 1- Peters 1997).

^ Air pollutants listed underneath each other were analysed jointly in the regression analyses.

ii. Effects of ultrafine and fine particles in urban air on peak expiratory flow among children with asthmatic symptoms - (Pekkanen et al., 1997)

- *Location and period* – The study was conducted in Kuopio, Finland. The main particle sources of air pollution in the area are traffic, a peat-fired power plant connected to the municipal district heating system, and a corrugation cardboard mill, which is located 10 km north from the centre of the town. Over 80% of the buildings around the downtown area are heated by the municipal district heating system, but 25% of the homes use in addition wood burning to help to heat their house during the coldest period of the winter. Data analysis covered the period from February to April (57 days), 1994.

- *Population* – A screening questionnaire was sent to parents of 2995 children aged 7-12 years in five schools in the centre of Kuopio and three schools in two suburbs of Kuopio. A total of 2554 (86%) questionnaires were returned. All children from the selected schools reporting chronic respiratory symptoms were asked to participate in the study. These schools were selected because they were located closest to the air monitoring sites. A total of 197 children agreed to participate and were characterised with skin prick tests and spirometry. Only 39 asthmatic children (7-12 years) living in the centre of the town, who had filled in the diary on more than 60% of the possible days, were included in the analyses, since particle size distributions were measured only in the centre of Kuopio.

- *Health effect measurement* – During the follow-up, the children measured their peak expiratory flow (PEF) every day three times in the morning and in the evening, before taking any medication, with a mini-Wright peak flow meter. All three PEF readings were noted in a diary, and the largest of these three readings was used for the analyses.

- *Exposure measurement* – Air quality was monitored continuously in the centre of Kuopio, at least 50 m from any of the surrounding streets. Ultrafine particles were measured with an aerosol spectrometer (EAS). PM_{10} was collected with a single stage Harvard impactor. BS was sampled according to the OECD protocol (OECD, 1964). Gaseous pollutants were measured with continuously recording monitors: NO and NO_2 with chemiluminescence method, SO_2 with UV fluorescence, and CO with a nondispersive infrared monitor. Concentrations of black carbon (BC) were measured with a computer-controlled aethalometer.

  All the schools were located within 2.5 km from the monitoring site. During a typical day, the children spent 3 hour outdoors. Although the residential locations were not reported, considering age of the children, we would expect that those were relatively close to the schools.

- *Confounding factors* - Wind speed, wind direction, and temperature, were obtained from the municipal weather station network and the data on relative humidity from the weather station at the National Public Health Institute, Kuopio.

- *Model terms* - Wind speed, wind direction, and temperature, relative humidity; day weighted by number of children with peak expiratory flow for that day; 24 hr

(noon to noon) averages, weekend (coded as indicator variable - weekend vs not weekend).

- *Statistical methods* – Time series and regression models performed using SAS statistical software. Regression coefficients calculated for interquartile range of data.

- *Air pollution data* – Daily variations in PM_{10}, BC, and particle number concentrations in size range between 0.032 and 0.32 μm and between 1.0 and 10.0 μm were highly intercorrelated as they all originated primarily from traffic. Average daily levels of the pollutants during the study period (57 days) were: 18 $\mu g/m^3$ for PM_{10}, 13 $\mu g/m^3$ for BC, 44 300 particles/ m^3 for $NC_{0.01–0.1}$, 19 $\mu g/m^3$ for NO, 28 $\mu g/m^3$ for NO_2, 6 $\mu g/m^3$ for SO_2, and 0.6 mg/m^3 for CO.

Main findings:

- All lags of PM_{10} were negatively associated with morning PEF with the 2-day lag and the 4-day average reaching statistical significance (Table 1 - Pekkanen et al 1997). For BC, the regression coefficient of the 2-day lag was significant and negative, but for lags 1 and 3 it was positive. The regression coefficients of the 2-day lag of all particle number concentrations were negative, but statistically insignificant. Regress on coefficients of particles in size ranges between 0.032 and 0.32 μm and 1.0 and 3.2 μm was close to statistical significance and of similar magnitude as the regression coefficients of PM_{10} and BC. The regression coefficients were smallest in the smallest size fraction and in the size range between 0.10 and 1.0 μm.

- Lag 1 of all indicators of particulate pollution was negatively associated with evening PEF (Table 2 - Pekkanen et al 1997). The associations were strongest, but not significant for size ranges between 0.032 and 0.32 μm.

- During the study period, there were some days when particle levels were above the usual levels. Analysis of data revealed that these days had a large influence on the estimated regression coefficients. The three highest values of PM_{10}, BS, and all particle number concentrations were therefore excluded from the analysis. As highest values in most particle fractions were observed during the same days, this meant excluding 5 days from the analysis. After this exclusion, no significant associations were observed (Table 3 - Pekkanen et al 1997). Regression coefficients were also close to zero, except for the 4-day average of PM_{10} (Table 3 - Pekkanen et al 1997).

Conclusions:
All pollutants tended to be associated with declines in morning peak expiratory flow (PEF). The concentration of ultrafine particles was less strongly associated with variations in PEF than PM_{10} or BC. Different time lags of PM_{10} were most consistently associated with declines in PEF.

Table 1 - Pekkanen et al 1997. Adjusted[a] associations of 24-hour levels of particles and morning PEF deviations.

		Lag 0	Lag 1	Lag 2	Lag 3	4-day average
PM_{10} (µg/m3)	Coefficient	-0.727	-0.704	-1.13*	-0.530	-2.24**
	SE	0.556	0.512	0.478	0.540	0.796
Black smoke ($\mu g/m^3$)	Coefficient	0.067	0.105	-0.928*	0.203	-0.762
	SE	0.495	0.448	0.442	0.463	1.06
$PN_{0.10\text{-}0.032}$ ($1/cm^3$)	Coefficient	-0.085	-0.369	-0.577	0.111	-0.728
	SE	0.897	0.695	0.624	0.633	1.23
$PN_{0.032\text{-}0.10}$ ($1/cm^3$)	Coefficient	0.116	-0.160	-0.970***	0.292	-0.483
	SE	0.700	0.618	0.582	0.607	1.09
$PN_{0.10\text{-}0.32}$ ($1/cm^3$)	Coefficient	0.012	0.132	-0.651	0.376	0.059
	SE	0.478	0.481	0.464	0.453	0.861
$PN_{0.32\text{-}1.0}$ ($1/cm^3$)	Coefficient	0.136	0.072	-0.379	0.405	0.348
	SE	0.530	0.577	0.571	0.576	1.05
$PN_{1.0\text{-}3.2}$ ($1/cm^3$)	Coefficient	-0.057	-0.238	-0.901***	0.217	-0.711
	SE	0.658	0.573	0.536	0.566	1.03
$PN_{3.2\text{-}10}$ ($1/cm^3$)	Coefficient	-0.062	-0.363	-0.903	0.183	-0.912
	SE	0.733	0.607	0.559	0.594	1.11

Note: Regression coefficients and standard errors (SE) are multiplied by the interquartile range of the concentration of the pollutant.
[a] Adjusted for autocorrelation and time trend, minimum temperature, relative humidity, and weekend.
* $P < 0.05$.
** $P < 0.01$.
*** $P = 0.10$.

Table 2 - Pekkanen et al 1997. Adjusted[a] associations of 24-h levels of particles and evening PEF deviations.

		Lag 0	Lag 1	Lag 2	Lag 3	4-day average
PM_{10} ($\mu g/m^3$)	Coefficient	-0.091	-0.174	-0.287	0.595	0.036
	SE	0.524	0.473	0.468	0.477	0.944
Black smoke ($\mu g/m^3$)	Coefficient	0.073	-0.351	0.029	0.520	0.440
	SE	0.426	0.392	0.407	0.386	1.03
$PN_{0.010-0.032}$ ($1/cm^3$)	Coefficient	-0.606	-0.167	0.264	0.098	0.352
	SE	0.827	0.597	0.559	0.588	1.17
$PN_{0.032-0.10}$ ($1/cm^3$)	Coefficient	-0.188	-0.815	0.030	0.722	0.102
	SE	0.649	0.516	0.533	0.526	1.07
$PN_{0.10-0.32}$ ($1/cm^3$)	Coefficient	0.304	0.691[*]	0.230	0.656[*]	0.590
	SE	0.438	0.394	0.408	0.387	0.843
$PN_{0.32-1.0}$ ($1/cm^3$)	Coefficient	0.365	-0.312	0.554	0.719	1.17
	SE	0.486	0.490	0.482	0.511	0.985
$PN_{1.0-3.2}$ ($1/cm^3$)	Coefficient	-0.358	-0.683	0.033	0.646	-0.514
	SE	0.603	0.479	0.483	0.489	1.02
$PN_{3.2-10}$ ($1/cm^3$)	Coefficient	-0.361	-0.611	0.144	0.344	0.003
	SE	0.673	0.510	0.501	0.525	1.09

Note: Regression coefficients and standard errors (SE) are multiplied by the interquartile range of the concentration of the pollutant.
[a] Adjusted for autocorrelation and time trend, minimum temperature, relative humidity, and weekend.
[*] $P < 0.10$.

Table 3 - Pekkanen et al 1997. Adjusted[a] associations of 24-h levels of particles and morning PEF deviations excluding 5 high days of ultrafine particles or PM_{10}.

		Lag 2	4-day average
PM_{10} ($\mu g/m^3$)	Coefficient	0.179	-0.914
	SE	0.760	1.48
Black smoke ($\mu g/m^3$)	Coefficient	-0.111	0.278
	SE	0.695	1.72
$PN_{0.010-0.032}$ ($1/cm^3$)	Coefficient	0.265	0.215
	SE	0.789	-0.130
$N0._{032-0.10}$ ($1/cm^3$)	Coefficient	0.105	-0.130
	SE	0.851	1.45
$PN_{0.10-0.32}$ ($1/cm^3$)	Coefficient	0.237	0.116
	SE	0.620	1.18
$PN_{0.32-1.0}$ ($1/cm^3$)	Coefficient	0.124	0.290
	SE	0.617	1.15
$PN_{1.0-3.2}$ ($1/cm^3$)	Coefficient	0.263	-0.069
	SE	0.791	1.41
$PN_{3.2-10}$ ($1/cm^3$)	Coefficient	0.278	0.162
	SE	0.791	1.47

Note: Regression coefficients and standard errors (SE) are multiplied by the interquartile range of the concentration of the pollutant.
[a] Adjusted for autocorrelation and time trend, minimum temperature, relative humidity, and weekend.

iii. Fine particulate air pollution, resuspended road dust and respiratory health among symptomatic children - (Tiittanen et al., 1999)

This study assessed a short-term association of PM with peak expiratory flow (PEF) and respiratory symptoms in children with chronic respiratory symptoms

- *Location and period* – Study was conducted in Kuopio, a town of 85, 000 residents in Finland. The main sources of ambient pollution are traffic, a peat-fired power plant connected to a municipal district heating system, and a corrugated cardboard mill. The children were followed-up for 6 weeks in spring 1995.

- *Population* – The procedure for selecting the participant was similar to that used in Pekkanen et al. (1997) study. Fifty-eight children with chronic respiratory symptoms (8-13 years old) agreed to participate. Only forty-nine children, who had completed the diary on > 60 % days, were included in the analysis.

- *Health effect measurement* – The children measured their peak expiratory flow (PEF) three times every morning and evening before taking any respiratory medication. They also recorded daily respiratory symptoms.

- *Exposure measurement* – Air pollution data were collected at a single sampling site in the centre of town, 50 m away from the roads. Number concentrations of particles within the size range of 0.01 – 10 μm were measured with the electrical aerosol spectrometer (EAS). PM_{10} and $PM_{2.5}$ were collected with single stage Harvard impactors. PM_{10} was also measured with a TEOM 1400A monitor. TSP was collected with a high-volume sampler.

  Daily concentrations of black carbon (BC) and gaseous pollutants NO_2, SO_2, and CO were measured continuously at the same site as PM. Concentrations of ozone were measured at the site located 3.5 km west of the centre of town.

  Daily pollen counts collected with a Burkard sampler located at the University of Kuopio, 3.5 km away from the centre.

- *Confounding factors* – Meteorological data (wind speed, wind direction and temperature) were obtained from municipal weather station network, and relative humidity was measured at Kuopio airport.

- *Statistical methods* – Time series analysis, generalised linear models, multiple linear regression and logistic regression were performed using SAS and S-Plus software.

- *Air pollution data* – Apart from four days, the concentrations of air pollutants did not exceed the national air quality guidelines. Specifically, the average daily levels of the pollutants during the study period ranged: from 5 to 234 $\mu g/m^3$ for TSP, from 5 to 122 $\mu g/m^3$ for PM_{10}, from 3 to 55 $\mu g/m^3$ for $PM_{2.5,}$ from 291 to 2120 ng/m^3 for BC, from 6980 to 40200 particles/ m^3 for $NC_{0.01–0.1}$, from 0 to 50 $\mu g/m^3$ for O_3, from 5 to 46 $\mu g/m^3$ for NO_2, from 0 to 5.2 $\mu g/m^3$ for SO_2, and from 0.1 to 1.0 mg/m^3 for CO. The intercorrelation between indicators of

particle air pollution and CO were high. In addition, NO_2 and O_3 were highly correlated with numbers of ultrafine particles and BC.

Main findings:

- There was more cough and ORS in the two highest tertiles of fine particles (Table 1- Tiittanen et al 1999). Bronchodilator use seemed to increase over the tertiles of fine and ultrafine particles. However, the results for bronchodilator and preventive medication use, and for LRS suffer from a low number of cases. Also, most of the cases were children who had LRS or used medication every day. The increase in fine particles was associated with a decrease in morning PEF, whereas evening PEF decreased along the tertiles of both fine and ultrafine particles.

- The results of the multiple linear regressions suggested that increases in the concentrations of particulate air pollutants for lag 1 were associated with a decrease in the morning PEF (Table 2 - Tiittanen et al 1999). Although both fine and coarse particles had a negative effect on PEF, only the estimate for $PM_{2.5}$ reached statistical significance ($p<0.05$). Evening PEF was also negatively associated with all indicators of particulate air pollutants at lag 1 (Table 2 - Tiittanen et al 1999). Fine particles, especially particles in the accumulation mode, tended to have a stronger effect on evening PEF than coarse particles. However, only the estimate for $PN_{0.1\text{-}1.0}$ was statistically significant. The results of the aggregated data analysis were similar to the results in Table 2 - Tiittanen et al 1999, except that at lag 1 PM_{10} was also statistically significantly associated with morning PEF (results were not shown).

- To check the sensitivity of the results on the selected confounders, the association between $PN_{0.1\text{-}1.0}$ and evening PEF, as well as $PM_{2.5}$ and morning PEF was thoroughly examined. The crude regression coefficient for $PN_{0.1\text{-}1.0}$ was –1.53 and for $PM_{2.5}$ –0.26. Adding confounders strengthened the association of $PM_{2.5}$ with morning PEF, but had little effect on the coefficient, for $PN_{0.1\text{-}1.0}$. Including indicator variables for the Easter holiday, the use of bronchodilators, preventive medication or exposure to tobacco smoking at home in the regression model did not change the results. Replacing 1-day lagged values of minimum temperature, relative humidity and pollen with the same day values in the regression models for evening PEF did not alter the results.

- The results of two pollutant models, *i.e.* having an indicator for particle mass concentration ($PM_{2.5}$ or PM_{10}) and an indicator for particle number concentration ($PN_{0.01\text{-}0.1}$ or $PN_{0.1\text{-}1.0}$) in the same model, were the following. The association between morning PEF and 1-day lagged $PM_{2.5}$ decreased when $PN_{0.01\text{-}0.1}$ was in the same model ($p=0.09$ for $PM_{2.5}$). Also, all the other associations between morning PEF and 1-day lagged particles decreased ($p>0.10$). The sign of the regression coefficients still varied by lag. The association between evening PEF and 1-day lagged $PN_{0.1\text{-}1.0}$ was still statistically significant when PM_{10} (for $PN_{0.1\text{-}1.0}$, B = -2.18, SE=0.93) or $PM_{2.5}$ (for $PN_{0.1\text{-}1.0}$, B = -2.43, SE=1.06) was included in the model. Lag 2 for $PN_{0.1\text{-}1.0}$ was still positive, while other lags were negative. Four-day average of $PN_{0.1\text{-}1.0}$ (B = -5.15, SE=2.48) also reached statistical significance when $PM_{2.5}$ was included in the model.

- Adding gaseous pollutants one at a time in the model, i.e. having both a particulate and gaseous pollutant in the model at the same time, changed the results for lag 1 as follows. In the case of morning PEF, O_3 strengthened the observed associations and all indicators of particulate air pollution reached statistical significance. Introducing either NO_2 or SO_2 in the model did not change the results markedly. In the analysis of evening PEF, SO_2 did not alter the results. There were no statistically significant associations remaining between particulate air pollutants and evening PEF when NO_2 or O_3 was included in the model. However, NO_2 or O_3 were highly correlated with $PN_{0.01\text{-}0.1,}$ thus causing multi-colinearity in the model. For the same reason, CO was not used at all in two-pollutant models. None of the gaseous pollutants at any lag, except the 4-day average of SO_2 and the 4-day average of $O_{3,}$ were statistically significantly associated either with morning or evening PEF, when a gaseous pollutant was the only pollutant in the model.

- Cough had a positive, statistically significant association with PM_{10}, $PM_{2.5\text{-}10}$, $PM_{2.5}$, and resuspended PM_{10} at lag 2 and with 4-day average $PM_{2.5}$ (Table 3 - Tiittanen et al 1999). Adding gaseous pollutants one at a time in the model did not change the results for cough.

- Phlegm had a positive, but not statistically significant association with PM_{10}, $PM_{2.5\text{-}10}$, $PM_{2.5,}$ and resuspended PM_{10} for lag 2. Particulate air pollutants at any lag did not seem to affect URS. Random effects models for LRS and use of medication (bronchodilator and preventive medication, separately) did not converge, possibly due to the low number of cases.

Conclusions:
No consistent effect of particles was found as the associations varied by lag. Of lags examined, only 1-day-lagged $PM_{2.5}$ was statistically significantly associated with decreased morning peak expiratory flow (PEF). Evening PEF was significantly associated with the 1-day-lagged number of particles in the size range of 0.1-1.0 μm. One-day-lagged PM_{10}, $PM_{2.5}$, and $PM_{2.5\text{-}10}$, and the 4-day average of $PM_{2.5}$ were significantly associated with increased risk of cough. Given the short duration of the study, separating the effects of different types of particles was difficult.

Table 1 - Tiittanen et al 1999. Adjusted* daily prevalences of respiratory symptoms and medication use, and the adjusted daily average peak expiratory flow (PEF) deviations (L/min) in the tertiles of particle air pollutants at lag 1.

	Range	No of Days	Preventive medication	Bronchodilator medication	Cough	Phlegm	URS	LRS	Morning	Evening
$PM_{2.5}$	-8.9	11	0.15	0.03	0.32	0.18	0.51	0.02	1.63	2.0
	9-20.2	12	0.16	0.03	0.36	0.20	0.52	0.04	1.17	0.72
	20.2-	12	0.16	0.05	0.37	0.19	0.52	0.04	-1.11	0.28
$PN_{0.01-0.1}$	-11300	11	0.15	0.03	0.35	0.19	0.50	0.03	0.46	1.55
	11300-20500	12	0.16	0.04	0.35	0.19	0.52	0.04	0.90	0.49
	20500-	12	0.16	0.05	0.35	0.20	0.55	0.04	-0.01	0.22
$ResPM_{10}$	-5	11	0.16	0.03	0.34	0.20	0.52	0.03	0.86	1.64
	5-22	12	0.15	0.04	0.35	0.19	0.51	0.03	1.16	0.53
	22-	12	0.16	0.04	0.36	0.19	0.53	0.04	-0.39	0.75

*: evening PEF, symptoms and medication use adjusted for day, day 2, weekend, minimum temperature (lag 1), relative humidity (lag 1) and pollen (lag 1), and morning PEF adjusted for day, day 2, weekend, minimum temperature (lag 0), relative humidity (lag 0) and pollen (lag 0). URS: upper respiratory symptom; LRS: lower respiratory symptom.

Table 2 - Tiittanen et al 1999. Adjusted associations of daily average levels of particulate air pollutants with morning* and evening+ PEF deviations (L/min).

	Lag 0	Lag 1	Lag 2	Lag 3	4-day average
Morning PEF					
n	1353	1307	1260	1213	1213
PM_{10}	0.74 (0.51)	-1.01 (0.53)^	0.46 (0.59)	-0.53 (0.52)	-0.77 (1.46)
$PM_{2.5-10}$	0.78 (0.49)	-0.86 (0.49)^	0.35 (0.56)	-0.39 (0.47)	-0.46 (1.34)
$PM_{2.5}$	0.62 (0.50)	-1.06 (0.52)+	0.54 (0.57)	-0.70 (0.54)	-1.08 (1.45)
$PN_{0.1-1.0}$	0.19 (0.71)	-1.24 (0.72)^	0.82 (0.76)	-0.07 (0.97)	-0.75 (2.34)
$PN_{0.01-.01}$	0.20 (0.59)	-0.68 (0.56)	0.88 (0.61)	-0.46 (0.55)	-0.43 (1.80)
Res. PM_{10}	0.84 (0.50)	-0.85 (0.51)^	0.47 (0.56)	-0.46 (0.49)	-0.18 (1.53)
Black carbon	0.28 (0.42)	-0.74 (0.42)^	0.69 (0.46	-0.19 (0.44)	-0.18 (1.38)
Evening PEF					
n	1362	1362	1315	1268	1268
PM_{10}	0.44 (0.42)	-0.33 (0.54)	0.05 (0.53)	0.27 (0.59)	1.42 (1.54)
$PM_{2.5-10}$	0.45 (0.39)	-0.21 (0.51)	0.02 (0.49)	0.33 (0.56)	1.61 (1.38)
$PM_{2.5}$	0.39 (0.43)	-0.43 (0.52)	0.08 (0.53)	-0.15 (0.57)	0.85 (1.55)
$PN_{0.1-1.0}$	0.38 (0.73)	-1.56 (0.72)+	0.28 (0.73)	-0.50 (0.76)	-3.07 (2.09)
$PN_{0.01-.01}$	0.69 (0.53)	-1.00 (0.60)^	0.49 (0.57)	-0.01 (0.62)	1.05 (1.88)
Res. PM_{10}	0.37 (0.40)	-0.11 (0.51)	0.10 (0.51)	0.42 (0.56)	2.13 (1.53)
Black carbon	0.35 (0.39)	-0.67 (0.43)^	0.28 (0.43)	-0.04 (0.47)	-0.38 (1.39)

The data are presented as the regression coefficient (B) and standard error (SE; in parenthesis) which are multiplied by the interquartile range of the pollutant.

*: adjusted for day, day2, weekend, minimum temperature (lag 0), pollen (lag 0) and first-order autocorrelation;

+: $p<0.05$;

^: $p<0.10$

Table 3 - Tiittanen et al 1999. Adjusted* associations of daily average levels of particulate air pollutants and cough.

	Lag 0 (n=1441)		Lag 1 (n=1441)		Lag 2 (n=1393)		Lag 3 (n=1345)		4-day average (n=1345)	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
PM_{10}	1.00	(0.92-1.10)	0.96	(0.86-1.08)	1.15	(1.03-1.28)^	1.01	(0.88-1.14)	1.33	(0.93-1.91)
$PM_{2.5-10}$	0.99	(0.91-1.08)	0.95	(0.85-1.05)	1.15	(1.04-1.27)^	1.00	(0.88-1.12)	1.20	(0.87-1.66)
$PM_{2.5}$	1.02	(0.92-1.11)	0.98	(0.88-1.10)	1.13	(1.01-1.26)^	1.02	(0.90-1.15)	1.48	(1.02-1.13)^
$PN_{0.1-1.0}$	1.06	(0.91-1.24)	1.04	(0.89-1.21)	1.01	(0.86-1.18)	1.06	(0.90-1.25)	1.71	(0.98-2.95)^
$PN_{0.01-.01}$	0.96	(0.86-1.08)	1.07	(0.95-1.22)	1.05	(0.93-1.18)	0.98	(0.86-1.12)	1.24	(0.76-2.00)
Res. PM_{10}	1.01	(0.92-1.09)	0.94	(0.84-1.05)	1.16	(1.04-1.29)^	0.98	(0.87-1.10)	1.25	(0.88-1.77)
Black carbon	1.99	(0.91-1.08)	1.03	(0.94-1.12)	1.05	(0.96-1.15)	0.99	(0.89-1.09)	1.29	(0.91-1.82)

Odds ratios (OR) and 95% confidence intervals (CI) refer to an increase in the pollutant by one interquartile range.
*: adjusted for day, day2, weekend, minimum temperature (lag 1), pollen (lag 1) and first-order autocorrelation;
+ : p<0.05;
^ : p<0.10.

iv. Ultrafine particles in urban air and respiratory health among adult asthmatics - (Penttinen et al., 2001).

- *Location and period* – The study was conducted in Helsinki, Finland, during the winter and spring season (November 1996 - April 1997). Characteristic features of air pollution in Helsinki are low ozone levels, occasional episodes of meteorological inversion situations with high levels of other pollutants, and seasonal episodes of resuspended road dust. The road dust phenomenon is seen particularly during spring when the streets are dry, the snow and ice on the ground have melted away, and the particulate matter deposited on the street is resuspended mechanically by traffic or wind. This particulate matter consists mainly of sand spread on the icy roads during the winter and matter ground from road surface by studded tires.

- *Population* - The study group consisted of 54 non-smoking adult asthmatic subjects from urban Helsinki, which complied with the requirements of participating at least 60% (125 participation days) of possible days. All participants resided within 2 km of the air quality monitoring site in attempt to ensure that the point measurement of pollutants reflects the personal exposure. The group was recruited with newspaper announcements, direct mail, and through the local association of pulmonary disabled.

- *Health effect measurement* – The respiratory health of the subjects was monitored with daily self-monitored peak flow measurements and a supervised biweekly spirometric lung function test. In addition, the subjects recorded their daily symptoms and medication use in a diary.

- *Exposure measurement* – Air pollutants were monitored on a fixed monitoring site in central urban Helsinki. Particle number concentration (NC) was measured continuously in 12 size ranges from 10 nm to 10 μm with an Electric Aerosol Spectrometer (EAS). For quality control purposes, NC was also monitored continuously with a condensation nucleous counter (CNC; TSI Inc, St. Paul, MN, USA). The correlation coefficient between particle number concentrations measured by CNC and EAS was 0.98. Twenty-four-hour, noon-to-noon particle mass concentrations (PM_1, $PM_{2.5}$, and PM_{10}) were monitored with single-stage Harvard impactors (Air Diagnostics and Engineering, Naples, ME, USA).

- *Confounding factors* – The data for meteorological parameters (wind speed, wind direction, relative humidity, and minimum temperature) were obtained from the metropolitan monitoring network of the Helsinki Metropolitan Area Council.

  Pollen count data were collected with the Burkard volumetric pollen trap and were provided by the Finnish Aerobiology Group. Because pollen counts were negligible during the whole study period, they were not considered confounders.

  The data on influenza activity were obtained from the health authorities of Helsinki City. Influenza activity increased during the end of January and the beginning of February. However, no serious epidemics were reported. Fever reporting was not increased during that period in the study group.
  .

- *Statistical methods* – Time series analysis and multivariate, auto-regressive linear regression were used to examine the associations between daily health end-points and indicators of air pollution.

- *Air pollution data* – The average daily levels of the pollutants during the study period were: 13.5 μg/m^3 for PM_{10}, 8.4 μg/m^3 for $PM_{2.5,}$ 3.4 μg/m^3 for PM_1, 14 500 particles/ m^3 for $NC_{0.01\text{-}0.1}$, 16.7 μg/m^3 for NO, 25.3 μg/m^3 for NO_2, and 0.4 mg/m^3 for CO. The particle mass concentrations (PM_1, $PM_{2.5}$, and PM_{10}) were highly intercorrelated. A high correlation was also observed between $PM_{2.5}$, PM_1, and $NC_{0.1\text{-}1.0}$. No high correlations were observed between meteorological parameters and particle mass or particle number concentrations.

***Main findings**:*

- The spirometric lung function indices (FVC, FEV_1, and PEFR) tended to be inversely, but mostly insignificantly, associated with ultrafine particle number concentrations measured on the same day, the previous day, and with a mean concentration of the past 5 days (Table 1 - Penttinen et al. 2001). The strongest associations were observed in the size range of 0.1-1μm. These associations were, however, predominantly insignificant. The spirometric PEFR also tended to be inversely associated with $PM_{2.5}$ and PM_1 concentrations.

- The regression coefficients from the models for self-monitored PEFR were smaller than the regression coefficients from the models for spirometric PEFR and FEV_1 (Table 2 - Penttinen et al. 2001).

- Spirometric PEFR was most strongly associated with the particle number concentrations in size classes between 0.10 and 1.0 μm (Table 3 - Penttinen et al. 2001).

Conclusions:
Daily mean number concentration, but not particle mass (PM_{10}, $PM_{2.5}$), was negatively associated with daily peak expiratory flow (PEF) deviations. The strongest effects were seen for particles in ultrafine range. The PEF decreased by - 0.84% for an interquartile range increase in $NC_{0.1\text{-}1}$ measured on the previous day. The corresponding effect estimates for PM_1 and $PM_{2.5}$ were somewhat smaller: - 0.15% and - 0.12%, respectively. No significant effect of particle pollution on symptoms or bronchodilator use was seen.

Table 1 - Penttinen et al. 2001. Associations of biweekly spirometric lung function indices and particle number concentrations measured on previous days.

	FVC[a]		FEV_1		PEFR	
	β[b]	SE	β	SE	β	SE
$PNC_{0.01-0.1}$						
Lag 0	0.00	0.45	-0.40	0.44	-0.52	0.50
Lag 1	-0.25	0.27	-0.37	0.27	-0.27	0.30
Lag 2	0.31	0.36	0.59	0.35	0.34	0.41
5-Day average	-0.68	0.75	-0.91	0.72	-0.72	0.84
$PNC_{0.1-1}$						
Lag 0	-0.06	0.42	0.14	0.42	-0.29	0.47
Lag 1	-0.60	0.32	-0.44	0.32	-0.84	0.36*
Lag 2	0.14	0.44	0.45	0.43	-0.17	0.50
5-Day average	-1.20	0.93	-0.86	0.90	-2.27	1.04*
PM_1						
Lag 0	-0.04	0.10	-0.04	0.10	-0.23	0.12*
Lag 1	-0.07	0.08	0.00	0.08	-0.15	0.09
Lag 2	0.12	0.08	0.18	0.08	0.04	0.09
5-Day average	0.15	0.16	0.21	0.16	-0.22	0.18
$PM_{2.5}$						
Lag 0	0.00	0.07	0.04	0.07	-0.06	0.08
Lag 1	-0.06	0.05	-0.02	0.05	-0.12	0.06*
Lag 2	0.07	0.04	0.10	0.05*	0.02	0.05
5-Day average	0.03	0.10	0.00	0.10	-0.17	0.11
PM_{10}						
Lag 0	0.05	0.04	0.07	0.04	0.04	0.04
Lag 1	-0.01	0.04	0.01	0.04	-0.03	0.04
Lag 2	0.05	0.03	0.06	0.03	0.04	0.03
5-Day average	0.05	0.06	0.06	0.06	0.02	0.06

Regression coefficients (β) and standard errors (SE) are adjusted for time trend, temperature, relative humidity, and diurnal variation.

[a] Lung function indices (FVC, FEV_1, and PEFR) are defined as deviation (%) from personal median.

[b] Regression coefficients and SEs were calculated per interquartile range of each particle measurement.

* $p < 0.05$.

Table 2 - Penttinen et al. 2001 Associations of daily self-monitored PEFRs and particle number concentrations measured on previous days.

	Morning PEFR[a]		Afternoon PEFR		Evening PEFR	
	β[b]	SE	β	SE	β	SE
$PNC_{0.01-0.1}$						
Lag 0	-0.017	0.094	-0.231	0.085**	-0.151	0.080
Lag 1	-0.240	0.090**	0.019	0.081	-0.002	0.078
Lag 2	0.068	0.099	0.057	0.087	-0.119	0.084
5-Day average	-0.307	0.283	-0.770	0.254**	-0.596	0.252*
$PNC_{0.1-1}$						
Lag 0	-0.061	0.104	-0.164	0.094	-0.125	0.089
Lag 1	-0.086	0.104	0.070	0.094	0.045	0.091
Lag 2	0.033	0.110	-0.095	0.097	-0.204	0.093*
5-Day average	0.053	0.321	-0.521	0.289	-0.528	0.287
$PM_{2.5}$						
Lag 0	0.113	0.112	0.049	0.100	-0.072	0.096
Lag 1	-0.076	0.112	0.134	0.100	0.129	0.097
Lag 2	-0.001	0.110	-0.059	0.100	0.100	0.096
5-Day average	0.146	0.142	0.063	0.138	0.019	0.132

Regression coefficients (β) and standard errors (SE) are adjusted for long-term time trend, temperature, relative humidity, weekends, and autocorrelation.

[a] PEFR is defined as deviation (%) from personal median.

[b] Regression coefficients and SEs were calculated per interquartile range of each particle measurement.

* $p < 0.05$;

** $p < 0.01$.

Table 3 - Penttinen et al. 2001. Association of PEFR[a] (spirometry) with the size classes of the particle number concentration (5-day mean).

Size class (μm)	β[b]	SE
0.010-0.018	0.03	0.93
0.018-0.032	-0.96	0.90
0.032-0.056	-1.23	0.86
0.056-0.100	-1.68	1.01
0.10-0.18	-2.13	1.05*
0.18-0.32	-2.49	1.06*
0.32-0.56	-2.89	1.12*
0.56-1.00	-2.46	1.19*

Regression coefficients (β) and standard errors (SEs) are adjusted for long-term time trend, temperature, relative humidity, and diurnal variation.

[a] PEFR is defined as deviation (%) from personal median.

[b] Regression coefficients and SEs were calculated per interquartile range of particle number concentration.

* $p < 0.05$.

v. Acute respiratory effects of particles: mass or number - (Osunsanya et al., 2001).

- *Location and period* – Aberdeen, Scotland UK; 90 days during winter 1998/1999

- *Population* – Participants were recruited from chest clinics at the Royal Hospitals. 44 patients (aged older than 50 years) agreed to participate. They had airflow obstruction associated with either asthma or chronic obstructive pulmonary disease and answered positively when asked if the weather affected their chest. All were resident within 5 miles of the air monitoring sites. They completed a questionnaire inquiring about their house, general indoor and outdoor activities, and exposure to dust, fumes, and cigarette smoke. They were then asked to keep a daily diary of chest symptoms, use of inhalers, and twice daily peak expiratory flow (PEF) for 3 months.

- *Health effect measurement* – Symptom scores, bronchodilator use, and peak flow rate (PEF) were recorded daily for 3 months. The Ferraris Medical Pocketpeak peak flow meter was used, recording between 90 and 710 L/min. Each subject was asked to record the best of three blows twice a day. Scores for cough and shortness of breath were graded once daily on a 6 point scale 0-5 (0 for no symptoms at all and 5 for the worst possible symptoms). Total number of puffs on an inhaler was recorded. Night-time scores were recorded, as were those the next morning at about 8 am before the use of any drugs. Daytime symptoms were recorded at about 8 pm.

- *Exposure measurement* – Continuous measurements of ultrafine particles were conducted by the TSI model 3934 scanning mobility particle sizer (SMPS) and PM_{10} measurements by the tapered element oscillating microbalance (TEOM) located at a background site in the city centre. Data were compressed to daily 24-hour mean, minimum, and maximum values.

  As the patients spent much time indoors, a separate facet of the study was performed to compare particle counts inside and outside the laboratory with windows closed. Only background counts were considered as indoor counts vary with combustion of domestic fuels. Seventy-nine concurrent measurements of indoor and outdoor counts were run for 2 months of this 3 month study.

- *Confounding factors* – Data on daily mean, minimum, and maximum temperature and relative humidity were obtained from one site located in central Aberdeen. Also, wind speed and wind direction were measured continuously. Wind direction was summarised as the number of days with wind from directions falling in eight sectors.

- *Model terms* - Temperature (24 hr mean, minimum, and maximum), relative humidity, wind speed, wind direction, ultrafine particles, PM_{10}

- *Statistical methods* – Time series and regression methods, and generalised estimating equations for binary outcomes, eg. presence/ absence of symptoms, etc. Analyses performed using STATA statistical software.

- *Air pollution data* – Some data were lost over the Christmas period. There were 78 valid daily counts of ultrafine particles and 85 of PM_{10} over the 90 days measurement period. The distribution of pollution measurements was highly positively skewed, so natural logs of these were taken before they were considered in regression models.

  The levels of pollution were very low in historic terms (PM_{10} did not rise higher than 40 $\mu g/m^3$), which allowed authors to conclude that no episode of pollution occurred over the period of the study. The outdoor concentrations of the pollutants ranged: from 740 to 60636 particle/ m^3 (with average of 10241 particle/ m^3) for $NC_{0.01-0.1}$ and from 6 to 34 $\mu g/m^3$ (with average of 13 $\mu g/m^3$) for PM_{10}.

  Number concentrations of ultrafine particles indoors and outdoors were significantly correlated, those indoors being about half of those outdoors.

Main findings:

- Ultrafine particle counts indoors and outdoors were significantly correlated, with the indoor counts being about half of those outdoors.

- PM_{10} particles significantly correlated with ultrafine particle numbers.

- The correlations between the original scores of cough and shortness of breath and use of medicine were all positive and greater than 0.5. There were the expected negative associations between both daytime and night time PEF and symptom scores and use of medication ($p<0.001$). No significant correlations were found between PEF and any of the particle or meteorological measurements. The results displayed in Tables 1-3-Osunsanya et al. 2001 are odds ratios for changes of 1 unit of measurement (for example, 1 ^oC in temperature, 1 m/s in wind speed, 1 % in relative humidity, and a change of 1 in the log of PM_{10}).

- In regression models for lung function, allowing for first order autocorrelation, none of the meteorological variables was associated with daytime or night time PER. No significant associations were found between daytime or night time PEF and the two measures of pollution after adjustment for confounders.

- No association was found between ultrafine particles and decrements of 10% in daytime or night time PER. However, associations were found between the decrements of 10% in daytime PEF and increasing values of log PM_{10} on the same day, which were of borderline significance after adjusting for mean temperature, wind speed, humidity, and serial association. The odds ratio for a change from 10 to 20 $\mu g/m^3$ was 1.19 (95% confidence interval (95% CI) 1.00 to 1.42).

- No significant associations between symptoms and ultrafine particles were found. High levels of symptoms of cough were significantly associated with lower mean temperatures ($p=0.02$) and higher log of PM_{10} over the previous 3 days ($p=0.02$)

after adjusting for autocorrelation, mean wind speed, and humidity (Table 2 - Osunsanya et al., 2001). The odds ratio shown is for one unit change in log of PM_{10} as a 3 day average.

- There was a significantly positive association between high levels of symptoms of shortness of breath and log same day PM_{10} ($p=0.003$). This association remained significant after adjusting for wind speed, humidity, and temperature (Table 3- Osunsanya et al., 2001). The odds ratio shown is for one unit change in log of PM, over the previous 3 days.

Conclusions:
Evidence was not found to support hypothesis that ultrafine particles affect respiratory health of subjects with asthma or chronic obstructive pulmonary disease.

Table 1 - Osunsanya et al. 2001. Mean peak flow results.

	Mean	SD	Min	Max
PEF-day (1/min, mean)	264	5	253	277
PEF-night (1/min, mean)	267	5	256	277

Table 2 - Osunsanya et al. 2001. Model for 10% decrements in day time peak flow rate.

	p Value	OR for change of one unit of measurement	95% CIs
Mean temperature (^oC)	0.18	0.97	0.928 to 1.014
Mean wind speed (m/s)	0.10	1.097	0.981 to 1.225
Mean humidity (%)	0.44	1.006	0.991 to 1.020
Log PM_{10}	0.05	1.284	0.996 to 1.656

Table 3 - Osunsanya et al. 2001. Model for high cough score.

	p Value	OR for change of one unit of measurement	95% CIs
Mean temperature (^oC)	0.02	0.965	0.936 to 1.994
Mean wind speed (m/s)	0.05	1.078	0.999 to 1.164
Mean humidity (%)	0.41	0.996	0.987 to 1.005
Log PM_{10}	0.02	1.470	1.066 to 2.028

Table 4 - Osunsanya et al. 2001. Model for high breathlessness score.

	p Value	OR for change of one unit of measurement	95% CIs
Mean temperature (^{o}C)	0.16	0.983	0.959 to 1.007
Mean wind speed (m/s)	0.90	1.004	0.940 to 1.011
Mean humidity (%)	0.30	1.004	0.997 to 1.011
Log PM_{10}	0.003	1.214	1.068 to 1.380

Table 5 - Osunsanya et al. 2001. Model for use of medication.

	p Value	OR for change of one unit of measurement	95% CIs
Mean temperature (^{o}C)	0.11	-0.018	-0.039 to 0.004
Mean wind speed (m/s)	0.11	0.048	-0.011 to 0.107
Mean humidity (%)	0.14	0.005	-0.002 to 0.011
Log PM_{10}	0.04	0.118	0.005 to 0.232
Constant	<0.001	3.667	2.905 to 4.430

vi. Increased asthma medication use in association with ambient fine and ultrafine particles - (Von Klot et al., 2002).

This study investigated the association between fine and ultrafine ambient particles and asthma medication use and symptoms in a panel of asthmatic adults.

- *Location and period* – The study was conducted from September 1996 to March 1997 in Erfurt, a Germany city with 200,000 residents. The main sources of air pollution are a power plant, traffic, and domestic heating.

- *Population* – Participants were recruited by their physicians. Eligible participants had to be treated with asthma medication during the previous year and to be non-smokers. Details on socio-demographic indicators of the study subjects were obtained through a questionnaire. Fifty-three persons who had participated > 30 days (37-77 years old) were included in the analysis.

- *Health effect measurement* – The subjects were asked to record the severity of symptoms and medication use every evening. They also recorded hours spent outside the study area.

- *Exposure measurement* – Air pollutants were measured continuously at a single sampling site located in urban area, 50 m away from the vehicle traffic.

  Number concentrations of ultrafine particles and fine particles were monitored with DMPS (to cover a range 0.01 - 0.5 μm) and LAS-X (0.1 - 2.5 μm). PM_{10} and $PM_{2.5}$ measurements were conducted with Harvard Impactors. The coarse

particle mass fraction ($PM_{2.5-10}$) was calculated as the difference of these two measurements. Number and mass concentrations were determined concurrently.

Daily concentrations of SO_2 were measured at the same site, but NO_2, and CO were monitored 2 km away from the central measurement station.

- *Confounding factors* – Weather data (temperature and humidity) were measured concurrently with PM at the same site.

- *Statistical methods* – Time series and logistic regression modelling controlling for trend, temperature, weekend, holidays and autocorrelation.

- *Air pollution data* – The correlation between ultrafine particle numbers and PF mass was moderate (r=0.45), as well as its correlation with PM_{10} and SO_2.The correlation of ultrafine particles with CO and NO_2 was stronger (r=0.66). Temperature was correlated negatively with all pollutants; relative humidity in contrast was positively correlated.

Main findings:

- Table 1 - von Klot et al 2002 summarises the results of the prevalence of short-acting β_2-agonists. There was no association of same day values of all pollutants with the prevalence of short-acting β_2-agonists. An association was present with the 5-day running means of almost all subfractions of particles. Only CO and the coarse particle fraction ($PM_{2.5-10}$) did not achieve statistical significance. Fourteen-day means of the pollutants had mostly lower, and often not statistically significant effect estimates.

- In the two-pollutant models, the 5-day mean ultrafine particle number concentration and fine particle mass concentration appeared to have independent effects on the use of inhaled short-acting β_2-agonists (Table 2 - von Klot et al 2002). NO_2 seemed to be the best predictor.

- Regression results of inhaled corticosteroid use are shown in Table 3 - von Klot et al 2002. Positive statistically significant associations were observed between inhaled corticosteroids and same day exposure to ultrafine particle number concentrations, fine particle number concentrations and the gases NO_2 and CO. Significant effects were found for 5-day running means of the particle fractions as well as of the gases. Noticeably higher effects were observed in association with the 14-day mean particulate air pollutants. SO_2 showed a similar, but not equally strong tendency to longer accumulated effects. CO and NO_2 demonstrated higher effects for the 5-day mean than for the 14-day mean. The magnitude of the associations of inhaled corticosteroid use with fine or ultrafine particles was similar.

- In the two-pollutant models, 14-day mean $NC_{0.01-0.1}$ showed a weaker effect than $MC_{0.01-2.5}$ (Table 2 - von Klot et al 2002). $MC_{0.01-2.5}$ showed a stronger association with inhaled corticosteroid use than CO and NO_2.

- Regression results of wheezing are shown in Table 4 - von Klot et al 2002. Little evidence was seen for an association between same-day pollutant measures and the prevalence of wheezing. Statistically significant associations between the 5-day mean number concentration of ultrafine particles, CO and NO_2 and the prevalence of wheezing were observed. Positive, but weaker associations were seen with mass concentrations. The observed effects of ultrafine particle and accumulation mode number concentrations increased when considering the 14-day means, but were little changed for particle mass, CO and NO_2.

- Two pollutant models, that considered the 5-day means of the pollutants, supported a strong ultrafine particle effect on the prevalence of wheezing (Table 2 - von Klot et al 2002). Ultrafine particles competed for the effect with the gases and CO and NO_2, whereas the evidence of a fine particle mass effect remained poor. In the two-pollutant model with the 14-day means, there was even stronger evidence of an association of the ultrafine particles with the prevalence of wheezing.

- Table 5 - von Klot et al 2002 shows the main results of the other symptoms. Shortness of breath and its combination with wheezing both showed a similar pattern as wheezing, whereas for "waking up with breathing problems" in addition a 5-day mean $PM_{2.5}$ effect and an immediate NO_2 effect were found. Cough and phlegm showed consistent and significant associations with ultrafine and fine particles, as well as with NO_2. The effect of 5-day and 14-day running means was comparable.

Conclusions:
Corticosteroid use and bronchodilator use both increased in association with cumulative exposure over 14 days of ultrafine particles and fine particless. A comparable effect was found for cumulative exposure over 5 days. The data suggest that asthma medication use increases with particle air pollution. The effect might be more delayed but more pronounced on anti-inflammatory medication than on bronchodilators.

Table 1 - von Klot et al 2002. Effect estimates[#] for the association between the prevalence of inhaled short-acting β_2-agonists use and particulate and gaseous pollution (odds ratios (OR) and 95% confidence intervals (CI) are given for an increase of one interquartile range (IQR).

	Same day			5-day mean			14-day mean		
	IQR	OR	CI	IQR	OR	CI	IQR	OR	CI
Number of concentrations									
$NC_{0.01-0.1}$	15000	0.97	0.90-1.04	10000	1.11	1.01-1.21	7700	1.08	0.96-1.21
$NC_{0.1-0.5}$	1800	0.99	0.92-1.05	1500	1.10	1.03-1.19	1450	0.95	0.86-1.05
$NC_{0.5-2.5}$	26	0.99	0.93-1.05	22	1.09	1.01-1.17	17	1.08	1.02-1.15
Mass concentration									
$MC_{0.1-0.5}$	21	0.98	0.92-1.04	21	1.11	1.02-1.20	17	1.01	0.93-1.10
$MC_{0.01-2.5}$	28	0.96	0.90-1.04	26	1.10	1.01-1.20	20	1.03	0.95-1.12
$PM_{2.5-10}$	12	1.01	0.95-1.06	11	1.01	0.94-1.09	6.7	0.92	0.86-1.00
Gases									
NO_2	22	1.00	0.94-1.06	16	1.08	1.02-1.14	12	1.03	0.97-1.08
CO	0.6	0.98	0.93-1.03	0.6	1.04	0.97-1.12	0.54	0.93	0.86-1.01
SO_2	21	1.03	0.98-1.08	17	1.06	1.00-1.12	13	1.07	1.02-1.12

NC: number concentration of particles (numbers represent diameter of particles in µm); MC: mass concentration of particles (numbers represent diameter of particles in µm); $PM_{2.5-10}$: particulate matter with a 2.5-10 µm aerodynamic diameter; NO_2: nitrogen dioxide; CO: carbon monoxide; SO_2: sulfur dioxide. [#]: Adjusted for cubic trend, temperature, weekend, Christmas holidays, first order autocorrelation. Analysis period October 29, 1996 – March 30, 1997 (153 days).

Table 2 - von Klot et al 2002. Effect estimates[#] of the association between two pollutants, jointly in one model, and the health outcomes (odds ratios (OR) and 95% confidence intervals (CI) are given for an increase of one interquartile range (IQR) as specified in Table 1 - von Klot et al 2002).

Prevalence of	Pollutant		$MC_{0.01-2.5}$		Pollutant		$NC_{0.01-0.1}$	
	OR	CI	OR	CI	OR	CI	OR	CI
Inhaled short-acting β_2-agonist use								
$NC_{0.01-0.1}$	1.07	0.97-1.18	1.07	0.98-1.18				
NO_2	1.10	1.02-1.19	1.00	0.89-1.12	1.09	1.02-1.18	1.01	0.90-1.13
CO	1.00	0.91-1.11	1.10	0.98-1.22	1.01	0.91-1.11	1.10	0.98-1.25
SO_2	1.05	0.99-1.11	1.07	0.98-1.17	1.06	1.00-1.12	1.08	0.99-1.19
Inhaled corticosteroid use[+]								
$NC_{0.01-0.1}$	1.01	0.87-1.18	1.53	1.39-1.69				
NO_2	1.02	0.95-1.10	1.51	1.35-1.68	1.19	1.07-1.32	1.06	0.85-1.32
CO	0.89	0.81-0.98	1.63	1.49-1.78	0.81	0.72-0.91	1.82	1.54-2.15
SO_2	Correlation coefficient larger than 0.8				1.24	1.19-1.29	1.28	1.15-1.44
Wheezing								
$NC_{0.01-0.1}$	1.12	1.01-1.24	1.02	0.92-1.12				
NO_2	1.05	0.97-1.14	1.01	0.89-1.15	1.01	0.94-1.09	1.12	0.99-1.26
CO	1.15	1.04-1.27	0.96	0.86-1.08	1.09	0.98-1.22	1.05	0.92-1.19
SO_2	0.97	0.91-1.04	1.09	0.99-1.20	0.97	0.92-1.03	1.14	1.04-1.26

NC: number concentration of particles (numbers represent diameter of particles in μm). NO_2: nitrogen dioxide; CO: carbon monoxide; SO_2: sulfur dioxide; MC: mass concentration of particles (numbers represent diameter of particles in μm); adjusted for cubic trend, temperature, weekend, Christmas holidays, first order autocorrelation; air pollution exposures: 5-day means; [+]: air pollution exposures: 14-day means.

Table 3 - von Klot et al 2002. Effect estimates[#] for the association between the prevalence of inhaled corticosteroid use and particulate and gaseous pollution (odds ratios (OR) and 95% confidence intervals (CI) are expressed for an increase of one interquartile range (IQR).

	Same day			5-day mean			14-day mean		
	IQR	OR	CI	IQR	OR	CI	IQR	OR	CI
Number of concentrations									
$NC_{0.01-0.1}$	15000	1.07	1.00-1.15	10000	1.22	1.12-1.33	7700	1.45	1.29-1.63
$NC_{0.1-0.5}$	1800	1.06	0.99-1.14	1500	1.23	1.14-1.32	1450	1.51	1.37-1.67
$NC_{0.5-2.5}$	26	1.13	1.06-1.21	22	1.28	1.19-1.37	17	1.44	1.36-1.53
Mass concentrations									
$MC_{0.1-0.5}$	21	1.09	1.02-1.17	21	1.28	1.18-1.39	17	1.49	1.38-1.61
$MC_{0.01-2.5}$	28	1.10	1.02-1.18	26	1.28	1.18-1.39	20	1.54	1.43-1.66
$PM_{2.5-10}$	12	1.03	0.98-1.08	11	1.12	1.04-1.20	6.7	1.27	1.18-1.37
Gases									
NO_2	22	1.15	1.09-1.23	16	1.29	1.22-1.37	12	1.21	1.15-1.28
CO	0.6	1.05	1.00-1.11	0.6	1.25	1.17-1.34	0.54	1.06	0.97-1.15
SO_2	21	1.03	0.98-1.09	17	1.21	1.14.1.28	13	1.28	1.22-1.33

NC: number concentration of particles (numbers represent diameter of particles in µm); MC: mass concentration of particles (numbers represent diameter of particles in µm); $PM_{2.5-10}$: particulate matter with a 2.5-10 µm aerodynamic diameter; NO_2: nitrogen dioxide; CO: carbon monoxide; SO_2: sulfur dioxide. [#]: Adjusted for cubic trend, temperature, weekend, Christmas holidays, first order autocorrelation. Analysis period October 25, 1996 – March 30, 1997 (157 days).

Table 4 - von Klot et al 2002. Effect estimates[#] for the association between the prevalence of wheezing and particulate and gaseous pollution (odds ratios (OR) and 95% confidence intervals (CI) are given for an increase of one interquartile range (IQR).

	Same day			5-day mean			14-day mean		
	IQR	OR	CI	IQR	OR	CI	IQR	OR	CI
Number of concentrations									
$NC_{0.01-0.1}$	15000	0.94	0.8601.01	10000	1.13	1.03-1.24	7700	1.27	1.13-1.43
$NC_{0.1-0.5}$	1800	1.00	0.93-1.07	1500	1.08	1.00-1.17	1450	1.11	1.00-1.24
$NC_{0.5-2.5}$	26	1.03	0.95-1.10	22	1.05	0.97-1.13	17	1.03	0.96-1.10
Mass concentrations									
$MC_{0.1-0.5}$	21	1.01	0.94-1.08	21	1.08	0.99-1.17	17	1.05	0.96-1.15
$MC_{0.01-2.5}$	28	1.01	0.93-1.09	26	1.07	0.98-1.17	20	1.07	0.98-1.17
$PM_{2.5-10}$	12	0.97	0.91-1.02	11	1.06	0.98-1.15	6.7	1.05	0.96-1.15
Gases									
NO_2	22	1.01	0.95-1.08	16	1.06	1.01-1.12	12	1.09	1.03-1.15
CO	21	1.00	0.95-1.06	17	0.99	0.93-1.05	13	0.99	0.95-1.04

NC: number concentration of particles (numbers represent diameter of particles in µm); MC: mass concentration of particles (numbers represent diameter of particles in µm); $PM_{2.5-10}$: particulate matter with a 2.5-10 µm aerodynamic diameter; NO_2: nitrogen dioxide; CO: carbon monoxide; SO_2: sulfur dioxide. [#]: Adjusted for cubic trend, temperature, weekend, Christmas holidays, first order autocorrelation. Analysis period November 4, 1996 – March 30, 1997 (147 days).

Table 5 - von Klot et al 2002. Effect estimates[#] for the association between the prevalence of respiratory symptoms and particulate air pollution and NO_2 (odds ratios (OR) and 95% confidence intervals (CI) are given for an increase of one interquartile range (IQR).

	Same day			5-day mean			14-day mean		
	IQR	OR	CI	IQR	OR	CI	IQR	OR	CI
Attack of shortness of breath and wheezing									
$NC_{0.01-0.1}$	15000	1.01	0.91-1.12	10000	1.08	0.96-1.21	7700	1.26	1.08-1.48
$MC_{0.01-2.5}$	28	0.99	0.89-1.10	26	1.02	0.91-1.14	20	1.13	1.02-1.26
$N0_2$	22	1.03	0.95-1.11	16	1.06	0.99-1.14	12	1.08	1.00-1.16
Waking up with breathing problems									
$NC_{0.01-0.1}$	15000	1.04*	0.96-1.13	10000	1.09	0.99-1.19	7700	1.26	1.13-1.41
$MC_{0.01-2.5}$	28	1.03*	0.96-1.10	26	1.16	1.06-1.25	20	1.15	1.06-1.24
NO_2	22	1.07*	1.01-1.13	16	1.10	1.04-1.15	12	1.12	1.07-1.18
Shortness of breath									
$NC_{0.01-0.1}$	15000	0.98	0.90-1.06	10000	1.09	0.99-1.19	7700	1.24	1.11-1.40
$MC_{0.01-2.5}$	28	1.04	0.96-1.13	26	1.05	0.96-1.15	20	1.03	0.94-1.12
NO_2	22	1.00	0.94-1.06	16	1.05	0.99-1.11	12	1.11	1.05-1.17
Phlegm									
$NC_{0.01-0.1}$	15000	1.01	0.94-1.09	10000	1.11	1.02-1.21	7700	1.11	0.99-1.25
$MC_{0.01-2.5}$	28	1.05	0.98-1.13	26	1.10	1.01-1.19	20	1.06	0.97-1.15
NO_2	22	1.05	0.99-1.11	16	1.09	1.04-1.15	12	1.05	1.00-1.11
Cough									
$NC_{0.01-0.1}$	15000	1.07	0.98-1.16	10000	1.17	1.07-1.28	7700	1.20	1.06-1.35
$MC_{0.01-2.5}$	28	1.07	0.99-1.17	26	1.04	0.95-1.13	20	1.01	0.92-1.10
NO_2	22	1.03	0.97-1.09	16	1.06	1.00-1.12	12	1.09	1.03-1.15

NC: number concentration of particles (numbers represent diameter of particles in µm); MC: mass concentration of particles (numbers represent diameter of particles in µm); NO_2: nitrogen dioxide; [#]: adjusted for cubic trend, temperature, weekend, Christmas holidays, first order autocorrelation; *for the symptom "waking up with breathing problems" the effect of the concentration of pollutants on the previous day was estimated.

Summary on the asthma panel studies

From the studies described above the following is suggested:

- There are more definite effects of particles on adults with asthma than on children with asthma symptoms.
- There are health effects of both ultrafine particles and fine particles, independently of each other.
- Because inflammatory events in the lungs take several days to develop it is likely that a lag time exists between exposure to ultrafine particles and observable health effects. Cumulative effects over 5 days (for medication use up to 14 days) are stronger than same-day effects.
- In two pollutant models, the effect on the same day is stronger for fine particles, whereas the cumulative effect is stronger for ultrafine particles.
- Inhaled asthma medication use and asthma symptoms in adults increase in association with ultrafine particles and fine particles and with gaseous pollutants such as NO_2.

STUDIES ON CARDIOVASCULAR MORBIDITY

i. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: The exposure and risk assessment for fine and ultrafine particles in ambient air - (Pekkanen et al., 2002).

- *Location and period* – Study was performed in Helsinki, Finland in 1998/1999 over a period of 6 month.

- *Population* – A panel of 45 adult subjects with stable coronary artery disease. The subjects were characterised by a questionnaire and recording of a 12-lead standard resting ECG. The main inclusion criteria for the study were a self-report or a doctor-diagnosed coronary artery disease, being a non-smoker, and age >50 years. All subjects lived within a 5-kilometre radius of the monitoring site.

- *Health effect measurement* – The subjects were followed up with biweekly clinical visits and daily symptom diaries. The clinical visit included recording of ambulatory ECG using a standardised protocol, which included a 6-minute submaximal exercise module with a bicycle ergometer. Only exercise-induced ST depressions were considered. For each subject, the visit was scheduled for the same weekday and time of day with no changes in daily medication. If the subject had symptoms of angina pectoris or moderate or severe respiratory symptoms during the clinical visit or fever during the preceding week, no exercise test was performed. A common cold or respiratory infection was a relative contraindication for the exercise.

- *Exposure measurement* – The number concentrations of ultrafine and accumulation-mode particles were measured continuously with an Electric Aerosol

Spectrometer (EAS) on a fixed urban background-monitoring site. Six missing days of ultrafine particle number concentrations were imputed based on total particle counts (CPC 3022A, TSI Inc) measured at the same site (correlation 0.98 with $NC_{0.01-0.1}$). $PM_{2.5}$ and PM_1 were monitored with single-stage Harvard impactors. Thirteen missing days of $PM_{2.5}$ were imputed using data from a dynamic mass monitor (ESM Eberline, FH 62 I-R) (correlation 0.97 with $PM_{2.5}$). $PM_{2.5-10}$ was calculated by subtracting $PM_{2.5}$ from PM_{10}. All variables were 24-hour means from noon to noon. The data on PM_{10} were provided by the Helsinki Metropolitan Area Council.

Although data provided were on gaseous pollutants NO_2 and CO, the methods and the site of their monitoring were not reported. It is assumed that those were also provided by the Helsinki Metropolitan Area Council.

- *Confounding factors* – The data on average temperature were provided by the Helsinki Metropolitan Area Council and relative humidity by Finnish Meteorological Institute.

- *Statistical methods* – Time series analysis, logistic regression and generalised additive models were performed using S-Plus software.

- *Air pollution data* – All measures of particles reflecting mostly the accumulation-mode particles ($NC_{0.1-1}$, PM_1, and $PM_{2.5}$) were highly inter-correlated but were less correlated with ultrafine particles ($NC_{0.01-0.1}$) and coarse particles ($PM_{2.5-10}$) NO_2 was mainly correlated with $NC_{0.01-0.1.}$

***Main findings**:*

- Consistent and statistically significant associations were observed between increased risk of exercise-induced ST- segment depression >0.1 mV and exposure to measured air pollutants, except $PM_{2.5-10}$ (Table 1 - Pekkanen et al. 2002**)**. The associations were strongest for $NC_{0.01-1}$ and accumulation-mode particles ($NC_{0.01-1}$, PM_1, and $PM_{2.5}$) measured 2 days before the clinic visit. Associations were more consistent when horizontal or downward-sloping ST-segment depressions during the exercise test were considered but smaller and not as consistent with ST-segment depression >0.05 mV.

- The association of $NC_{0.01-1}$ with risk of ST-segment depression was sensitive to selection of lag of temperature. The association was strongest with lag 3 used in the basic model and weakest with short lags (odds ratio [OR] 1.82; 95% CI, 1.09 to 3.03 for lag 0). Different lags of temperature had little effect on the association with $NC_{0.01-1.}$ Current day pollen decreased the size of the effect (with pollen OR 2.38 for $NC_{0.01-1,}$ OR 3.18 for $NC_{0.01-1}$, and OR 3.55 for PM_1, all lag 2), but the associations remained statistically significant. Relative humidity and ambient air barometric pressure had little effect on any of the associations analysed. This was also true for influenza epidemics, occurrence of supraventricular ectopic beats during exercise test, and excluding those days with pollution levels above the 95^{th} percentile of pollution.

- All of the associations were strengthened when subjects with left bundle-branch block, left ventricular hypertrophy, or anterolateral infarction were excluded (Table 2 - Pekkanen et al. 2002). All associations, but especially associations with accumulation mode particles, tended to be stronger among subjects who did not use β-blockers. Associations tended to be stronger among women and those without history of past myocardial infarction for $NC_{0.01\text{-}1}$ (NO_2), whereas the opposite was true for $PM_{2.5}$ and PM_1.

- In two-pollutant models (lag 2 of all pollutants), the effects of $NC_{0.01\text{-}1}$ (OR 2.55; 95% CI, 1.25 to 5.19) and $PM_{2.5}$ (OR 2.34; 95% CI, 1.14 to 4.80) were independent of each other. Otherwise the pollutants were quite correlated, so their independent effects were more difficult to separate. However, there was a suggestion that the effects of PM_1 and especially $NC_{0.01\text{-}1}$ were least changed in two pollutant models with other pollutants, like with ultrafine particles (OR 2.70; 95% CI, 1.04 to 6.99 for $NC_{0.01\text{-}1}$ and OR 1.34; 95% CI, 0.54 to 3.36 for $NC_{0.01\text{-}1}$ or with $PM_{2.5}$ (OR 3.75; 95% CI, 1.13 to 12.41 for $NC_{0.01\text{-}1}$ and OR 0.84; 95% CI, 0.23 to 3.02 for $PM_{2.5}$).

Conclusions:
The association was most consistent for measures of particles reflecting accumulation mode particles, but ultrafine particles also had an effect, which was independent of $PM_{2.5}$. Gaseous pollutants NO_2 and CO were associated with an increased risk for ST-segment depressions. No consistent association was observed for coarse particles. The associations tended to be stronger among subjects who did not use β-blockers.

Table 1 - Pekkanen et al. 2002. ORs* between daily levels of air pollution and occurrence of ST-segment depressions during repeated mild exercise tests.

		>0.05 mV (n=125 +)		>0.1 mV (n=72 +)		>0.1 mV + Slope ^ (n=51 +)	
	n	OR	95% CI	OR	95% CI	OR	95% CI
$NC_{0.01-0.1}$							
Lag 0	342	0.72	0.46-1.11	1.12	0.72-1.76	1.11	0.70-1.76
Lag 1	342	0.93	0.54-1.60	1.21	0.64-2.29	1.03	0.54-1.95
Lag 2	342	1.73	1.01-2.97	3.14	1.56=6.32	1.98	0.99-3.95
Lag 3	342	1.38	0.80-2.38	1.45	0.79-2.64	1.35	0.71-2.55
$NC_{0.1-1}$							
Lag 0	330	0.50	0.25-0.99	1.04	0.50-2.17	1.29	0.62-2.67
Lag 1	327	1.06	0.58-1.94	1.10	0.52-2.30	1.28	0.59-2.77
Lag 2	322	1.53	0.88-2.67	3.29	1.57-6.92	2.88	1.38-6.01
Lag 3	327	1.41	0.82-2.44	1.19	0.64-2.21	1.38	0.70-2.70
PM_1							
Lag 0	342	0.78	0.30-2.02	1.35	0.44-4.10	1.43	0.46-4.45
Lag 1	342	1.07	0.49-2.35	1.46	0.58-3.71	1.57	0.63-3.90
Lag 2	342	1.51	0.74-3.07	4.56	1.73-12.03	5.50	1.89-16.04
Lag 3	342	1.41	0.66-3.01	1.72	0.74-3.99	1.53	0.65-3.60
$PM_{2.5}$							
Lag 0	339	0.72	0.37-1.41	1.12	0.49-2.56	1.26	0.55-2.91
Lag 1	340	0.97	0.57-1.65	1.11	0.58-2.13	1.34	0.70-2.57
Lag 2	342	1.51	0.91-2.52	2.84	1.42-5.66	3.65	1.69-7.90
Lag 3	342	1.29	0.76-2.17	1.40	0.78-2.53	1.28	0.71-2.33
$PM_{2.5-10}$							
Lag 0	339	0.83	0.44-1.53	1.48	0.70-3.15	1.00	0.48-2.05
Lag 1	340	2.02	0.94-4.38	0.78	0.35-1.74	0.40	0.13-1.23
Lag 2	342	0.96	0.41-2.26	1.99	0.80-5.67	1.89	0.66-5.44
Lag 3	342	1.60	0.67-3.81	0.46	0.16-1.34	0.79	0.28-2.22
NO_2							
Lag 0	342	0.79	0.58-1.07	1.04	0.74-1.45	1.03	0.73-1.45
Lag 1	342	1.08	0.77-1.52	1.21	0.80-1.82	1.02	0.67-1.53
Lag 2	342	1.23	0.89-1.69	2.02	1.34-3.04	1.54	1.03-2.29
Lag 3	342	1.31	0.93-1.84	1.16	0.80-1.66	1.17	0.78-1.73
CO							
Lag 0	334	0.99	0.73-1.33	1.38	0.98-1.95	1.35	0.94-1.94
Lag 1	330	1.19	0.93-1.51	1.20	0.89-1.63	1.20	0.89-1.63
Lag 2	334	1.37	1.06-1.78	1.73	1.26-2.39	1.60	1.14-2.23
Lag 3	339	1.23	0.97-1.58	1.13	0.88-1.46	1.20	0.91-1.58

* ORs calculated for a change of 10 000 particles/cm^3 in $NC_{0.01-0.1,}$ 1000 particles/cm^3 in $NC_{0.1-1}$), 10 µg/m^3 in PM_1, $PM_{2.5}$, $PM_{2.5-10}$, NO_2 and 0.1 µg/m^3 in CO.

+ Number of events for 342 visits. Minimum number of events (322 visits, lag 2 of $NC_{0.1-1,}$) is 116, 63, and 46 for ST depressions >0.05 mV, and horizontal or downward sloping depressions >0.1 mV, respectively.

^ Horizontal or downward sloping ST-segment depression >0.1 mV.

Table 2 - Pekkanen et al. 2002. ORs* of pollutants (lag 2) with ST depressions >0.1 mV during the exercise test in different subgroups.

		$NC_{0.01-01}$		$PM_{2.5}$		CO	
	n	OR	95% CI	OR	95% CI	OR	95% CI
All	342	3.14	1.56-6.32	2.84	1.42-5.66	1.73	1.26-2.39
No LVH, LBBB, Antero-lateral MI +	312	4.14	1.91-9.00	3.64	1.66-7.98	1.94	1.36-2.77
Women	157	7.59	2.75-20.94	2.57	1.15-5.72	1.91	1.30-2.82
Men	185	0.73	0.23-2.32	6.32	1.31-30.45	1.48	0.77-2.83
No MI ^	144	3.24	1.29-8.11	2.13	0.94-4.84	1.41	0.97-2.05
MI	198	2.91	0.97-8.70	7.89	1.67-37.20	3.27	1.54-6.97
No β-blockers	114	3.68	0.88-15.39	8.40	1.76-40.21	4.95	1.58-15.50
β-Blockers	228	3.11	1.35-7.18	2.12	0.99-4.56	1.39	0.97-1.97

* ORs calculated for a change of 10 000 particles/cm^3 in $NC_{0.01-0.1}$, 10 μg/m^3 in $PM_{2.5}$, and 0.1 μg/m^3 in CO.

+ Subjects with left bundle-branch block (Minnesota code 7-1-1), left ventricular hypertrophy (3-1, 3-3), or anterolateral infarction (1-1 or 1-2 in leads I, aVL, V6) in the resting ECG excluded.

^ History of myocardial infarction.

Summary, critique, and conclusions on morbidity studies

Exposure assessment plays a key role in the risk assessment process. In order to quantify the associated risk and health impact of a possible pollutant, it is necessary to obtain a description of the full-range of exposure distribution. To achieve these goals, measurements of pollutant concentrations on personal and air pollution levels in homes, at work and outdoors must be combined with information on time spent in indoor and outdoor locations. It is however typical for epidemiological studies to use ambient particle concentrations as a measure for personal exposure to PM.

While the results of the reviewed studies are described as providing exposure-response relationships, they actually provide information on ambient concentration-response relationships across the population studied. The studies did not aim to address the health effects of ultrafine particles from indoor sources. Thus, it is not clear whether the observed (or unobserved) associations between PM and daily morbidity were due to biases in the measurement of exposure to air pollutants caused by the monitoring ambient levels of PM rather than measuring personal exposure of each member of the study population. Unfortunately, it has not been shown how well ambient ultrafine particles correlate with personal exposure to ultrafine particles from ambient origin. This is a real gap in the existing knowledge. It has been estimated that people in most Western societies spend up to 90% (95% in Australia, ABS 1996) of their time indoors (Fishbein and Henry, 1991; Jenkins et al., 1992; Byrne, 1998) so that the relationship between personal exposure and ambient concentrations warrants further attention.

Furthermore, the relationship between personal exposures and PM as measured by central outdoor monitors was not established. Even if ambient exposure data were an adequate surrogate for personal exposure, it is unlikely that the measurements taken at a

fixed-point monitoring site, which is the case in all the reviewed studies, will give a reasonable estimate of overall outdoor pollutant exposure.

Similarly, approaches used in these studies, eg. regular measurements by subjects themselves, measurements in a clinical setting, and daily diary entries, are open to problems of compliance, either due to subjects forgetting to make the entries or do the tests, or because subjects may be temporarily indisposed and unable to perform the required tests without risk of triggering a health crisis. In turn, the lack of compliance represents missing data, with some studies defining at least 60% or 80% complete data as the criterion for inclusion in the final dataset. This has implications for the techniques that are used in the analysis, as well as for the ultimate findings of the study. The use of generalised estimation equations in some studies represents a means of accommodating these problems. Incorporating the individual's measurement for the previous day as a term in the model in another study was another way of more thoroughly assessing the relationships between the health outcome of interest and the factors that are thought to influence it.

To illustrate the effects of ultrafine particles across the studies reviewed, Figure 5.2 presents a set of odds ratios and 95% confidence intervals as reported in these studies. It can be seen that all the odds ratios are greater than or equal to 1.00, which suggests some increased risk, while the 95% confidence intervals indicate that these increases do not reach statistical significance since they all include 1.00 within their ranges. These results may reflect the small sample sizes on which they are based and support the argument for further research, especially with larger samples.

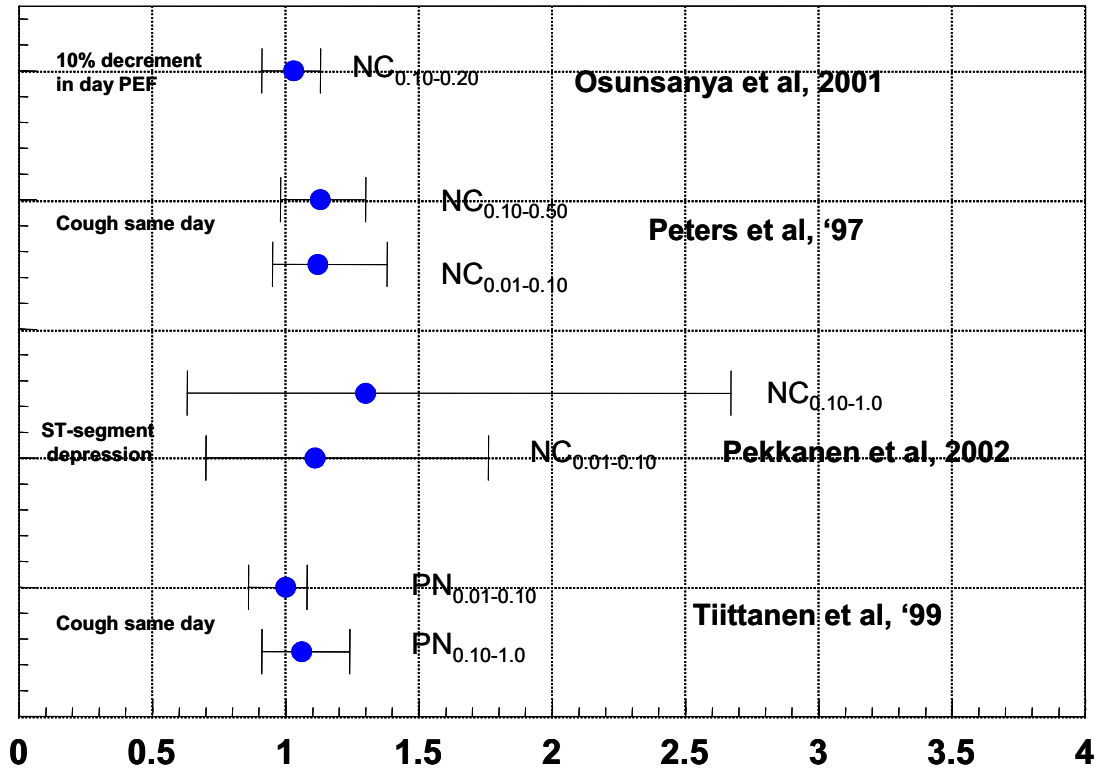

Figure 5.2. Summary of the results of the epidemiological studies on association of ultrafine particles with health outcomes: odds ratios (dots) and 95% confidence interval (bars) for ultrafine particles by study. All confidence intervals include the value 1.00.

ONGOING STUDIES

A case-crossover study on survivors of acute myocardial infarction in association with fine and ultrafine particles

Investigators:

- GSF-Institute of Epidemiology: Annette Peters (PI), Stephanie von Klot, Hannelore Löwel, Margit Heier, Ines Trentignalia, Katrin Zetzsche, Josef Cyrys, Mike Pitz, H.-Erich Wichmann;
- GSF-Institute of Medical Computer Sciences: Almut Hörmann, Ursula Kaup;
- KORA-Coronary Event Registry: Petra Pitschi, Gabriele Zimmermann, Christine Winter, Gabriele Orlik, Anita Schuler, Christa Meisinger;
- Collaboration: Pater Alkuin, St. Benediktinerkloster, Augsburg, Germany; Michael Hauptmann, National Cancer Institute, Bethesda, USA; Murray A. Mittleman, Douglas W. Dockery, Harvard School of Public Health, Boston, USA.

Study objective: The influence of fine and ultrafine particles on triggering of myocardial infarction will be analysed in a case-crossover study.

Specific aims: To test the following hypotheses:

- Myocardial infarctions are associated with the acute exposure to particulate air pollution (two hours before the onset).
- Myocardial infarctions are specifically associated with the acute exposure to the number of ultrafine particles rather than the mass of fine particles.

Study design: Case-crossover design based on the coronary event registry Augsburg from January 1999 until July 2001. The activity of myocardial infarction survivors is ascertained 4 days before the event. Clinical data will be obtained from medical records and from the interview of coronary event registry. Measurements of $PM_{2.5}$ and of the number concentration of particles will be conducted for the study.

Study population: Survivors of myocardial infarction age 25 to 74 who had their event within the study area of the Coronary Event Registry (see below). Eligible subject were registered in the coronary event registry, who participated in the interview and who had a myocardial infarction according to the criteria of MONICA.

Study area: City of Augsburg, District of Augsburg (rural), District of Aichach-Friedberg.

Study methods:

- Assessment of activities: A diary was developed to assess the four days preceding the myocardial infarction on an hourly basis. The diary includes information on the time of the myocardial infarction, sleeping periods, activity levels during the day, time spent outdoors, means of transportation used, whereabouts within the study area, angina pectoris symptoms,

occurrence of extreme anger or joy, and dust or solvent exposures. A semi-structured interview is conducted by a trained research nurse.

- Assessment of ambient air pollution: Air pollution is measured by a fixed monitoring site in Erfurt, Germany. The measurements consist of (1) outdoor hourly total number concentrations of particles with a condensation nucleus counter (CPC 3022A, TSI, Aachen, Germany); (2) outdoor hourly $PM_{2.5}$ concentrations with a Tapered Element Oscillating Microbalance (TEOM 1400A, Rupprecht und Pataschnik, MLU, Essen, Germany); (3) outdoor hourly concentrations of O_3, NO_2, SO_2 and CO concentrations collected through the local air quality network

Study Period: February 1999 to July 2001.

Current status: Statistical analyses are ongoing.

Study on inflammatory responses and cardiovascular risk factors in elderly subjects with angina pectoris or COPD in association with fine and ultrafine particles 2000/2004

Principal Investigators: H.-Erich Wichmann and Annette Peters, GSF-Institute of Epidemiology.

Investigators: GSF-Institute of Epidemiology: H.-Erich Wichmann, Annette Peters, Angela Ibald-Mulli, Gabriele Wölke, Regina Rückerl, Katrin Zetzsche, Sabine Kött, Josef Cyrys, Mike Pitz

Collaboration: University of Rochester – Department of Environmental Medicine, Rochester, USA Günter Oberdörster, Mark Utell, Wojciech Zareba, Betty Jane Mykins; UCLA School of Medicine – Vascular Medicine Program, Los Angeles, USA: Victor J. Marder, Joel Kanouse, Elizabeth Vandeventer; University of Ulm – Abteilung für Innere Medizin II, Ulm, Germany: Wolfgang Koenig; Praxis für Hygiene und Umweltmedizin, Erfurt, Germany: Olaf Manuwald, Gisela Bock, Brigitte Manuwald, Cornelia Schlegelmilch

Study objectives: The objective of the study is to characterize the association between ambient particle exposures and changes in biomarkers of inflammation in the airways and the blood of patients with stable coronary artery disease (CAD) and patients with chronic obstructive pulmonary disease (COPD).

Specific aims: To assess: (1) Whether the concentration of fine and ultrafine particles is associated with an inflammatory response in the airways and/or a rise of plasma viscosity and the concentration of fibrinogen and other acute phase proteins in the blood; (2) Whether an increased blood coagulation is connected to a change in the autonomic control of the heart in association with fine and ultrafine particles; (3) Whether the impact of ultrafine particles on health is stronger compared to the impact of fine particles; (4) Whether there is an association between the autonomic control of the heart and ultrafine particles.

Study design: Prospective panel study with repeated clinical examinations.

Study population: Panel 1: (Coronary Artery Disease): 60 male non-smokers, aged between 50 and 80 years, recruited from local practitioners. Further participants have physician diagnosed coronary artery disease or stable angina pectoris or take angina pectoris medication. People with fresh (less than three months) cardiac events (e.g. MI, stroke, by-pass, PTCA), pace makers, instable AP, right/left bundle branch block, insulin dependent diabetes mellitus and patients that are taking anticoagulants were excluded from the panel.

Panel 2 (Chronic Obstructive Pulmonary Disease): 40 males, recruited from local practitioners with physician diagnosed chronic obstructive pulmonary disease (COPD), chronic asthma, or chronic bronchitis. People with recent (less than three months) cardiac events (e.g. MI, stroke, by-pass, PTCA), pace makers, instable AP, right/left bundle branch block, insulin dependent diabetes mellitus and patients that are taking anticoagulants or need permanent oxygen supply were excluded from the study. Patients, who were on antibiotics more than 4 times in the preceding summer or winter were not allowed to enrol.

Study area: Erfurt, Germany

Study methods: Health assessment: Each subject will be followed over a period of 6 months. Clinical examinations include:

- a total of 12 examinations (every 2 weeks) with lung function testing (only COPD-patients), ECG-recording (to determine e.g. heart rate, heart rate variability, heart rate turbulence, ST-segments and PCA) including a sub-maximal exercise challenge on a bicycle ergometer and pulse oximetry for the COPD-panel, urine sampling, blood sampling (to determine plasma viscosity, fibrinogen, prothrombin, D-Dimer, CRP, IL-6, ICAM 1 and E-selectin), blood pressure measurements and breathing frequency
- monthly 24 hour ECG-recordings
- information on respiratory and cardiovascular symptoms collected through a questionnaire at each clinical visit
- diary information on respiratory and cardiovascular symptoms as well as medication use
- daily blood pressure measurements for a period of 1 month.

Assessment of ambient air pollution: air pollution is measured by a fixed monitoring site in Erfurt, Germany. The measurements consist of:

- outdoor number concentrations of particles sized between 0.01 µm - 2.5 µm measured with an aerosol size spectrometer
- outdoor 24 hour $PM_{2.5}$ and PM_{10} measured by Harvard Impactors
- outdoor concentrations of O_3 and NO_2
- assessment of chemical composition (through PIXE)
- SO_2 and CO concentrations collected through the local air quality network.

Study on Health Effects of Air Pollution on Susceptible Subpopulation: Traditional air pollutants, ultrafine particles, and myocardial infarction.

Investigators: GSF-Institute of Epidemiology: Annette Peters, Hannelore Löwel, Stephanie von Klot, Claudia Greschik, Christa Meisinger, Josef Cyrys, Margit Heier, Allmut Hörmann, Ursula Kaup, Ines Trentinaglia, Katrin Zetzsche

Contractors: GSF-National Research Centre for Environment and Health, Munich, Germany: Annette Peters; Institut Municipal d'Investigacio Medica (IMIM), Barcelona, Spain: Jordi Sunyer; National Public Health Institute (KTL), Kuopio, Finland: Juha Pekkanen; Division of Environmental Epidemiology at the National Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden: Göran Pershagen; University of Helsinki (UHEL), Finland: Markku Kulmala.

Study objectives: The objective of this study is to assess the risk of hospitalisation and death due to air pollution, with a focus on airborne ultrafine particles, in individuals with coronary heart disease.

Specific aims: (1) To provide a database of cross-European data on ultrafine particles; (2) To determine dose-response relationships between environmental factors (particulate matter/gaseous air pollutants) and the incidence of first myocardial infarctions in the general population; (3) To determine dose-response relationships between environmental factors (particulate matter/gaseous air pollutants) and hospitalisation and death among survivors of a myocardial infarction; (4) To assess interactions between biological and social characteristics of the individuals and environmental factors in the development of hospitalisation or death.

Study design: The study comprises two components. The first part is a population based acute exposure study (incident cases of myocardial infarctions in the general population) and the second part is a cohort-based follow up (hospital admissions and mortality of post MI patients).

Study population: Population based part: All male and female inhabitants of the defined study areas, aged between 35 and 75 years; Cohort based part: Subjects, aged 35 - 75, who had a nonfatal MI, who were admitted to a hospital of the study area, and were enrolled in the population registries during the years defined for each city (Augsburg 1995-1999).

Study location: The study will be performed in five European cities – Augsburg, Barcelona, Helsinki, Rome, Stockholm – chosen so that to include a variety of geographical surroundings and air pollution characteristics.

Study methods: Data collection: incident cases of non-fatal MI are studied through established myocardial infarction population registries (Augsburg, Barcelona, Stockholm) or from available records of hospital admissions (Helsinki, Rome) in five European cities. Each subject will be followed for at least one year to

ascertain hospital readmission for a second MI and mortality, especially sudden death.

Assessment of ambient air pollution: Monitoring of the 'classical' air pollutants (e.g. gases SO_2, NO_2, CO, TSP, BS and/or PM_{10}) has been carried out throughout the whole follow-up period in the five study areas. Measurement of ultrafine particles will be done in the participating cities over a one-year period. These two data sources will provide the basis for retrospective estimation of exposure to particles in the ultrafine range for the entire follow-up period.

Study period: In Augsburg the date of the index event will be between 1995 and 1999, the follow up will be done until the end of 2000.

Study status: Collection of the cohort and follow up data as well as measurements of total number concentrations of ambient particles are currently ongoing.

A study of air pollutants and acute asthma exacerbations in urban areas

Principal Investigators: Daniel Luttinger and Lloyd Wilson, New York State Department of Health, Bureau of Toxic Substance Assessment, 547 River Street, Troy, NY 12180-2216, USA

Study location: New York City (NYC), USA

Study objectives: (1) To evaluate whether ambient levels of certain hazardous air pollutants (HAPs), criteria pollutants or pollens and spores differ in two NYC neighbourhoods that have different rates of hospital admissions for asthma and different socio-economic status (SES) characteristics; (2) To compute the overall rates of air contamination attributable asthma emergency room visits among residents of the two communities and test whether the magnitude of the air pollution effect differs in the two communities; (3) To investigate which air contaminant, or mix of air contaminants, is most associated with acute asthma exacerbations in each community.

Study design: This study will evaluate temporal associations between air contaminants and acute asthmatic exacerbations as measured by emergency room visits for asthma in communities in the Bronx and Manhattan in NYC. The ambient air data and hospital data will be analysed by time-series analysis to determine whether and to what extent various air contaminants, or mixture of air contaminants, contribute to emergency room visits for asthma and whether air pollution effects differ in the two communities. In addition, the study will evaluate whether ambient levels of various air pollutants differ in two communities.

Study population: The communities studied were those residences within approximately 1.5 miles of the monitoring site based on zip code boundaries. Twenty-three hospitals (6 public and 17 private) were identified by the criteria that the hospital had greater than 10 asthma hospitalizations per year for residences of

the study area zip codes. Emergency room billing data were extracted for identifying study area patients.

Study methods: The study measured 24-h average ambient air concentrations of aldehydes, chromium, iron, nickel, manganese, hydrogen ion, sulfate, pollens and mould spores. In addition, hourly concentrations for ozone, sulfur dioxide, nitrogen oxides, number of particles between 7 nm and 2.5 μm, particulate matter ($PM_{2.5}$, PM_{10}) were measured, and 3-h concentrations for elemental and organic carbon. The hourly data will be used for calculating daily averages, maximum concentrations, and for ozone an 8-h moving average. Meteorological (temperature, wind speed and direction, humidity) data were also collected. Some limited (1 year) additional analyses were measured daily for nitrous, nitric and hydrochloric acids and ammonia. The health effects measure is a visit to the emergency room (ER) resulting in a diagnosis of asthma, other respiratory conditions, or for several other health conditions not thought to be related to ambient air contaminants (e.g. non-infectious gastrointestinal disorders).

Sampling locations for the study were at NYS Department of Environmental Conservation monitoring sites that were already established as part of their ambient air-monitoring network. The monitoring site in the Bronx represented a mixed residential/industrial area, whereas the Manhattan site is primarily residential and commercial.

Concentrations of air contaminants in the two study areas will be compared using a paired *t*-test, or a non-parametric statistic (if appropriate). The association, within each community, between daily ER visits for asthma, and air contaminant and meteorological variables will be assessed using multiple regression methods for time-series data. Variables will be added to the multiple regression models in four groups to determine the overall explanatory power of each group in terms of variance explained. The variable groupings will include HAPs, criteria air pollutants, pollens and spores and meteorological variables.

Study period: 1999-2003

Study status: Data collection is complete, and data analyses are underway for evaluating the three principal objectives.

Study on Health Effects of Air Pollution on Susceptible Subpopulation - HEAPSS (http://airnet.iras.uu.nl/inventory/projects/p010.html).

Principal investigator: Francesco Forastiere, Department of Epidemiology, Health Authority RM/E, Rome, Italy

Study objectives: To determine whether exposure to ambient air pollution increases the risk of acute hospitalization and the risk of mortality among population-based cohorts of patients who had survived a myocardial infarction (MI).

Study design: Incident cases of non-fatal MI are recruited through ad-hoc population registries or from available records of hospital admissions in five European cities (Augsburg, Barcelona, Helsinki, Rome, and Stockholm) with different air pollution levels and climate. Each subject will be followed for at least one year;

outcomes of specific interest will be subsequent hospitalization for secondary MI, arrhythmia, congestive heart failure, or sudden deaths.

The study is carried out in the five cities using a common core protocol for data collection; standardised and simultaneous measurement of ultrafine particulate matter is foreseen in order to provide reliable retrospective imputation of exposure to particles in the ultrafine range for all the follow-up period. The extent of the association between daily concentration of particulate matter (fine and ultrafine particles)/gaseous air pollutants and the acute health outcomes will be evaluated using a case-crossover approach. Since the unit of observation will be the individual, the study will be able to explore which characteristics of these patients confer a major susceptibility to air pollution. In order to maximise the power of the study, joint analysis of the data from the national studies will be carried out. The results are relevant for European initiatives to implement targeted prevention strategies on air pollution, to release evidence-based guidelines for susceptible subgroups, and to develop dedicated early warning systems.

Study period: 2001 - 2003.

Exposure and dose-response calculations of particulate matter in Stockholm - number, surface area, and mass concentrations.
(http:// airnet.iras.uu.nl/inventory/projects/p046.html)

Principal investigators: Tage Jonsson, Slb-analys, Stockholm Environment and Health Administration, Sweden; Christer Johansson, Institute of Applied Environmental Research - ITM, Stockholm University

Study description: The aim of this project is to study how short-term exposure to air pollution affects the risk for hospitalization (for e.g. MI) and mortality in population based cohorts of survivors of a first time MI, compared to e.g. the risk for MI in the general population. Specific objectives:

- To quantify the particle size distribution, its temporal and geographic variation in and around the urban area of Stockholm. Specifically the following questions will be addressed: (1) How large is the influence of local emissions on particle number concentrations in different size ranges? (2) How large is the geographical and temporal variation of particle size distribution in the city? (3) What is the relative contribution of different sources to the particle concentration in different size ranges? (4) Which physical properties of the aerosol are best related to health effects?
- To develop and validate models that may be used to assess the temporal and geographical distribution of particulate matter in urban air in Stockholm.
- To assess the health effects of particulate matter in urban air by evaluating dose-response relationships between particle numbers, particle surface area and particle mass.

Approximately 29,000 cases of non-fatal MI will be identified through existing MI-registers (in Augsburg, Germany, Barcelona, Spain and Stockholm, Sweden) or through data on hospital admissions (in Helsinki, Finland and

Rome, Italy). Each patient is followed for at least one year with regard to renewed hospital admission for MI or death, especially "sudden death". Since the study is based on an individual level, we will be able to investigate which properties in these patients increase their sensitivity to air pollution. A common protocol in the five cities will be used for data collection and standardised simultaneous measurements of ultrafine particles will be performed using the CPC TSI 3022 instrument. The measurements will be the basis for retrospective estimation of the exposure to ultrafine particles for the entire study period. The main method for data analysis will be the case-crossover design, originally developed to study triggers for disease onset, which has recently been applied in studies of the effects of air pollution. The design allows both confounding control and effect modification.

Study period: 2001-2004.

SUMMARY AND CONCLUSIONS ON EPIDEMIOLOGICAL STUDIES

A number of recent epidemiological studies addressed the associations between ambient ultrafine particle concentrations and mortality or morbidity of urban populations. These studies are limited to the investigation of the acute health effects of short-term exposure by evaluating the impact of day-to-day variation in ambient pollution on health through correlating morbidity and mortality with daily pollution levels. The general approach of these studies was to compare the effects of ultrafine particles with those of fine particles. Studied parameters spanned the range from population mortality to changes in specific parameters or biomarkers in individuals.

The measurements were taken on a fixed monitoring site in the central urban area in all studied cities. Although instrumentation used for measuring the ultrafine particles in Helsinki was different from that used in other cities, the preliminary side-by-side measurements have demonstrated a good agreement between all instruments employed. The data on the pollutant levels measured in the epidemiological studies are summarised in Table E-1 (Appendix E). Mean concentrations of ultrafine particles in all locations were comparable whereas the concentrations of fine particles differed substantially between the cities (Table E-1). In addition to particle number and mass distributions, elemental composition was determined in order to identify potential sources of particles.

The main findings of the reviewed studies can be summarised as the following:

- A study (Wichmann et al., 2000b) conducted in Germany on daily mortality showed comparable and independent increases in mortality in association with fine and ultrafine particles.

- The mortality data suggest that fine particles have immediate health effects whereas ultrafine particles have more delayed effects. Immediate effects seem to be attributable to respiratory disease mortality whereas delayed effects are based on an increase in cardiovascular disease mortality.

- Panel morbidity studies with asthmatic subjects indicate that both fine and ultrafine particles are associated with the respiratory health of the exposed population. A decrease of respiratory functions (e.g., peak expiratory flow) and an increase in symptoms and medication use are associated with elevated particle concentrations of ultrafine particles, independently from fine particles.

- There is an indication that the acute effects of the number of ultrafine particles on respiratory health are stronger than that of the mass of the fine particles.

- The acute effects of ultrafine particles on respiratory health of adult asthmatics are more profound than that for children with asthma symptoms.

- Inflammatory events in the lungs take several days to develop. It is likely that a lag time exists between exposure to ultrafine particles and the acute respiratory health effects of the exposed population. Cumulative effects over 5 days seem to be stronger than same-day effects.

- There is association between exposure to ultrafine particles and cardiovascular morbidity in the population with chronic heart diseases. A panel study among subjects with coronary heart disease indicate that there are independent associations between both fine and ultrafine particles and the risk of ST-segment depression, which is used as indicator of myocardial ischemia, in the exposed population. The study report increased odds ratios for 45 subjects ranging from 1.03 to 3.29, with 95% confidence intervals ranging from 0.54 to 6.32.

Conclusions

Both fine and ultrafine particles appear to affect health outcomes such as mortality and respiratory and cardiovascular morbidity and appear to do so independently of each other. However, the database at present is too limited (both in numbers of studies and numbers of subjects) and geographically restricted, to allow clear conclusions on the mode of action or generalizations to other settings. Given that there is a poor correlation between ultrafine particles (measured by number) and fine particle mass, observed statistical independence (in the multiple regression models) is interesting. However, there is also epidemiological as well as toxicological evidence of similar biological responses to fine and ultrafine particles, although the size of the effects is often larger for ultrafine than for fine particles (at least on a per mass basis). Furthermore, given that fine and ultrafine particles often originate from common sources, given the dynamics of particle formation and accumulation, and given the different observed lead-lag relationships between exposure and observed health responses, it is currently difficult to make very strong inferences about independent effects. Further studies are currently under way but more studies in other settings need to be initiated to improve understanding of ultrafine particles and health outcomes.

Prevailing uncertainties in the epidemiological evidence of the effect of ultrafine particles.

Exposure Assessment

Outdoor measures versus. actual human exposure

The reviewed epidemiological studies investigating the association between exposure to particulate matter and mortality and morbidity have used ambient particle concentrations as a surrogate for personal exposure. The assumption that ambient exposure data are an adequate surrogate for personal exposure to ultrafine particles has however not yet been validated. To do so, one must understand how the data for ambient levels relate to personal exposure, which involves varying amounts of time spent outdoors by individual members of the population. Until then, it is not clear whether the observed (or not observed) associations between ultrafine particles and daily morbidity were due to the uncertainties in the measurement of the exposure caused by monitoring only the ambient levels of ultrafine particles rather than measuring personal exposure of the members of the study population.

The uncertainties in regard to the correlation of ambient particle concentrations with personal human exposure is further amplified by the fact that the studies used a fixed-point sampling strategy for the exposure assessment. However, as was mentioned previously, it is unlikely that the measurements taken at a fixed-point monitoring site will give a reasonable estimate of overall outdoor pollutant exposure. For example, as discussed in chapter 4 of this report, differences in particle number concentrations between urban background locations and in the vicinity of the roads could be more than ten times.

Averaging time

The measurement of ambient particulate matter is challenging because particle composition and size distribution varies from one location to another and from one time to another in the same location. The reviewed studies of acute effects assessed PM values over 24 hour periods. Though the hourly means available from continuous monitoring do characterise the pollution exposures, the role of rate of change of concentrations of particles and other pollutants may also be important. It may be that high concentrations over shorter periods of time may have more profound health effect but this aspect has not been investigated. Consequently, the results did not establish whether it was the mean exposure or peak exposure or a combination of the two that caused the elevated risk. There is thus a need to determine whether effects of short-term, high peak concentrations of ultrafine particles have more pronounced health effects than similar daily concentrations without such peaks. This has been identified as the most urgent research need and this question might be able to be addressed through quasi-experimental studies.

Particle size versus mixture of pollutants

The authors of the reviewed studies have noticed that the analysis of particle pollution data indicate that automobile traffic was the major source of the ultrafine particles.

However, engine exhaust is known to be a complex mixture of hundred of components in both particle and gas phases. Among the gaseous components that are of toxicological relevance are numerous organic compounds such as benzene, aldehydes, 1,3-butadiene, polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs, in addition to the CO, NO_x, and SO_x. The particles present in the engine exhausts are composed of elemental carbon, adsorbed organic compounds, and small amount of sulfate, nitrate, metals, and other trace elements. The most toxicologically relevant organic compounds that are adsorbed onto these particles include PAHs, nitro-PAHs, and oxidised PAH derivatives. Thus, the observed health effects may be due to ultrafine particles but also to some specific components of the complex mixture. Therefore a good characterisation and quantification of the composition of the mixtures would be important to decouple the effects related to ultrafine particles from the effects of other components of the mixtures. This would also help to identify possible synergistic and antagonistic effects of the different components of the mixture.

Study design issues

Panel studies have been used in all of the morbidity studies cited and provide a means of following a specific group of subjects who are assessed or measured for the outcome variable(s) at the same regular intervals. The important feature of this design is the systematic nature of the assessments; however, the problems with compliance in the assessment schedule can lead to problems with missing data. Where studies involved daily diaries of symptoms or activities or regular clinical presentations and assessments, complete data were not available for all subjects and investigators had to make a decision as to the minimum data set (e.g. 60% or 80% completeness) that would be included in the analyses. In this situation, issues arise as to whether data are missing at random or in some systematic pattern that would affect the results of the analysis.

A design that could be used for population-based mortality studies is the case crossover design, which provides a means of controlling for possible confounding by allowing subjects or cases to serve as their own controls. The bi-directional form of this design can control for time trends in the data by using information on study subjects before and after the event of interest. This design has been applied to examination of short-term studies of coarse particulate air pollution on health outcomes.

Subject selection and definition of health outcomes to be measured can also be problematic. Recruitment through clinics may result in overrepresentation of subjects with poor outcomes. Recruitment by invitation through a survey can result in a self-selected sample that may be biased towards those individuals with better outcomes as opposed to those too unwell and therefore unwilling to participate. Similarly, where the outcome of interest is likely to be precipitated through the assessment process itself, its occurrence may be unintentionally minimised when investigators modify the assessment so that not to jeopardize the health of subjects. The specific criteria for inclusion of subjects in these studies may also contribute to the relatively small numbers of subjects recruited. Such numbers are likely to represent relatively heterogeneous groups of subjects and make assessments of the contribution of different age groups, co-morbidities and other factors to the models extremely difficult.

Statistical methods

The associations between air pollution and acute respiratory or cardiovascular health outcomes in these studies were examined using time series analysis, a popular technique for studies of environmental exposure and morbidity and mortality over time (see appendix B for an overview and description of this technique). Controlling for trends with this method is advantaged, however, the results are model dependent which can be a disadvantage. Another disadvantage is that the associations revealed could be sensitive to the length of the window used in the data filter function and there is an associated risk that certain patterns of exposure may be overfiltered.

Regression techniques, where the outcome of interest is explained as some combination, either linear or non-linear, of predictor variables, were used to develop models of the associations between the health outcomes and air pollution (see appendix B). Model building strategies included Poisson regression, linear regression and logistic regression. Considerable subjectivity can enter into the model building process and investigators are faced with choosing amongst several different ways of describing the same phenomenon. The primary objective of this type of analysis is identification and adjustment of confounders rather than statistical hypothesis testing. In this setting, a number of correlated variables could provide good confounder control although statistical tests of their parameters might be misleading. The complexity of the model building process and the individuality of methods that can be used for that process is likely to contribute to the variability of results in these studies.

Analyses in these studies have been performed using SAS or S-Plus software and some investigators report on results derived using generalised additive models. The use of S-Plus software in the generation of these models has recently been critically reviewed and there are reports of overestimation of model parameters due to a fault in the GAMs algorithm. While this fault has been corrected in the software since the original report in May 2002, and subsequent analyses have indicated that the magnitude of the difference between uncorrected and corrected estimates is quite low (<1%), some investigators and organizations are undertaking a re-analysis of their data (Colburn & Johnson, 2003). It is possible that studies in this review that used S-Plus for this purpose may have unknowingly overestimated some of the parameters, although the conclusions are unlikely to be significantly altered.

5.3.2 TOXICOLOGICAL STUDIES

Although there is substantial literature on animal studies of ultrafine particles, the majority of reports relate to particles generated in diesel exhaust. These studies have been examined as part of this literature review, but because they represent a mixed exposure to particles and to other materials adsorbed onto the particles they do not have the capacity to provide information on the effects of ultrafine particles on their own. This mixed exposure includes the particle and other compounds such as benzenes, polyaromatic hydrocarbons and many other compounds and elements. By virtue of the way the experimental work was carried out, it is not possible to dissect the different components of this mixed exposure. Consequently, these studies have not been included in the current evaluation.[3]

Examination of the other literature on the subject shows that there are four headers under which the toxicological investigations might be grouped. These are shown in Tables 5.3.2.1 through 5.3.2.4.

It is particularly notable that the most commonly investigated process is that of inflammation. These studies of the inflammatory process have involved both *in-vivo* and *in-vitro* studies.

Animal Haemostasis

Table 5.3.2.1 Summary of study on animal haemostasis

Ref	Groups studied	Effects studied	A. Study Description	Findings/ Conclusions
Nemmar et al. (2002b)	Hamster	Cardiovascular haemostasis	Studied the effect of ultrafine (60 nm) polystyrene particles on thrombus formation in a hamster model after intravenous and intratracheal administration of unmodified, carboxylase-polystyrene, or amine-polystyrene particles.	The results suggest that the presence of ultrafine particles in the circulation may affect haemostasis. The observed *in-vivo* prothrombotic tendency resulted from platelet activation by positively charged amine-polystyrene particles.

[3] Al-Humadi et al (2002a), Baeza-Squiban et al (1999), Bai et al (2001), Bion et al (2002), Boland et al (1999, 2000a, b), Bonvallot et al (2001), Bunger et al (2000a, b), Carero et al (2001a, b, 2002), Casillas et al (1999), Castranova et al (2001), Devalia et al (1999a,b), Doornaert et al (2003), Elder et al (2000), Fahy et al (1999, 2000), Fujimaki et al (2001, 2001a), Greenwell et al (2002), Han et al (2001), Hashimoto et al (2000), Heo et al (2001), Hiura et al (1999, 2000), Ito et al (2000), Juvin et al (2002a,b), Kawasaki et al (2001), Koike et al (2002), Kumagi et al (2002), Li et al (2002), Madden et al (2000), Maejima et al (2001), Marano et al (2002), Matsuo et al (2001), Minami et al (1999), Mori et al (2002), Murphy et al (1999, 1998), Pacheco et al (2001), Pandya et al (2002), Rengasamy et al (2003), Reynolds et al (2000, 2001), Rudra-Ganguly et al (2002), Saito et al (2002a & b), Sato et al (1999), Takano et al (20002a, b), Takenaka et al (2001), Takizawa et al (1999a, 2000), Taneda et al (2000, 2002), Tokiwa et al (1999), Tokiwa & Sera (2000), Tsuke et al (2002), Tsurudome et al (1999), Ushio et al (1999), van Zijverden et al (2000), Whitekus et al (2002), Yamazaki et al (2000), Yang et al (1999) (2001), Yin et al (2002b), Yoshida et al (1999, 2000), Yoshino et al (2002a, b), and Yoshino & Sagai (1999, 1999a).

The conclusion from this study must be that by an undefined mechanism the presence of the relatively inert polystyrene particle has been sufficient to induce clot formation in the hamster. A direct extrapolation of this to humans would be that the presence of a positively charged polystyrene particles (and by extrapolation other charged particles) could be instrumental in clot format *in-vivo*, providing a rationale for some of the cardiovascular and central nervous system effects of particles.

Animal Allergy Studies

Table 5.3.2.2 Summary of studies on animal allergy

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Granum et al. (2000)	Mice	Allergic responses	Investigate which physical properties (weight, size, number, and surface area) of particles might be important for the allergic effects. NIH/Ola mice were given 2 intraperitoneal injections with PSP plus OVA or OVA alone, over a 16-day period. The mice were exsanguinated at the end of each experiment, and the serum concentration of IgE anti-OVA nas measured.	The results indicate that the total number and total surface area of ultrafine particles, rather than the dose weight, are important parameters for the IgE adjuvant activity.
Granum et al. (2001)	Mice	Lung inflammation	Investigated immediate and delayed IgE adjuvant effects caused by particles in a mouse model.	The results indicate that individuals exposed to particulate air pollution at one point of time may develop an increased reaction towards allergens inhaled later that day or even several days after the particle exposure.

These studies cinducted by the same laboratory suggests both an increased sensitivity to immune response after exposure to ultrafine particles. The studies also demonstrate the important conclusion that the number and surface area of the particles are the determinants of this effect.

Inflammatory Processes: Animal In-vivo Studies

These *in-vivo* studies on inflammatory processes consistently demonstrate a greater propensity for inflammatory processes subsequent to exposure to ultrafine particles. As with other studies these effects are related to the increased surface area of the particles.

Summary of these studies are presented in Table 5.3.2.3 below.

Table 5.3.2.3 Summary on animal in-vivo studies

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Baggs et al., (1997).	Rats	Pulmonary inflammatory	Male Fisher 344 rats were exposed for 6 hours a day, 5 days a week, for 3 months to 1) filtered air (control); 2) TiO_2-D, 20 nm particle size, 23.5 mg/m; 3) TiO_2-F, 250 nm, 22.3 mg/m^3; or 4) crystalline SiO_2, a positive control particle (similar to 800 nm particle size, 1.3 mg/m^3). Groups of 3-4 animals were sacrificed at 6 and 12 months following the completion of exposure. Pulmonary effects of exposure were evaluated using standard hematoxylin and eosin-stain sections, histochemical stains for collagen, and immunohistochemical assays for cell turnover.	Six months after animals were exposed to SiO2, they had moderate focal interstitial fibrosis and moderately severe focal alveolitis. Animals exposed to TiO_2-D had slightly less fibrosis. The least fibrosis was seen in the TiO_2-F group. At 1 year after exposure, fibrosis was still present but decreased in the SiO_2 group. The amount of interstitial fibrosis in the TiO_2-D- and TiO_2-F-treated animals had largely returned to untreated. Although initially irritant, TiO_2-induced lesions regressed during a 1-year period following cessation of exposure. Inhaled ultrafine particles of TiO_2 (TiO_2-D, 20 nm particle size) lead to a greater pulmonary inflammatory response than larger pigment-grade particles (TiO_2-F, 250 nm).
Brown et al., (2001).	Rats	Respiratory	Investigated proinflammatory responses to various sizes of polystyrene particles as a simple model of particles of varying size including ultrafine.	There was a significantly greater neutrophil influx into the rat lung after instillation of 64 nm polystyrene particles compared with 202- and 535 nm particles and this was mirrored in other parameters of lung inflammation, such as increased protein and lactate dehydrogenase in bronchoalveolar lavage. Conclusions: the results suggest that ultrafine particles composed of low-toxicity material such as polystyrene have proinflammatory activity as a consequence of their large surface area.

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Cassee et al., (2002a)	Rats: healthy & with pulmonary hypertension	Pulmonary toxicity	Tested the hypothesis that secondary model aerosols exert acute pulmonary adverse effects in rats, and that rats with pulmonary hypertension (PH), induced by monocrotaline (MCT), are more sensitive to these components than normal healthy animals. In addition, tested the hypothesis that fine particles exert more effects than ultrafines. Healthy and PH rats were exposed to ultrafine (0.07-0.10 μm; 4 x 10^5 particles/cm^3) and fine (0.57-0.64 μm; 9 x 10^3 particles/cm^3) ammonium aerosols during 4 h/day for 3 consecutive days. The mean mass concentrations ranged from 70 to 420 μg/m^3), respectively, for ultrafine ammonium bisulfate, nitrate, and ferrosulfate and from 275 to 410 μg/m^3 for fine-mode aerosols. Bronchoalveolar lavage fluid (BALF) analysis and histopathological examination were performed on animals sacrificed 1 day after the last exposure.	Histopathology of the lungs did not reveal test atmosphere-related abnormalities in either healthy or PH rats exposed to the ammonium salts, or to a combination of CB + nitrate. Alveolar macrophages in rats exposed to CB only revealed the presence of black material in their cytoplasm. There were no signs of cytotoxicity due to the aerosol exposures (as measured with lactate dehydrogenase [LDH], protein, and albumin contents in BALF). Macrophages were not activated after MCT treatment or the test atmospheres, since no changes were observed in N-acetyl glucosaminidase (NAG). Cell differentiation profiles were inconsistent, partly caused by an already present infection with Haemophilus sp. The results show that at exposure levels of ammonium salts at least one order of magnitude higher than ambient levels, marked adverse health effects were absent in both healthy and PH rats.
Dick et al., (2003).	Rats, *in-vitro*	Toxicity, Inflammation	By using four types of ultrafine particles, carbon black (UFCB), cobalt (UFCo), nickel (UFNi), and titanium dioxide (UFTi), determined the attributes of the ultrafine particles (surface area, chemical composition, particle number, or surface reactivity) that contribute most to its toxicity and proinflammatory effects both *in-vivo* and *in-vitro*.	The results suggest that ultrafine particles may cause adverse effects via oxidative stress, and this could have implications for susceptible individuals. Susceptible individuals, such as those with COPD or asthma, already exhibit pre-existing oxidative stress and hence are in a primed state for further oxidative stress induced by PM.

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Johnston et al., (2000).	Rats	Inflammation	Used ultrafine particles Teflon (PTFE) fumes (count median particle size ~ 16 nm) to test three hypotheses: (i) ultrafine particles PTFE (polytetrafluoroethylene) fume particles are causally involved in the induction of acute lung injury, (ii) ultrafine particles PTFE elicit greater pulmonary effects than larger sised PTFE accumulation mode particles, and (iii) preexposure to the ultrafine particles PTFE fume particles will induce tolerance.	Teflon fumes at ultrafine particle concentrations of 50 mg/m^3 were extremely toxic to rats when inhaled for only 15 min. When generated in argon, the ultrafine Teflon particles alone are not toxic at these exposure conditions; neither were Teflon fume gas-phase constituents when generated in air. Only the combination of both phases when generated in air caused high toxicity, suggesting either the existence of radicals on the surface or a carrier mechanism of the ultrafine particles for adsorbed gas compounds. Aging of the fresh Teflon fumes for 3.5 min led to a predicted coagulation to >100 nm particles which no longer caused toxicity in exposed animals. This study shows the importance of pre-exposure history for the susceptibility to acute ultrafine particle effects.
Nemmar et al., (1999).	Guinea-pigs	Inflammation	The effects of ultrafine polystyrene carboxylate-modified (fluorospheres) on inflammatory processes are being investigated in rabbit lungs. One millilitre of sterile NaCl (0.9%) containing 4 mg of ultrafine particles (ultrafine particles) was intratracheally instilled into anaesthetised rabbits. The control animals were only instilled with sterile NaCl (0.9%).	The results indicate that chemically inert, electrically charged ultrafine particles induce a pulmonary inflammatory process during which the release of SP and histamine from C-fibres and mast cells was enhanced after various stimuli. These latter mediators can also modulate the inflammatory process.
Oberdorster (2000)	Rats	Pulmonary inflammation	Exposure of rats to laboratory-generated ultrafine carbonaceous (elemental, and organic, carbon) particles was carried out at a concentration of ca. 100 $\mu g/m^3$ for 6 h. Modulating factors of responses were prior low-dose inhalation of endotoxin in order to mimic early respiratory tract infections, old age (22- month old rats versus 10-week old rats) and ozone co-exposure.	Found that: (i) ultrafine carbon particles can induce slight inflammatory responses; (ii) LPS priming and ozone co-exposure increase the responses to ultrafine carbon; (iii) the aged lung is at increased risk for ultrafine particle-induced oxidative stress. Studies with ultrafine and fine TiO_2 showed that the same mass dose of ultrafine particles has a significantly greater inflammatory potential than fine particles. **Conclusions**: the increased surface area of ultrafine particles is a most important determinant for their greater biological activity. The propensity of ultrafine particles to translocate may result in systemic distribution to extrapulmonary tissues.

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Osier & Oberdorster (1997)	Rats	Inflammation	Compared the response of rats exposed by intratracheal inhalation to "fine" (similar to 250 nm) and "ultrafine" (similar to 21 nm) titanium dioxide particles with rats exposed to similar doses by intratracheal instillation.	Animals receiving particles through inhalation showed a decreased pulmonary response measured by bronchoalveolar lavage parameters, in both severity and persistence, when compared with those receiving particles through instillation. These results demonstrate a difference in pulmonary response to an inhaled vs an instilled dose, which may be due to differences in dose rate, particle distribution, or altered clearance between the two methods.
Takenaka et al., (2000).	Rats, *in-vitro* (macropha ge cells)	Inflammation	Investigated the fate of agglomerated ultrafine particles in macrophages *in-vitro* and *in-vivo*. Metallic silver (Ag) was used as a test particle. For the *in-vitro* study, J774 macrophage cell suspensions (200,000 cells in 400 µl medium) were plated in small chambers. Six hours later, 100 mu l of the silver-PBS suspension was added to each chamber. For the *in-vivo* study using F344 rats, 50 µg Ag particles were instilled intratracheally. On days 1, 4, and 7 following instillation, rats were sacrificed and the lungs were examined morphologically.	Both *in-vitro* and *in-vivo* studies suggested that agglomerated Ag particles remained in targets for a given period of time-at least up to 7 days.
Takenaka et al., (2001b)	Rats	Cardiovascular	Pulmonary and systemic distribution of inhaled ultrafine elemental silver (EAg) particles was investigated on the basis of morphology and inductively coupled plasma mass spectrometry (ICP-MS) analysis. Rats were exposed for 6 hr at a concentration of 133 µg EAg per m^3 (3 x 10^6 per cm^3), 15 nm modal diameter) and were sacrificed on days 0, 1, 4, and 7.	Found that although instilled agglomerates of ultrafine EAg particles were retained in the lung, Ag was rapidly cleared from the lung after inhalation of ultrafine EAg particles, as well as after instillation of $AgNO_3$, and entered systemic pathways.

Inflammatory Processes: In-vitro studies

Summary of in-vitro studies on inflammatory processes caused by ultrafine particles is presented in Table 5.3.2.4. The *in-vitro* studies described in these papers have examined a number of different cell types from both humans and animals. In all cases inflammation was the end point evaluated. In the ones in which diesel exhaust particles were included the effects appeared to be primarily associated with adsorbed compounds. Studies also showed that the particles persist in tissues as relatively large aggregates of the individual particles. The smaller the particles, the greater likelihood that they will pass through epithelial cells into the sub-epithelial space. The particles also have the capacity to augment the production of inflammatory cytokines. Compounds such as interleukin 8 and interleukin 10 are produced. However, *in-vitro* studies do not allow appropriate evaluation of the complex interaction of these cytokines since they generally deal with single cell types and do not evaluate the intra-cellular processes that define the operations of these compounds in a whole organism. As with the other studies, the primary determinant of the effects of ultrafine particles appears to be the surface area of the particles rather than their weight.

General Conclusions

The general conclusions from these studies are that there are complex interrelationships between ultrafine particles and materials adsorbed onto them. There are very few studies which examine the particles on their own. Studies have shown that cytokines are produced by these particles as mediators of the inflammatory response. The complex interrelationships between these cytokines have yet to be evaluated in appropriately designed experimental studies.

All of the studies available demonstrate that the primary determinant of the effect of ultrafine particles is their number and surface area and not the weight of particles present. This means that the traditional use of PM weight measures is inappropriate in evaluation of the likely biological effects of ultrafine particles.

Table 5.3.2.4 Summary of the in-vitro studies on inflammatory processes.

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Beck-Speier et al., (2001).	*In-vitro* (immune cells)	Physiologic responses of immune cells	Evaluated physiologic responses of immune cells on exposure to the agglomerates of 77 nm elemental carbon [(EC); specific surface area 750 m^2/g] and 21 nm titanium dioxide (TiO_2) particles (specific surface area 50 m^2/g) by the release of lipid mediators by alveolar macrophages (AMs). .	The results indicate that surface area rather than mass concentration determines the effect of ultrafine particles, and that activation of phospholipase A(2) and COX pathway occurs at a lower particle surface area than that of 5- LO-pathway.
Boland et al. (2000)	*In-vitro* (human bronchial epithelial cells)	Lung inflammation	Studied the mechanisms underlying the increase in GM-CSF release elicited by DEPs using the human bronchial epithelial cell line	DEP treatments increased GM-CSF mRNA levels. Comparison of the effects of DEPs, extracted DEPs, or extracts of DEPs revealed that the increase in GM-CSF release is mainly due to the adsorbed organic compounds and not to the metals present on the DEP surface. Conclusions-the increase in GM-CSF release is mainly due to the adsorbed organic compounds and that the effect of native DEPs requires endocytosis of the particles. Reactive oxygen species and tyrosine kinase(s) may be involved in the DEP-triggered signalling of the GM-CSF response.
Churg et al., (1998a)	*In-vitro* (rat tracheal explants)	Inflammation	Examined the relationship between particle uptake by pulmonary epithelial cells and particle size. Exposed rat tracheal explants to fine particles (FPs; 0.12 μm) or ultrafine particles (ultrafine particles; 0.021 μm) of titanium dioxide for 3 or 7 days.	The results suggest that the behaviour of particles of different size is complex: ultrafine particles persist in the tissues as relatively large aggregates, whereas the size of FP aggregates becomes smaller over time. Ultrafine particles appear to enter the epithelium faster, and once in the epithelium, a greater proportion of them are translocated to the subepithelial space compared with FPs. However, if it is assumed that the volume proportion is representative of particle number, the number of particles reaching the interstitial space is directly proportional to the number applied; i.e., overall, there is no preferential transport from lumen to interstitium by size.
Churg et al., (1999).	*In-vitro* (rat tracheal	Inflammation	Examined whether particle size affects mediator generation. Exposed rat tracheal explants, an	The results suggest that ultrafine particles are intrinsically able to induce procollagen expression even in the absence of

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
	explants)		inflammatory cell-free model of the airway wall, to various concentrations up to 500 μg/cm^2 of fine (0.12 μm) or ultrafine (0.021 μm) titanium dioxide (anatase), maintained the explants in an organ culture in air for 1-7 days, and used RT-PCR to examine the expression of fibrogenic mediators and procollagen.	inflammatory cells; that chronic exposure to PM_{10} may result in chronic airflow obstruction, in part because of ultrafine particle-mediated increases in airway wall fibrosis; and that chemically identical dusts of differing size can produce quite different patterns of gene expression in the airway wall.
Fahy et al. (1999)	*In-vitro* (human cells)	Inflammation	Investigated the effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells.	The results suggest that the chemokine pathways are modulated by DEP-PAHs at the transcriptional level, reinforcing the idea that the development of inflammatory reactions might be affected by diesel exhaust emission.
Hiura et al. (1999)	*In-vitro* (human cells)	Lung inflammation	Investigated the mechanism of inflammatory processes in the respiratory tract as well as the cellular targets for DEP.	Found that the phagocytosis of DEP by primary alveolar macrophages or macrophage cell lines, RAW 264.7 and THP-1, leads to the induction of apoptosis through generation of reactive oxygen radicals (ROR). The apoptotic effect on macrophages is cell specific, because DEP did not induce similar effects in nonphagocytic cells. DEP that had their organic constituents extracted were no longer able to induce apoptosis or generate ROR. The organic extracts were, however, able to induce apoptosis. DEP chemicals also induced the activation of stress-activated protein kinases, which play a role in cellular apoptotic pathways. Conclusions-organic compounds contained in DEP may exert acute toxic effects via the generation of ROR in macrophages.
Hiura et al. (2000)	*In-vitro* (human cells)	cytotoxic, inflammatory effects	Investigated the cytotoxic and proinflammatory effects of DEP in the respiratory tract.	Found that DEP chemicals induce apoptosis in macrophages via a toxic effect on mitochondria.
Kawasaki et al. (2001)	*In-vitro* (human cells)	Lung inflammation	Investigated proinflammatory effects of DEP in the respiratory tract. Studied the effects of several components extracted from DEPs on interleukin (IL)-8 expression in human bronchial epithelial cell line BEAS-2B and normal human airway epithelial	Found that DEPs augmented the production of inflammatory cytokines by human airway epithelial cells *in-vitro*. Benzene-extracted components showed effects mimicking DEPs on IL-8 gene expression, release of several cytokines (IL-8; granulocyte macrophage colony- stimulating factor and

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
			cells obtained from very peripheral airways by an ultra thin bronchoscope. Used several agents active on signal transduction pathways in cytokine expression, such as the protein kinase C inhibitor staurosporin, antioxidant agents including N-acetyl cysteine (NAC) and pyrrolidine dithiocarbamate (PDTC), and p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580.	regulated on activation, normal T cells expressed and secreted) and nuclear factor (NF)-kappaB activation. Also found that NAG, PDTC, and SB203580 suppressed the activities of DEPs and their benzene extracts, suggesting the roles of oxidants-mediated NF-KB activation and p38MAPK pathways. Finally, benzo[a]pyrene, one of the important compounds included in the benzene component, replicated the activities shown by DEPs.
Kim et al., (2003).	*In-vitro* (collagen gel model)	Inflammation	The three-dimensional collagen gel contraction model was used to assess that ultrafine carbon particles (UFC) could affect tissue repair.	The results demonstrate the ability of ultrafine particles to contribute to altered tissue repair and extend the known mechanisms by which these biologically active particles exert their effects.
Moller et al., (2002).	*In-vitro* (alveolar macrophages from beagle dogs (BD-AM); macrophages from the cell line J774A.1	Inflammation	Studied the influence of fine and ultrafine test particles (UFP), such as TiO_2, elemental carbon, commercial carbon black, diesel exhaust particulate matter, and urban dust (UrbD), on cytoskeleton-related functions of macrophages, such as phagocytosis, phagosome transport mechanisms, and mechanical cytoskeletal integrity. The diameter of the test particles ranged from 12 to 220 nm and the Brunauer-Emmet-Teller specific surface area ranged from 6 to 600 m^2/g. Macrophages were exposed *in-vitro* with 10-320 µg ultrafine particles /ml/10^6 cells up to 24 h.	While fine TiO_2 did not show any effect, macrophages were sensitive to ultrafine particles exposure. Urban dust and DEP (standard reference material 1650) caused comparable cytoskeletal dysfunctions to elemental carbon with high specific surface area. Cytoskeletal dysfunctions induced by DEP or UrbD could be reduced after washing the particles. All cytotoxic parameters showed only weak correlations with the specific surface area or the total number of ultrafine particles, which can result from the different types of particles and different surface compositions. Conclusions- ultrafine particles cause cytoskeletal toxicity *in-vitro* in macrophages, which can cause cellular dysfunctions, such as impaired proliferation, impaired phagocytic activity, and retarded intracellular transport processes as well as increased cell stiffness and can result in impaired defence ability in the lung.
Murphy et al. (1999)	*In-vitro* (human cells)	Inflammation	Investigated the mechanisms of particle toxicity to the lung. The bioreactivity of carbon black (CB; 50, 40, 30, and 20 nm) and diesel exhaust particles (DEP, 30 nm) were examined with primary cultures of Clara and type II epithelial cells. All particle	Bioreactivity was found to be related to CB particle size and hence surface area: the smaller the particle and larger the surface area, the more toxic the particles. Also, CB particles with the most complicated surface chemistry were the most bioreactive. Freshly prepared DEPs were equally toxic to type

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
			samples had different surface chemical compositions.	II and Clara cells and they became progressively less toxic to the type II cells with time. With all CB and DEPs, the primary epithelial cells internalised the particles, although this was noted most in cells of low functional competence.
Murphy et al., (1998).	*In-vitro* (lung epithelium Cells)	Inflammation	The comparative toxicological effects of diesel exhaust and other well-characterised particles (carbon black, amorphous and crystalline silica) on rat respiratory epithelium were investigated in the present study. The effects of small masses of particles (1 mg) delivered by intratracheal instillation were monitored by changes in components of lavage fluid.	Respirable, crystalline quartz, produced significant increases in lung permeability, persistent surface inflammation, and progressive increases in pulmonary surfactant and activities of epithelial marker enzymes up to 12 weeks after primary exposure. Ultrafine amorphous silica did not induce progressive effects but it promoted initial epithelial damage with permeability changes and these regressed with time after exposure. By contrast, ultrafine/fine carbon black had little effect on lung permeability, epithelial markers or inflammation, despite being given at a dose, which readily translocated the epithelium. Similarly, diesel exhaust particles produced only minimal changes in lavage components. It is concluded that diesel exhaust particles are less damaging to respiratory epithelium than silicon dioxide and that the surface chemistry of a particle is more important than ultrafine size in explaining its biological reactivity.
Reibman et al., (2002).	*In-vitro* (bronchial-epithelial-cells)	Inflammation	Assessed hypothesis that ambient PM of different size fractions collected from an urban environment (New York City air) would activate primary culture human bronchial epithelial cells (HBECs). Because of the importance of granulocyte-macrophage colony-stimulating factor (GM-CSF) on inflammatory and immunomodulatory processes, the study was focused on this cytokine.	Demonstrated that the smallest size fraction (ultrafine/fine; <0.18 μm) of ambient PM (11 $\mu g/cm^2$), upregulated GM-CSF production (2-fold increase). The absence of effect of carbon particles of similar size, and the day-to-day variation in response, suggested that the chemical composition, but not the particle itself, was necessary for GM-CSF induction. Activation of the extracellular signal-regulated kinase and the p38 mitogen-activated protein kinase was associated with, and necessary for, GM-CSF release. These studies serve to corroborate and extend those on model particles. Moreover, they emphasise the role of the smallest size ambient particles in airway epithelial cell responses.

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Samet et al. (1999)	In vitro (human airway epithelial cells)	Inflammation	Examination of the effects of arsenic, zinc and vanadium ions on the intracellular catabolism of phosphorylated protein tyrosine of BEAS-2B (Subclone 6) and normal human bronchial epithelial cells; collectively "Human Airway Epithelial Cells" (HAEC).	Found HAEC intracellular accumulation of phosphorylated protein tyrosine due to inhibition of one or more tyrosine phosphatases in the case of zinc and vanadium ions and possible kinase activation in the case of arsenic ions. Accumulation of intracellular phosphorylated protein tyrosine cause by pulmonary toxicants such as arsenic, zinc and vanadium may activate HAEC cytokine expression signalling pathways by multiple mechanisms.
Stearns et al., (2001).	Rats	Inflammation	Used an *in-vitro* model of type II lung epithelium to evaluate the cells' ability to take up ultrafine particles (titanium dioxide [TiO_2], 50 nm diameter). The human epithelial cell line A549 was grown on aclar substrates and exposed to 40 μg/ml TiO2 particles for 3, 6, and 24 h before imaging with energy-filtering transmission electron microscopy.	After 3 h of TiO_2 exposure, cells internalised aggregates of the ultrafine particles, which were observed in cytosolic, membrane-bound vacuoles. After 24 h of exposure there were considerably more intracellular aggregates of membrane-bound particles, and aggregated particles were also enmeshed in loosely and tightly packed lamellar bodies. Throughout 24 h of exposure a preponderance of particles remained associated with the free surface of the cells and was not internalised. The majority of membrane-bound vacuoles contained aggregates of particles and only occasionally did they contain as few as two or three particles, despite the use of several different approaches to assure the possibility for individual particles to be ingested and detected. There was morphologic evidence of microfilament disturbance, but no evidence of a decrease in internalised particles in cells pretreated with cyto D.
Stone et al., (2000).	Rats	Inflammation	Investigated whether ultrafine particles could invoke alterations in calcium influx in both macrophage cell lines and primary macrophages.	Effects on calcium fluxes induced by thapsigargin were seen with two very different ultrafine particles ultrafine latex beads and ultrafine CB and were seen in both the human MM6 cell line and rat BAL cells. The induction of an oxidative stress by the ultrafine particles was supported by the ability of ultrafine latex beads to induce ROS production. Ultrafine carbon black was round to induce enhanced calcium influx, partly through oxidative stress.

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Timblin et al., (2002).	*In-vitro* (alveolar epithelial cells)	Inflammation	Demonstrated the development of dose-related proliferation and apoptosis after exposure of an alveolar epithelial cell line (C10) to PM or to ultrafine carbon black (UFCB), a component of PM	Found that the ultrafine particle component of PM is critical to its biological activity.
Veronesi et al (2002)	*In-vitro* (human tracheal-bronchial epithelial cells)	Inflammation	In this study, selected physicochemical characteristics (i.e., size, particle number, acidity, and surface charge) were measured on various polydispersed field PM, derived from urban ambient (St. Louis, Ottawa), residential (Woodstove), volcanic dust from Mt. St. Helen (MSH), and industrial [oil fly ash (OFA) coal fly ash (CFA)l sources. Levels of intracellular Ca2+ and IL-6 cytokine synthesis in human, immortalised, tracheal- bronchial epithelial cells (BEAS-2B) were measured to assess the biological effects of PM exposure.	Exposure of BEAS-2B cells to each field PM and/or their soluble and insoluble fractions produced immediate, but differential increases in intracellular Ca2+ and the release of the inflammatory cytokine IL-6 after 4 and 16 hours. The results indicate that the surface charge (i.e., zeta potential) carried on PM's visible field particles predicts their differential release of the inflammatory cytokine IL-6 in cultures of human respiratory epithelial cells.
Wilson et al., (2002).	*In-vitro*	Inflammation	Investigated interactions between transition metal salts and a surrogate environmental particle-ultrafine carbon black.	In all experimental systems employed, the UFCB was found to be more reactive than its fine counterpart (CB). The findings suggest that: (1) ultrafine particles and metals interact by chemical potentiation in a cell-free environment to generate ROS; (2) potentiation between ultrafine particles and metal salts is not observed in the presence of macrophages as iron is sequestered or chelated by the cells; (3) in the lung, ultrafine particles and iron salts interact in a potentiative manner to generate inflammation.

ONGOING STUDIES

Cardiovascular effects of PM and ultrafine particles

Principal investigator: Prof. Dr. Paul J. A. Borm, Particle Research Core, Medical Institute of Environmental Hygiene (MIU), Heinrich Heine University, Düsseldorf, Germany

Study design: The purpose of this research is to investigate both direct and indirect mechanisms that could be responsible. Among direct effects, the potential translocation of ultrafine model particles in the isolated perfused rat lung (IPL) is evaluate under conditions that allow modification of epithelial and endothelial permeability. In addition, the study is focused on indirect effects of particle pre-exposure in spontaneous hypertensive rats (SHR) on vascular function as well as cardiac performance in a Langendorff model. Rats after pre-treatment with different PM are sacrificed and aorta rings and hearts are taken for ex-vivo experiments. Additional experiments with isolated cardiomyocytes and endothelial cell are directed to solve specific mechanisms for observed in vivo effects.

Study period: 2001-2004.

Study on chemical and biological characterisation of ambient air coarse, fine and ultrafine particles for human health risk assessment in Europe.

(http://airnet.iras.uu.nl/inventory/projects/p003.html).

Principal investigator: Dr. Raimo O. Salonen, National Public Health Institute (KTL), Department of Environmental Health, Kuopio, Finland

Study design: The coarse, fine and ultrafine fractions of ambient particles are sampled with High-Volume Cascade Impactor (HVCI) in six European countries. The large-capacity samplings in 3 - 4-day periods provide relatively large PM masses for chemical and biological characterisation of the selected contrasting PM pollution situations. Routine air quality monitoring data and extensive reference PM samplings are used for further physico-chemical and source characterisation of the PM subfractions. Inorganic analyses of the total elemental content and readily bioavailable ions and elements are made as well as analyses of elemental and organic carbon, endotoxin and PAH compounds. In-vitro tests in human and murine respiratory cells are made to identify the causative characteristics and chemical constituents in cytotoxic, proinflammatory and genotoxic responses. The key findings from in-vitro studies are investigated by exposing healthy and compromised animals intratracheally to selected PM samples. All toxicology results are compared with previously collected epidemiological data among subjects with chronic cardiopulmonary disease.

Study period: 2002 - 2004

5.3.3 CLINICAL STUDIES OR CONTROLLED HUMAN EXPOSURE STUDIES OF INHALED ULTRAFINE PARTICLES: DOSIMETRY AND EFFECT OF ULTRAFINE PARTICLES ON SYMPTOMS, LUNG FUNCTION, AND AIRWAY INFLAMMATION

Introduction and General Comments

In theory, controlled exposure experiments have significant advantages over observational epidemiological studies for assessing the health effects of ambient air pollutants. These advantages include the capacity to select the dose of the air pollutant of interest and to assess its effects by reference to an exposure using a benign control substance, usually clean air. Often randomised crossover trials are used where each individual is exposed firstly to the pollutant, followed by the control substance, following an appropriate washout period or vice versa in a random order. In this way subjects become their own controls to reduce the potential for the results to be influenced by between subject variability.

However, the theoretical advantages of controlled exposure experiments may not always be fully realised in practice for a number of reasons including the ubiquitous nature of ultrafine particle pollution in ambient air. For example it would be possible for a subject to report to the laboratory for a controlled exposure to an ultrafine particles (or clean air control) having experienced an uncontrolled ultrafine particles exposure in the ambient environment beforehand. This creates the potential for bias in the results. In addition confounding factors may not necessarily be eliminated by controlled exposures. For example, in the quasi-parallel controlled exposure trial by Ghio et al (2002), which is described in detail below and in Table 5.3.3.2, the concentration of particles delivered to the exposure chamber varied with the $PM_{2.5}$ concentration in the ambient air creating the potential for bias and/or confounding of the results. Interpretation of controlled exposure results may not therefore be straightforward.

There have been a number of controlled exposure studies that have examined the effects of short-term inhalation exposure to sulfuric acid or soluble sulfate aerosols on respiratory symptoms and/or lung function of healthy and asthmatic subjects. For example: sulfuric acid aerosol and asthmatic subjects (Koenig et al 1983); ferric sulfate and normal and asthmatic subjects (Kleinman et al 1981); ammonium sulfate, ammonium bisulfate and sulfuric acid and normal, sensitive and asthmatic subjects (Avol et al 1979); sodium bisulfate, ammonium sulfate, ammonium bisulfate, sulfuric acid and asthmatic subjects (Utell et al 1983). The mass mean aerodynamic diameters of the aerosol particles used in these studies ranged from 0.5 μm to 1.3 μm, i.e. above the ultrafine particles upper size limit. However as the aerosols comprised polydispersed particles the vast majority of particles would have been in the ultrafine range.

Koenig et al (1983) found that inhalation exposure of 100 $\mu g/m^3$ of sulfuric acid aerosol caused a reduction in several lung function parameters for a group of asthmatic subjects when compared with a sodium chloride solution aerosol exposure at the same concentration. Other studies cited above however found that sulfate aerosol inhalation exposures had no adverse effect on lung function at concentrations at or below 100 $\mu g/m^3$ even amongst asthmatic individuals who had previously been found to be sensitive to inhalation of sulfur dioxide or ozone.

Plausible biological mechanisms by which inhaled particles produce increased morbidity and mortality have not been elucidated. Frampton (2001) has proposed a pathophysiology for particulate pollution-induced cardiovascular effects that starts with injury to epithelial cells by reactive oxygen species followed by increased epithelial cell synthesis of pro-inflammatory cytokines that initiates a train of cellular interactions and biochemical events to create a hypercoagulable state. An increased risk of thrombus formation and sudden ischaemic injury or death would result. Frampton (2001) makes the point that the various events in the proposed pathogenic sequence can be assessed in the types of studies possible with controlled exposure experiments.

Controlled exposure experiments summarised in this section fall into two categories. The first category relates to dosimetry. The dosimetry studies are listed in Table 5.3.3.1. These studies have assessed various dosimetric aspects of ultrafine particles inhalation including the potential of ultrafine particles to cross the air-blood barrier of the lung with the associated possibility of disseminated pathological events.

The second category of studies are of the type suggested by Frampton to investigate whether and to what extent inhaled ultrafine particles cause acute injury and/or inflammation of the lung and systemic changes capable of producing apparent non-respiratory related adverse health effects. These studies are listed in Table 5.3.3.2.

A brief summary of the studies followed by a discussion and conclusion follow.

Dosimetry

i. Total Lung Deposition of inhaled ultrafine particles in Healthy Men and Women – (Jaques and Kim, 2000).

The inhalation studies by Jaques and Kim (2000) investigated the deposition of monodispersed particles with number mean diameters of 0.04 μm, 0.06 μm, 0.08 μm and 0.1 μm. Ultrafine particles were measured amongst a relatively large sample size of subjects comprising 11 healthy female and 11 healthy male subjects.

Results for the Mean Total Deposition Fraction for various particle sizes and for breathing patterns spanning the normal range of minute ventilation while resting were as follows:

Particle Diameter μm	Breathing Pattern 500ml Tidal Volume	
	9 breaths/ minute	15 breaths/ minute
0.04	0.53	0.44
0.06	0.44	0.35
0.08	0.40	0.30
0.1	0.34	0.26

The pattern of increasing deposition with decreasing particle size shown above was consistent across all breathing patterns. The results also showed the Total Deposition Fraction increased with increasing residence time for any given tidal volume. These

findings are consistent with a diffusion-like process governing deposition of particles with a size 0.1μm and below.

Total Deposition Fraction values were found to be higher in women than men for particles with a diameter of 0.04 μm.

ii. Possible underestimation of inhaled dose of ultrafine particles by models based on widely accepted assumptions of pulmonary aerosol transport – (Darquenne et al, 1997).

Although the particle sizes involved in this study were larger than the upper size limit for ultrafine particles the study is relevant because the near weightless environment on board the NASA Microgravity Research Aircraft has tested generally accepted assumptions concerning pulmonary transport of ultrafine particles.

The investigators measured total airway/lung deposition of aerosol particles with nominal particle size 0.5 μm, 1 μm, 2 μm and 3 μm inhaled under constant inspiratory and expiratory flows and tidal volume under accelerations of almost 0G (microgravity) and 1.6G (onboard the NASA aircraft) and at 1G on the ground.

A mathematical model based on the widely accepted assumption of reversible axial streaming gas flow in peripheral airways was found to consistently underestimate aerosol deposition for microgravity conditions. Furthermore, the model was found to underestimate deposition of the 0.5 μm particles under 1.0G and 1.6G as well. The investigators speculated that the discrepancy between the model and observations for microgravity conditions might be due to:

- Less particle deposition in the conducting airways because of the absence of gravity sedimentation effects that allowed a greater flow of particles to the alveolar regions to increase alveolar concentrations and deposition by diffusion (the investigators noted that this did not fully explain the discrepancy);

- A mixing effect due to irreversible airway gas flow in contradiction to the models assumption of reversible axial streaming flow, mixing that would be more prominent under microgravity conditions.

Henry et al (2002) demonstrated the possibility of irreversible acinar region gas flow using a computational fluid dynamic model of an alveolar duct. This model predicted longitudinal stretch-and-fold fractal like patterns of fresh and residual aerosol caused by cyclical breathing. Tsuda et al (2002) found a similar flow pattern using a physical model with an excised rat lung. These studies suggest that the rate of mixing and transport and the residence time and deposition in the gas exchange region of the lung for particle deposition is greater than is generally assumed. Modelling by Henry et al (2002) and Tsuda et al (2002) support the proposition by Darquenne et al (1997) of irreversible airway flows.

iii. Regional Deposition of ultrafine particles – (Kim and Jaques, 2000) and Brown et al, 2002)

Kim and Jaques (2000) examined fractional recoveries of inhaled aerosol boluses of particle number mean diameters of 0.04 μm, 0.06 μm, 0.08 μm and 0.1 μm with the aim of estimation regional deposition in the airway and lung. Bolus penetration volumes (ie the volume of air inhaled between the time of concentration peak of the bolus injection and the end of the inspiration) were varied to provide for bolus penetration volumes from 50 ml to 500 ml in steps of 50 ml. The inhalation procedure required subjects to inhale 500 ml of air from the functional reserve capacity during which time the bolus was injected followed by forced expiration to residual volume.

To calculate regional deposition efficiencies the investigators considered that the 500 ml of inhaled air occupied ten contiguous compartments each having a volume of 50 ml. Unrecovered aerosol from a bolus with a penetration volume of 500 ml was considered to have been deposited across all 10 compartments while unrecovered aerosol from a bolus with a penetration volume of 450 ml was deposited across the first 9 compartments from the mouth and so on. On this basis the investigators constructed 10 nonlinear equations relating the unknown regional deposition efficiencies for each compartment to the 10 measured fractional recoveries. The equations were solved to estimate the regional deposition efficiencies and to derive local deposition fractions for each compartment.

On the basis of the calculated local deposition fractions the investigators concluded that peak deposition occurred in lung regions situated between 150 ml and 200 ml from the mouth regardless of particle size. Local enhancement of dose therefore occurs in normal lungs.

The equations used to relate regional deposition efficiencies to aerosol bolus recoveries assume that aerosol bolus penetration volume is the same as actual airway/lung volume penetration. However, the Reynolds Numbers for the peripheral airways for quiet breathing are too low for this assumption to be valid. Some amount of unrecovered bolus aerosol would also be due to the increased aerosol mixing and residence time associated with increasing bolus penetration volumes, factors not allowed for in the equations. The implications for the findings on the precise details of regional deposition are not clear.

Brown et al (2002) studied deposition and clearance of technetium-99M labelled polydispersed ultrafine carbon aerosol with count mean diameter of 0.033 μm and activity mean diameter of 0.061 μm. Healthy, bronchitic and emphysemic subjects were studied. Aerosol deposition was assessed by monitoring radioactivity levels of inhaled and exhaled air. Scintigraphy was used to determine retained radioactivity within the central and peripheral regions of the lung immediately after exposure and 24 hours after exposure and regional deposition indices of large airway deposition, C/P_0 and C/P_{24} respectively, were calculated from these scans. Scans were also conducted during the two hour period following exposure to investigate whether there were any pulmonary clearance differences between healthy and COPD subjects. The liver region was also included in the scintigraphic scans.

Among healthy subjects mean fractional aerosol deposition was found to be 0.54 and mean C/P_0 and C/P_{24} indices were 1.01 and 0.92 respectively, confirming the higher rate of clearance of particles deposited in the central airways by the mucociliary elevator.

Mean fractional aerosol deposition, C/P_0 and C/P_{24} were found to be 0.67, 1.13 and 1.04 respectively in bronchitic subjects and 0.48, 1.06 and 0.90 respectively in emphysemic subjects. The results indicated increased aerosol deposition in the larger airways of bronchitic subjects.

Although the mean deposition fraction of aerosol was found to be lower for emphysemic subjects than for healthy subjects, the actual dose received from any given concentration would be higher due to higher minute ventilation. This would also apply to bronchitic cases.

iv. Translocation of Inhaled Insoluble Particles to the Circulation – (Nemmar et al., 2002).

Nemmar et al (2002) examined whether, and if so, at what rate, insoluble inhaled ultrafine particles can cross the pulmonary air-blood barrier to enter the blood stream. Subject inhaled Technegas, which is an aerosol suspension of technetium-99M labelled carbon particles with a size of the order of 0.005 μm to 10 μm. Immediately after exposure scintigraphic static images were recorded of lungs and thyroid followed by dynamic imaging of the abdomen, including liver, stomach, and bladder. Whole body images were made on completion of the dynamic imaging.

Blood samples were collected 1, 5, 10, 20, 30, 45, and 60 minutes after exposure and radioactivity in blood determined. Thin layer chromatography (TLC) was carried out on each blood sample and also on urine sample collected 60 minutes after exposure.

The investigators detected radioactivity in each blood sample collected, including the sample collected one minute after exposure. Blood sample activity rose progressively to a plateau between the 10 and 20-minute samples.

Blood TLC showed one gamma peak at the origin and a second that moved with the solvent front for all blood samples indicating the presence of both elutriating and non-elutriating radioactive species. The TLC of the urine sample produced only one gamma peak corresponding to elutriating species.

Radioactivity recorded for the liver region was stable from 5 minutes after exposure at around 8 percent of the initial lung radioactivity. Radioactivity recorded over the bladder rose progressively to 25 percent of the initial lung radioactivity.

Nemmar et al (2002) proposed that the experimental results provide plausible evidence for translocation of ultrafine particles from the lung to the circulation based principally on the finding of a non-TLC elutriating radioactive species in blood samples.

Brown et al (2002) found no accumulation of radioactivity in the liver following inhalation technetium-99M labelled UF carbon particles and suggested that the

disparity between their findings and those of Nemmar et al (2002) might be due to soluble pertechnetate contamination of the Technegas used by Nemmar et al (2002).

Providing tissues are healthy, the respiratory epithelium forms a continuous layer of cells with tight junctions that would present a significant barrier to insoluble particles gaining access to the blood. Stearns et al (2001) did not observe 50 nm TiO_2 particles moving through tight junctions using an in-vitro epithelial lung model. Churg (1996) commented that findings of asbestos particles passing between alveolar epithelial cells appeared to be a relatively rare event.

Clinical Trials relating to the Inflammatory Effects of Inhaled Ultrafine Particles

i. Sulfuric Acid Aerosol – (Frampton et al, 1992)

In the study by Frampton et al (1992) healthy non-smoking subjects were exposed to inhalation of 1000 $\mu g/m^3$ of sulfuric acid aerosol in a randomised double blind cross over manner. The control exposure used sodium chloride aerosol.

Lung inflammatory response was assessed by relative cell counts and by total protein and albumin concentrations in bronchoalveolar lavage fluid collected 18 hours after sulfuric acid and sodium chloride exposures.

Neutrophils as a proportion of total cells in bronchoalveolar lavage fluid did not differ significantly for exposure to sulfuric acid when compared with sodium chloride exposure. Concentrations of total protein and albumin were no different between sulfuric acid and sodium chloride exposures.

The authors concluded that brief exposures to sulfuric acid aerosol at a concentration of 1000 $\mu g/m^3$ did not cause an influx of inflammatory cells into the alveolar space.

ii. Zinc Oxide Fume – (Fine et al, 1997 and Kuschner et al, 1995)

Metal fume fever is an illness that can result from the inhalation of the fumes produced when particular metals are raised to very high temperatures in an air environment. This occurs mostly in workplace settings. Zinc and brass (an alloy of zinc) are particularly associated with metal fume fever. Boilermakers engaged in welding or oxy-acetylene cutting of galvanised steel seem especially at risk of developing the disease.

Incidents of metal fume fever were known amongst brass foundry workers more than a century ago when the illness was called "Brass Ague". The symptoms are flu-like and include cough, fever, muscle ache and fatigue. Symptoms persist for about 24 to 48 hours and resolve spontaneously.

The study by Kuschner et al (1995) followed earlier studies that found that fumes generated while welding galvanised steel, when inhaled, produced a dose dependent inflammatory response in the lung (Blanc et al 1991, Blanc et al 1993). The aim of the study was to test whether inhalation exposure to ultrafine particles of purified zinc oxide at lower levels than for the welding studies would produce a similar dose dependent inflammatory response. The study also examined whether the inflammatory response would different between smokers and non-smokers.

The study was a single blind randomised cross over design with medical grade air used as the control. A furnace system was used to generate zinc oxide fume particles with a typical median primary particle diameter between 0.008 and 0.04 μm and mass median diameter of 0.17 μm. The concentration of zinc in the test air ranged from 2.76 to 37.0 mg/m^3. End points measured were various lung function parameters, and analyses for selected biological markers of inflammation in bronchoalveolar lavage fluid.

The study found that the test inhalation of zinc oxide fume did not produce any clinical signs or symptoms of metal fume fever, nor were there any differences in peripheral blood neutrophil counts.

No differences were reported in lung function between baseline and post exposure measurements except for FEV_1. A measured fall in FEV_1 was attributed to normal diurnal variation. Presumably, lung function measurements for both control and zinc oxide exposures showed reductions of similar magnitude that were consistent with diurnal variation.

Concentrations of TNF-α, IL-8 and neutrophil- and lymphocyte count in bronchoalveolar lavage liquid were higher for zinc oxide fume exposure. The data suggested a dose dependent response for TNF-α, IL-8 concentrations and neutrophil count with a threshold. Macrophage counts and IL-1 and IL-6 concentrations were higher for zinc oxide exposure. IL-10 and MIP-α were not detected in bronchoalveolar lavage liquid for either control or zinc oxide exposures.

Fine et al (1997) used a similar system for generating and exposing normal healthy subjects to ultra fine zinc oxide particles to test whether IL-6 and TNF-α would be found in the circulation at the same time or before the onset of metal fume fever. The control exposure was furnace gas composed of 97% filtered air and 3% argon. Zinc oxide concentrations for the test exposures were 2.5 and 5 mg/m^3. The study found that IL-6 was significantly elevated in peripheral blood six hours after exposure for both zinc oxide concentrations. Peripheral blood TNF-α concentrations were not affected. Metal fume fever symptoms were apparent following the zinc oxide exposure at 5 mg/m^3 and peaked 9 hours following the exposure.

IL-6 is a known pyrogen, (a fever causing substance). Based on their own and other studies Fine et al (1997) suggested that inhaled zinc oxide stimulated cellular production and release of TNF-α in the lungs, and that this in turn stimulated production and release of IL-6 by cells located in the lung, liver or circulation consistent with a cytokine driven inflammatory process. Fine et al (1997) did not speculate as to the cells or tissues involved in the increased cytokine release.

iii. Magnesium Oxide Fume – (Kuschner et al, 1997)

Kuschner et al (1997) aimed to characterise human pulmonary responses to high-dose inhalation of fine and ultrafine magnesium oxide particles and in particular to test whether metal fume fever was a specific chemical effect of zinc oxide or a potentially generic response to metal oxide particles. The same furnace system as used by Kuschner et al (1995) to produce zinc oxide fume was used to generate fine and

ultrafine magnesium oxide particles. More than 98% by weight of the magnesium oxide particles produced by the furnace were less than 2.5 μm.

Subjects were their own controls and were separately exposed to magnesium oxide particles at a concentration between 5.8 mg/m^3 and 143 mg/m^3 or medical grade air for the control.

No differences were found between magnesium oxide and control exposures in respect of inflammatory cell counts/proportions or proinflammatory cytokine/chemokine IL-1, IL-6, IL-8, TNF-α or total protein concentrations found in bronchoalveolar lavage fluid collected 20 hours after exposure.

Kuschner et al (1997) concluded that the toxicology of metal oxide particles is not determined by particle size characteristics alone and that air pollution effects associated with inhalation of fine particles (<2.5 μm diameter) should give great weight to chemical-specific mechanisms that may modify the dose response depending on the particle inhaled.

iv. Concentrated Ambient Particles - Ghio et al (2000)

Ghio et al (2000) conducted a quasi-parallel design trial with groups of healthy normal non-smoking subjects exposed by inhalation to either filtered air (control group) or concentrated ambient particles (CAPS). The device used to concentrate the ambient particles (rejected particles) with a size greater than 2.5 μm and did not concentrate particles with sizes 0.1 μm and below. CAPS were therefore composed mainly of particles smaller than 2.5 μm with the fraction below 0.1μm consisting of particles at their ambient concentration at the time of the exposure.

Subjects were assigned to one of four quartiles depending on the concentration of CAPS they were exposed to. Quartile 1 consisted of subjects exposed to filtered air. Equal numbers of CAPS exposed subjects were classified into quartiles 2 to 4 according to ascending particle mass of the exposure. CAPS exposure concentrations ranged from 23.1 μg/m^3 to 311.1 μg/m^3. The concentration of CAPS to which a subject was exposed depended on the ambient air concentration at the time of the exposure and was not known beforehand . The investigation is therefore not strictly a controlled exposure experiment.

A comparison of the means of the combined CAPS exposed quartile groups and the control group bronchoalveolar lavage samples showed a mild increase in neutrophils in bronchial and alveolar fractions amongst the CAPS exposed subjects. Peripheral blood samples collected 18 hours after exposure showed a higher concentration of blood fibrinogen in the CAPS exposed subjects.

Ghio et al concluded that ambient air particles are capable of inducing a mild inflammation in the lower respiratory tract, as well as an increased concentration of blood fibrinogen.

v. Diesel Exhaust Particulate Matter – (Nightingale et al, 2000)

The study by Nightingale et al (2000) assessed the lower airway inflammatory response of normal healthy subjects to inhalation of diesel exhaust particulate matter (DEP). DEP was collected from the exhaust of a stationary diesel engine. A randomised double blind crossover procedure was followed with a DEP exposure concentration of 200 $\mu g/m^3$. Clean air was used as the control.

Airway inflammation markers selected for the assessment were IL-8, TNF-α and MPO concentrations and differential cell counts for induced sputum collected 4 hours after exposure. Peripheral blood plasma assays were also carried out to determine circulating concentrations of IL-6, TNF-α and P-Selectin.

Neutrophils as a percentage of nonsquamous cells and MPO concentrations in induced sputum were higher for DEP exposure than for controls. There were no differences in peripheral blood concentrations of IL-6, TNF-α or P-Selectin.

Nightingale et al (2000) concluded that exposure to DEP at high ambient concentration leads to an airway inflammatory response in normal subjects.

vi. Diesel Exhaust – (Salvi et al, 1999, Salvi et al, 2000 and Holgate, 2002)

These studies used diesel exhaust exposure controlled for PM_{10} mass. The studies by Salvi et al (1999) and Salvi et al (2000) report on different aspects of the same diluted diesel exhaust (DE) controlled exposure trial. Holgate et al (2002) followed a similar experimental protocol to that of Salvi et al (1999) trial study but with a lower exposure concentration of DE and with groups of normal healthy subjects and mild asthmatics to study the asthmatic response. These trials were of a randomised crossover design, double blind in the case of Holgate et al (2002) and at least single blind in the case of Salvi et al (1999) and Salvi et al (2000).

Salvi et al (1999) and Salvi et al (2000)

The subjects for the Salvi et al (1999) and Salvi et al (2000) investigations were normal healthy individuals. Diesel exhaust for the test exposure was produced by an idling diesel engine and diluted with air to maintain a particulate matter concentration of 300 $\mu g/m^3$ PM_{10}. Air was used for control exposure.

Salvi et al (1999) reported on inflammatory markers in: bronchial and bronchial-alveolar fractions of bronchoalveolar lavage fluid; bronchial biopsy tissue samples and peripheral blood samples. Samples were collected 6 hours after exposure.

Significant increases in neutrophils and B lymphocytes were found in airway lavage fluids along with increases in histamine and fibronectin. Bronchial biopsies showed a significant increase in neutrophils, mast cells $CD4^+$ and $CD8^+$ T lymphocytes along with upregulation ICAM-1 and VCAM-1 and increases in the numbers of $LFA\text{-}1^+$ cells in bronchial tissue.

Significant increases in peripheral blood neutrophils and platelets were observed following DEP exposure.

Salvi et al (2000) reported the effects of the inhaled diluted diesel exhaust on the regulation of gene transcription for several proinflammatory proteins by quantifying mRNA levels cytokine/chemokine levels in bronchial biopsy tissue samples and cells collected from bronchoalveolar lavage. The study found that diesel exhaust exposure upregulated bronchial tissue and lumen cellular levels of mRNA for IL-8 synthesis together with an increase in the levels of IL-8 and GRO-α in the epithelium.

Salvi et al (1999) and Salvi et al (2000) interpreted the findings of the studies as demonstrating a well defined and marked systemic and pulmonary response in healthy subjects and a possible explanation for the association observed between ambient levels of particulate matter and various respiratory health outcome indices noted in epidemiological studies.

Holgate et al (2002)

To test the hypothesis that inhalation exposure to DE would cause a more pronounced airway inflammatory response amongst asthmatics than healthy individuals, Holgate et al (2002) carried out randomised double blind crossover DE exposure trials with separate groups of healthy and mild asthmatic subjects. The control exposure was filtered air.

Both asthmatic and healthy groups exhibited small increases in airway resistance immediately after the exposure to DE when compared with air exposure. Other measurements of lung function did not show any differences between DE and air exposure for either the asthmatic or the healthy groups.

Bronchial tissue samples from the healthy group showed increases in cellular and biochemical markers for inflammation for DE exposure when compared to air including: neutrophil infiltration to the proximal airways; up regulation of IL-8 mRNA; and, endothelial adhesion molecules. DE exposure also increased IL-8 concentrations in airway lung fluid. The asthmatic group showed none of the above differences between DE and air exposures.

Baseline markers of allergic airway inflammation were elevated amongst the asthmatic group as would be expected. However, markers of allergic inflammation did not change for the worse following DE exposure.

Discussion and Conclusions

Dosimetry

Figure 5.3.1 has plotted data for airway/lung particulate matter deposition calculated by Bloch et al (2001) using the LUDEP computer program. LUDEP is an implementation of the particle depositional model proposed by the International Commission on Radiological Protection (International Commission on Radiological Protection, 1994). The ICRP model is semi-empirical with parameters tuned to reproduce data on which the model is based (Lazaridis et al, 2001). The output of the LUDEP program in Figure 5.3.1 is therefore a useful summary of controlled exposure experiments considered in

the model development. The LUDEP output also provides a useful picture of regional particle deposition across the spectrum range of particles sizes likely to be inhaled.

The clinical studies of groups in normal respiratory health cited in this review are generally consistent with the LUDEP results, in particular:

- Deposition of ultrafine particles increases with decreasing particle size;
- Fractional deposition of inhaled monodispersed particles while mouth breathing at rest is of the order of 26 to 34 % and 44 to 55 % for particles of with diameters of 0.1 μm and 0.05 μm respectively; and
- Ultrafine particles are deposited across all regions of the airways and lungs with particles in the size range 0.02 μm to 0.10 μm being deposited predominantly in the respiratory bronchioles and alveoli (acinar region of the lung);

The mucus blanket protecting and clearing the airways of deposited insoluble particles thins out around the distal end of the terminal bronchioles (Clark and Pavia 1991). Therefore, there is a strong likelihood that particles deposited in the acinar region will be internalised by alveolar macrophage and epithelial cells. Contact and uptake of insoluble or sparing soluble ultrafine particles by epithelial cells probably occurs along the whole of the respiratory tract but rates of uptake are likely to be far higher in the acinar region because of the absence of the protective mucous blanket (Churg 1996).

In contrast to healthy people, people with chronic bronchitis have shown greater airway deposition of ultrafine particles in the conducting airways than in the acinar region. The data also ultrafine particles relative to healthy people due to their higher minute ventilation. The implications of these findings for the health impacts on COPD patients are not clear.

There are inconsistent findings in respect of insoluble ultrafine particles crossing the air-blood barrier of the lung to join the circulatory system. In-vitro and histological studies do not in general support findings of extracellular transport of inhaled insoluble particles to the circulation for healthy tissue (Churg, 1996; Stearns et al, 2001).

Theoretical aerosol deposition models that rely on an assumption of reversible axial streaming flow in the peripheral airways are likely to underestimate total ultrafine particles deposition in the distal regions of the lung. The ICRP model (International Commission on Radiological Protection, 1994) has a semi-empirical basis and does not entirely rely on any particular theory of pulmonary gas flow.

Lung Function

Holgate et al (2002) found a modest but statistically significant increase in Airway Resistance across groups of healthy and asthmatic subjects following diesel exhaust inhalation with no corresponding significant change in FVC and FEV_1. Other studies summarised in Table 5.3.3.2 found no changes in lung function parameters following exposure to ultrafine particles. Results of controlled exposure studies of effects of ultrafine particles on lung function as measured by plethysmography and spirometry have not been consistent (Department of Health Committee on the Medical Effects of Air Pollutants, 1995). It is possible that lung function measurements that involve deep

inspiration to full lung capacity such as FEV_1 may not be sufficiently sensitive to detect ultrafine particles induced airway calibre reduction.

Inflammation

The studies included in Table 5.3.3.2 analysed samples of lung liquids, airway biopsies and/or peripheral blood samples for a large number of markers of airway inflammation to assess the biological effects of inhaled ultrafine particles. The inflammatory marker results, with the exception of those for the asthmatic group of the Holgate et al (2002) study, have been collated in Tables 5.3.3.3 - 5.3.3.5 to highlight areas of consistency and inconsistency across the studies. Exposure of the asthmatic group of the Holgate et al (2002) study to diesel exhaust, if anything, produced an anti-inflammatory response, and is discussed further below.

Magnesium oxide fume (Kuschner et al, 1997), sulfuric acid aerosol (Frampton et al, 1992) appears not to have generated an inflammatory response in healthy individuals.

The studies involving zinc oxide fume, concentrated ambient particles, diesel exhaust particles and diesel exhaust consistently found:

(a) Evidence of inflammation marked by neutrophil infiltration of the airway; and,

(b) No changes in total protein and albumin levels in lung fluids indicating an absence of airway injury.

Other markers of inflammatory changes however are not consistent across the studies. However several studies found increased synthesis of the chemokine IL-8 and increased expression of vascular endothelium adhesion molecules with particulate matter exposure. These findings are consistent with the findings of neutrophil migration from the blood to the airway.

The physical and chemical natures of inhaled particles are important factors in determining whether there will be an inflammatory response to inhaled ultrafine particles. This may explain the contrast between the absence of an inflammatory response found following sulfuric acid and magnesium oxide inhalation and the exposures of zinc oxide, CAPS and diesel exhaust.

Biological responses to inhaled sulfuric acid aerosol result from the delivery of hydrogen ions (H^+) to the cells of the airway and lungs (Schlesinger and Chen, 1994). However the mucous blanket which lines much of the respiratory tract has a substantial buffering capacity that will protect the airway from H^+ exposure (Holma, 1987).

In vitro studies with human bronchial epithelial cells have shown that the Zn ion as well as various vanadium ions can activate cellular signalling pathways to upregulate synthesis of proinflammatory cytokines (Samet et al, 1999). Exposure to fuel-oil ash that is rich in water-soluble vanadium in the respiratory size range is associated with symptoms of airway inflammation suggestive of upregulation of IL-8 and associated neutrophilic inflammation (Woodlin et al, 1998; Woodlin et al, 2000).

Biological responses to insoluble particles are far more complex than for pure soluble molecules such as the acid aerosols tested in controlled exposure experiments. Insoluble particles are persistent toxicants and have far higher residence times in intracellular and extracellular spaces and while in contact with cellular components. Reactive sites on the surface of particles can catalyse production of reactive oxygen species that induce synthesis of proinflammatory cytokines through cellular oxidative stress. The catalytic action of surface reactive sites means that surface area and surface chemistry is more important than bulk properties in assessing biological responses to insoluble particles. Donaldson and Tran (2002) have published a useful review of inflammatory responses to inhaled particles.

The study by Holgate et al (2002) found markers of elevated baseline allergic airway inflammation in samples of lung fluid and tissue taken from the asthmatic group. These markers indicated that allergic inflammation did not change for the worse following diesel exhaust exposure. Furthermore, unlike the group of healthy subjects, no change in the markers of neutrophilic inflammation followed diesel exhaust exposure amongst the asthmatic group. Significantly, the level of immunostaining for IL-10 in the airway biopsy samples increased amongst the asthmatic group following diesel exposure but not amongst the healthy group.

The cytokine IL-10 inhibits the expression of many pro-inflammatory cytokines and chemokines including the neutrophil chemoattractrant IL-8 (Barnes, 2002). Barnes (2002) has suggested that drugs that activate the signal transduction pathways for IL-10 synthesis may be developed as an asthma therapy. Increased expression of IL-10 following diesel exhaust exposure was not mirrored amongst the healthy group. The increased bronchial IL-10 expression may therefore explain the apparent calming of inflammation amongst the asthmatic group.

Inflammation is the body's response to tissue injury and/ defence against invasion by infectious organisms. Inflammation involves highly complex and dynamic cellular processes and interactions to control the activation, regulation, and termination of the inflammatory response. These processes are not completely understood. Blood, bronchial biopsies and bronchoalveolar lavage samples represent a snapshot of a time varying biological environment. Apparent inconsistencies across studies may be due therefore to differences in the elapsed time between exposure and sample collection.

Plausible biological mechanism for associations between ultrafine particles and health effects

Findings of upregulation of adhesion molecules on airway vascular endothelial cells amongst healthy subjects and elevated fibrinogen concentrations and thrombocyte counts in peripheral blood provide some support for cardiovascular mortality and morbidity associations.

The finding by Holgate et al (2002) of the absence of any worsening of asthma or the worsening of any other marker of inflammation amongst asthmatics is difficult to reconcile with epidemiological studies into acute effects of ultrafine particles. However Holgate et al's findings are not representative of ambient exposure.

On the basis of knowledge gained from animal experiments and models of pulmonary particle deposition, the following mechanisms would be plausible. None of these are mutually exclusive and all might apply.

- Since fine particles are deposited mainly in the conducting airways, one would expect to see effects there. For soluble particles these should be proportional to the volume (mass) deposited. For insoluble particles, where surface reactive sites are responsible for the toxic effect, surface area may be a more appropriate metric for assessing dose-response. Soluble toxic agents are a possible candidate for causation of effects as they are able to penetrate the mucous blanket and reach cellular targets but some amount of insoluble particles would also be able to react with lung fluids, contact airway cells and be internalised. For soluble particles dose would depend on the mass concentration deposited. These soluble compounds could initiate inflammation through cytokine driven processes and lead to an acute local inflammatory response in the lung and thereby may contribute to the exacerbation of pre-existing diseases (Bates 1992).

- Ultrafine particles are deposited mainly in the acinar region. Since the mass of ultrafine particles is negligible, mass-related effects are less probable. Current evidence is that effects are related to number of particles and thus to surface area. That is consistent with surface reactive sites catalysing production of reactive oxygen species in intracellular and extracellular spaces thus inducing pro-inflammatory protein elaboration through cellular oxidative stress. It is not the soluble but the insoluble compounds that might be expected to be most relevant in urban environments.

- Ultrafine particles are phagocytised less efficiently by alveolar macrophages and therefore are more likely to be internalised by epithelial cells and translocated to interstitial sites (Ferin et al, 1991; Stearns et al, 1994). At the same time inflammatory indicators may be upregulated, suggesting that the increased epithelial internalisation and access of ultrafine particles to the interstitium triggers an inflammatory response there.

- Ultrafine particles in lung lining fluids, internalised by epithelial cells and/or translocated to sites in the interstitium may activate cytokines, endothelial and circulating leukocytes adhesion molecules and alter blood coagulability (Utell & Frampton, 1999). The time for these processes to become effective can range from hours to years. These events could increase the risk of an ischaemic event amongst individuals with compromised arterial blood flow.

Do the epidemiological data support the described mechanisms?

At this point ecological epidemiological data do not identify possible mechanisms by which ultrafine particles might produce adverse health effects. The majority of medical opinion seems to be that the most likely mechanism by which ultrafine particles cause the adverse health effects is initiated by cytokine mediated inflammation triggered by oxidative injury to the respiratory epithelium and/or disruption of the cellular signalling pathways that regulate synthesis of the cytokines involved. Epidemiological data from

occupational exposure of zinc oxide fume, quartz, and residual oil fly ash provide considerable support for these particular mechanisms.

There are no existing in-vitro or animal models that can be used comprehensively to study the health impacts of ambient air ultrafine particles because of their complexity of the interactions involved. Given the current state of the art, in-vitro and animal models, and clinical trials can only provide fragments of information required, from any one experiment. Eventually studies will narrow down the mechanisms involved.

The finding by Holgate et al (2002) that an inhaled dose of diesel exhaust that was sufficient to induce neutrophilic inflammation in healthy subjects produced, if anything, a reduction in allergic airway inflammation of mild asthmatic subjects with no neutrophilic response is inconsistent with the epidemiological data. The Holgate et al (2002) findings may not be representative of the effects of actual ambient exposures and could possibly be: a chance event; an indication that inflammation in asthmatics follows a different time course to that of healthy people; a result that is applicable only to a subset of asthmatics. Either way this case illustrates that further experimental work is required.

Generation of the following data would be very important to test the hypotheses, but are missing:

- Data on panel studies with cardiovascular patients are missing; these would test whether or not delayed effects of ultrafine particles are present.

- Measurements of the soluble fraction of relevant components such as transition metals or hydrocarbons in fine particles and of the non-soluble fractions in ultrafine particles are missing, in the context of epidemiological studies.

- Given the difficulty in reproducing realistic ambient atmospheres, it would be useful to conduct a parallel placebo control trial in which the exposed group would live in an environment supplied with ambient air from which all particle matter had been removed. The control group would live in a similar environment but breathe ambient air in a particle polluted environment.

Table 5.3.3.1 Dosimetry

[1] Administration. [2] Exposure details.

Purpose of Study (Investigators)	Subject Details	Exposure Details	End Points Measured	Key Results
To characterise the deposition and clearance of technetium-99m-labeled ultrafine aerosol in subjects with COPD and healthy age matched volunteers. (Brown et al 2002)	**COPD Group** 6 female, 4 male 45-70 years age range classified into bronchitic and emphysema groups **Healthy Group** 6 female, 3 male (data for one subject discarded due to equipment fault) 40-67 years age range	[1] Inhalation from a mouthpiece. [2] Technetium-99m-labeled ultrafine carbon aerosol Activity Mean Diameter of 61 ± 4 nm and Count Mean Diameter was 33 ± 2 nm. Inhalation continued until 25 µCi deposited in the lung.	**Scintigraphy** Scans carried out immediately following exposure and 24 hours after exposure. Activity in the liver was quantified 2 hours after inhalation. Scan divided into three regions of interest (ROI) as follows: Central ROI being an area with dimensions equal to half the lung's width and one third of its height with one boundary roughly corresponding to the mediastinal surface and centred by lung height; Peripheral ROI, area enclosing the whole lung less the central ROI; Liver ROI being an area below the right lung. The ratio of the Central ROI activity to Peripheral ROI activity as an index of airway deposition immediately determined after exposure (C/P_0) and after 24 hours following exposure (C/P_{24}). **Deposition** A Deposition Fraction was calculated from inhaled and exhaled activity measurements.	**Scintigraphy** No accumulation of activity in the liver was observed. Clearance was not significantly different between healthy and COPD groups. The C/P_0 index was elevated in the bronchitic members of the COPD Group relative to the healthy group. **Deposition** Deposition Fraction was significantly greater for bronchitic subjects than healthy or emphysema subjects. Estimated Dose Rate for COPD subjects was found to be 70% greater than for healthy subjects mainly because of their higher minute ventilation.
To elucidate the effect of gravitational sedimentation on pulmonary aerosol deposition. (Darquenne et al 1997)	2 female, 2 male. 26-29 years age range. Normal health.	[1] Inhalation from a mouthpiece. [2] Monodispersed polystyrene latex particles. Separate tidal breathing exposures carried out for nominal particles sizes (concentrations particle count/ml) 0.5 µm (10^4/ml), 1µm (10^4/ml), 2 µm (5 x10^3/ml) and 3 µm (10^3/ml)	**Total Deposition** Total deposition (TD) calculated for each breath according to the following formula: $$TD = 1.0 - \frac{N_{ex}}{N_{in}} \times \frac{V_{in}}{V_{ex}}$$ Where: N_{ex} is the total number of particles exhaled;	**Total Deposition** Under accelerations of 1G and 1.6G TD was found to be strongly size dependent with TD increasing with particle size. TD ranged from 0.165 to 0.440 under 1G and 0.200 to 0.607 under 1.6G corresponding to particle sizes 0.5µm and 3µm respectively for both acceleration conditions. Under microgravity conditions TD hardly varied with particle size the range with a minimum TD value for the 1um particle size exposure and a range 0.129 to 0.149. Under for microgravity conditions TD was higher than predicted by a

		in micro, normal and hypergravity conditions.	N_{in} is the total number of particles inhaled; V_{ex} is the volume exhaled; and, V_{in} is the volume inhaled.	numerical model of aerosol transport-deposition except for the 3um particle size. TD was also higher than predicted by the model for the 0.5um particle size under 1.6G.
1. To measure total lung deposition of ultrafine particles using a large sample size of male and female subjects; 2. to investigate intersubject variability and potential for gender differences in deposition of UFP's; 3. to improve the database of exposure-dose relationships for use in health risk assessments. (Jaques and Kim 2000)	11 female, 11 male. 20-40 years age range No history of smoking or no smoking in previous 5 years.	[1] Inhalation from a mouthpiece. [2] Aerosol particles comprised sebacate oil condensed on metallic nuclei at a concentration of about 50,000 particles/cm^3. Separate tidal breathing exposures carried out for number particle median diameters of 0.04µm, 0.06µm, 0.08µm and 0.1µm and the following tidal breathing patterns: Vt = 500ml Q = 150ml/s Vt = 500ml Q = 250ml/s Vt = 750ml Q = 250ml/s Vt = 750ml Q = 375ml/s Vt = 1000ml Q=250ml/s Vt = 1000ml Q=500ml/s Vt is the tidal volume Q is the respiratory flow rate	**Total Deposition Fraction** Total deposition Fraction (TDF)was calculated for each breathing pattern and particle size according to the following formula: $$TDF = \frac{N_i - N_e}{N_i}$$ Where: N_e is the total number of particles exhaled; N_i is the total number of particles inhaled;	TDF increased with: • decreasing particle size regardless of breathing pattern; • increasing in tidal volume for a fixed flow rate; • decrease in respiratory flow rate for a fixed tidal volume; • being female for the 0.04µm particle size only (ie there were no male/female TDF differences for other particle sizes).
To obtain detailed site-dose relationships for the human lung. (Kim and Jaques 2000)	11 female, 11 male. 20-40 years age range No history of smoking or no smoking in previous 5 years.	[1] Inhalation from a mouthpiece. [2] Bolus delivery of aerosol with subjects breathing with a tidal volume of 500ml and flow rate of 250ml/s. Bolus halfwidth of about 45ml. Aerosol particles comprised sebacate oil condensed on metallic nuclei. Separate tidal breathing exposures carried out for number	**Recovery of bolus and Local Deposition Factor.** Bolus recovery (RC) calculated for each test inhalation according to the following formula: $$RC = \frac{N_{ex}}{N_{in}}$$ where: N_{ex} is the total number of particles exhaled; and, N_{in} is the total number of particles inhaled.	**Recovery of bolus and Local Deposition Factor.** Regional deposition varies widely along the depth of the lung regardless of particle size. The calculated Local Deposition Factor (LDF) peaked for compartments corresponding to 150ml and 200ml from the mouth.

		particle median diameters of 0.04µm, 0.06µm, 0.08µm and 0.1µm. The timing of the bolus injection after commencement of the inhalation cycle was varied to study deposition at different bolus penetration volumes.	Regional Deposition Efficiency (X_i) for each of 10 volumetric divisions of the maximum penetration volume called "compartments" was found by solving a system of non-linear equations. The Local Deposition Factor (LDF) defined as the fraction of total inhaled aerosol deposited in a compartment was calculated from the RDE's. In turn the LDF's were averaged into head, tracheobronchial and alveolar regions assuming that bolus penetration volume equated to airway/acinus volume.	
To Investigate whether the smallest particle fraction in an inhaled aerosol translocate from the lung to the circulation. (Nemmar et al 2002)	5 male non-smokers. 24 - 47 years age range	[1] Inhalation from a mouthpiece. [2] Technetium-99m-labeled ultrafine carbon aerosol with individual particles of the order of 5 - 10 nm. Subject inhaled approximately 100 MBq of aerosol in 5 breaths.	**Scintigraphy** Static acquisition (1 to 3 minutes) of lungs and thyroid followed by dynamic acquisition (5 to 25 minutes) of the abdomen and successive images of the whole body (50 to 60 minutes) **Peripheral Blood** Samples taken at 1, 5, 10, 20, 30, 45 and 60 minutes after exposure and for each time point sample gamma activity was measured. Thin Layer Chromatography conducted on each blood sample. **Urine** Thin layer chromatography conducted on a urine sample taken 60 minutes after exposure.	**Scintigraphy** 8% of deposited activity accumulated in he liver within 5 minutes after inhalation. Activity progressively increased in the bladder reaching about 25% of deposited activity by 45 minutes. **Peripheral Blood** Radioactivity measured in samples rose progressively with elapsed time to a plateau between the 10 minute and 20 minute samples. Thin Layer Chromatography results indicated that ^{99m}Tc bound to non-elutriating species were present in all blood samples together with a soluble ^{99m}Tc species. **Urine** Thin layer chromatography showed the presence of a soluble ^{99m}Tc species and the absence of any ^{99m}Tc bound carbon particles.

Table 5.3.3.2 Controlled Exposure Studies of Ultra Fine Particles

[1] Administration. [2] Exposure and control substances and concentration. [3] Exposure time and ventilation intensity. [5] Trial design

Purpose of Study (Investigators	Subject Details	Exposure Protocol .	End Points Measured	Results Differences between mean results for exposed and control groups unless otherwise stated
To test the hypothesis that IL-6 and TNF-α will be present in the circulation before the onset, or at the same time as the onset, of metal fume fever symptoms following inhalation exposure to purified zinc oxide fume. (Fine et al 1997)	5 female, 8 male healthy non-smokers. One subject dropped out for unspecified reasons. Mean age 30.8 years with standard deviation 7.7 years.	[1] Face mask. [2] Purified zinc oxide fume with median primary particle diameter ranging between 0.008 and 0.04 um and a mass median diameter of 0.3 um or air and 3%argon (control). Separate exposures at particle concentrations 0, 2.5 and 5 mg/m^3 of zinc oxide. [3] 120 minutes of exposure while at rest. [4] Subjects were their own controls. Random order, single blind design.	**Self Reported Symptoms** Each subject was asked to record and grade any symptoms (using a visual analogue chart) before each exposure and at 3, 6 and 9 hours after exposure and to record his/her body temperature at intervals not exceeding 2 hours following exposure until the subject went to sleep for the night. **Peripheral Blood** Venous blood samples taken before and immediately after exposure and at 3 and 6 hours after exposure. Plasma analysed for IL-6 and TNF-α.	**Self Reported Symptoms** Ten hours following exposure mean changes in body temperature from baseline for each zinc oxide exposure (ie 2.5 and 5 mg/m^3) were significantly higher than control exposure and estimated to be about 0.7°C. Eleven hours after zinc oxide exposure at 5 mg/m^3 mean increase in body temperature reached a peak at 0.75°C. The symptoms most consistently reported were cough, fatigue and muscle ache, mostly in the slight to mild range. Symptom scores were significantly different for the 5 mg/m^3 zinc oxide exposure after 6 and 9 hours. Symptom scores for the 2.5 mg/m^3 zinc oxide exposure were not significantly different from baseline. **Peripheral Blood** Mean plasma IL-6 levels showed a significantly and monotonic rise with elapsed time relative to the pre-exposure baseline for each zinc oxide exposure (ie 2.5 and 5 mg/m^3). IL-6 levels for the control did not rise from baseline. Differences between IL-6 levels and control were statistically significant for the 6 hour after exposure measurement. Mean TNF-α levels following zinc oxide exposure did not vary significantly from the control exposure.
To determine whether single exposures to H_2SO_4 aerosol cause cellular responses in the alveolar space important to host defence. (Frampton et al 1992)	2 female, 10 male, all healthy people lifetime non-smokers 20-39 age range	[1] Exposure chamber [2] 1000 $\mu g/m^3$ NaCl (Control) or H_2SO_4 aerosol. The aerosol generated had an average mass median aerodynamic diameter of 0.9 um [3] 2 hours of a cycle of 10 min of moderate exercise performed on a bicycle ergometer in each half-hour period. Alternate NaCl or H_2SO_4 exposures were	**Symptoms** Subjects were polled by questionnaire immediately after exposure and 18 hours after exposure regarding respiratory symptoms, nasal or eye irritation and odour. **Plethysmography** Thoracic gas volume, Airway Resistance reported as Specific Airway conductance (SGaw) with pneumotachograph used to measure FEV_1 and FVC before (baseline) and immediately after exposure to the NaCl (control) or H_2SO_4 aerosol and at 18 hours post	**Symptoms** Four subjects detected an odour or taste during H_2SO_4 exposure. No odour or taste was reported for NaCl exposure. Three subjects had cough and four subjects reported throat irritation during H_2SO_4 exposure. One subject had cough and three reported throat irritation during NaCl exposure. Subjects were asymptomatic 18 hours after exposure. **Plethysmography** No changes in FVC, FEV_1 or SGaw immediately after or 18 hours after exposure to NaCl or H_2SO_4 when compared to pre-exposure baseline measurements. There were no differences in lung function measurements between NaCl and H_2SO_4 exposures.

		carried out 2 weeks or more apart. [4] Randomised double blind cross over design.	exposure. **Bronchoalveolar lavage (BAL)** Lavage fluid instilled/collected from a segmental bronchus of the right middle lobe and from a segmental bronchus of the lingula 18 hours after the NaCl (control) or H_2SO_4 aerosol exposures. BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes expressing, CD3, CD4, CD8 antigens quantified. **Alveolar Macrophage Function** Tests of: • Antibody-dependent Cell Mediated Cytotoxicity • Superoxide ion release • Influenza virus inactivation	**Bronchoalveolar lavage (BAL)** No significant differences in cell differential counts in BAL. Neither NaCl nor H_2SO_4 exposure indicated inflammation for those markers measured in BAL with no evidence of neutrophil infiltration to the airway A lower (but not statistically significant lower) percentage of T lymphocytes found in BAL following H_2SO_4 exposure when compared with NaCl exposure. This was accounted for by lower counts of $CD4^+$ phenotype but not significantly so. There were no significant differences in other cell type counts. **Alveolar Macrophage Function** No statistically significant difference was found in Alveolar Macrophage function between NaCl and H_2SO_4 exposures.
To test the hypothesis that concentrated ambient particles (CAPS) can cause neutrophilic inflammation in the lungs of healthy humans. (Ghio et al 2000)	38 healthy people, non-smokers for at least 5 years. Subjects classified into one of four quartiles depending on air/CAPS exposure as follows: Quartile 1 - Exposed to filtered air - 8 subjects Quartiles 2, 3, 10 - each quartile comprised 10 subjects classified according to individual exposure level	[1] Exposure Chamber [2] Filtered air or concentrated ambient air particles (CAPS) size range 0.1 - 2.5 µm. Quartile 1 - Filtered air. Quartile 2 - 47.2 µg/m^3 Quartile 3 - 107.4 µg/m^3 Quartile 4 - 206.7 µg/m^3 [3] 2 hour of a cycle of 15 min moderate exercise followed by 15 min of rest. [4] Parallel design. Subjects allocated to Quartiles 2 to 4 inclusive after exposure	**Symptoms** **Plethysmography** Airway Resistance (Raw) immediately before and after exposure. **Spirometry** FEV_1, FVC, PEF immediately before and after exposure **Peripheral Blood** Samples taken immediately before and 18 hours after exposure. Erythrocyte, neutrophil, lymphocyte, monocyte, platelet, counts; haemoglobin, hematocrit, ferritin, fibrinogen levels; blood viscosity **Bronchoscopy with Lavage**	**Spirometry, Plethysmograph, Symptoms** Subjects did not report symptoms after either air or CAPS exposure. No significant differences in FEV_1, FVC, PEF or Raw across the Quartiles. All spirometry measurements were normal. **Peripheral Blood** No changes between pre-exposure and post exposure were recorded for any marker except for fibrinogen concentration amongst the CAPS exposed groups. A significant difference was found in fibrinogen levels between airs exposed (Quartile 1) and CAPS exposed (combined Quartiles 2 - 4). A similar magnitude change was recorded for each of the CAPS exposed Quartiles indicating that the response was independence of dose. **Bronchoscopy with Lavage** **BL fraction**

		known. It is not clear how the subjects for Quartile 1 (the control group) were selected.	Lavage sample instilled to a segmental bronchus of the lingula and collected in two fractions reported as Bronchial Lavage (BL) and BAL. BL and BAL were considered to reflect the environments of the bronchial and distal airways respectively. Lavage samples were taken 18 hours after the air or CAPS exposure. Total cell count and percentages of macrophage, neutrophil, lymphocyte, monocyte and epithelial cells were determined for BL and BAL fractions. BL and BAL fractions were also analysed for concentrations of protein, IL-6, IL-8, PGE_2, α_1-antitrypsin and fibronectin. The concentration of Fibrinogen additionally determined for BAL fraction.	No significant difference between CAPS exposed groups and air-exposed group for total cell count, or proportions of macrophage, lymphocyte or epithelial cells. CAPS exposed groups had significantly higher numbers and proportions of neutrophils in the BL sample. Monocytes were also significantly higher in the CAPS exposed group. The concentration of protein was significantly lower in the CAP exposed group. **BAL fraction** Total cell count in BAL was significantly higher for CAPS exposed groups compared with air exposed group. Except for the proportions and counts of macrophages and neutrophils, which were significantly higher in the CAPS exposed group, the counts of other cells were not significantly different. Monocytes counts were also higher on average amongst the CAPS exposed individuals but the difference between the group means was not sufficient to support a statistically inferred effect. Fibrinogen concentrations were lower in the CAPS exposed group. Concentrations of protein, IL-6, IL-8, PGE_2, α_1-antitrypsin and fibronectin were not significantly different between air and CAPS exposed groups although amongst the individuals of the two higher CAPS exposed quartiles IL-8 concentrations were lower than for the air exposed group but not sufficiently so to infer a statistical difference.
To assess the impact of short-term exposure to diluted diesel exhaust on inflammatory parameters in human airways. The study was designed to assess whether observed increased sensitivity to air pollutants of asthmatics can be explained by acute	**ASTHMATIC GROUP** 5 female, 10 male 23-52 years age range mild atopic asthma Positive skin tests to at least one common airborne allergen Non-smokers Not every asthmatic subject completed the full experimental program.	[1] Exposure Chamber Diluted fresh diesel exhaust [2] 100 µg/m[3] [3] 2 hour of a cycle of 15 min moderate exercise followed by 15 min of rest. Random sequence 3 weeks or more apart. [4] Randomised double blind cross over design.	**Lung Function** Lung Function measured before exposure, one hour after start of exposure and at end of exposure for Airway Resistance, FVC and FEV_1. Procedure not reported. **Peripheral blood** Venous blood samples taken:	**Lung Function** For diesel exhaust exposure compared to air for Control and Asthma Groups: • A modest but statistically significant increase in Airway Resistance at the end of exposure amongst the Asthmatic Group. • A modest but statistically significant increase in Airway Resistance was found after one hour and at the end exposure amongst the Control Group. • No significant changes in FVC or FEV_1. **Peripheral blood before and after exposure** Only the results for before exposure and 6 hours following exposure to air

neutrophilic inflammation or and increase in allergic airway inflammation resulting from diesel exhaust exposure. (Holgate et al 2002)	**CONTROL GROUP** 9 female, 16 male 19-42 years age range Normal lung function. Negative skin prick tests to common airborne allergens Not all control subjects completed the full experimental program.		• before exposure • one hour after start of exposure • at the end of exposure • 6 hours after end of exposure Samples analysed for leukocytes, neutrophils, lymphocytes and monocyte counts and haemoglobin levels. **Bronchial Wash and Bronchoalveolar Lavage** performed 6 hours after exposure to air and diesel exhaust lung liquid, assessed for: • total cell count and proportions of neutrophil, lymphocytes, eosinophils, mast cells and macrophages; • albumin and total protein concentrations • BW sample only concentrations of MPO, ECP, methyl histamine, soluble ICAM-1, IL-8, GM-CSF, extracellular Superoxide Dismutase • BW sample only total RNA extracted from cells and RT-PCR ELISA used to quantify relative changes in mRNA for IL-1β, IL-5, IL-8, TNF-α, IFN-γ and GM-CSF. **Endobronchial biopsy**	and diesel exhaust are presented. Neither diesel exhaust nor air exposures produced any significant changes. **Bronchial Wash** For diesel exhaust exposure compared to air for Control and Asthma Groups: • Significant higher neutrophil relative count for Control Group but no significant difference for Asthma Group; • No significant differences in other cell types except relative macrophage count for the Control Group which is reported as lower for diesel exhaust exposure; • No significant differences in total protein or albumin; • MPO and ECP concentrations were measured to assess degranulation of neutrophils and eosinophils respectively but these were not significantly different. • Soluble ICAM-1 concentration was measured as an indicator of epithelial cell activation but was not significantly different. • Significantly higher IL-6 and IL-8 levels for the Control Group; no significant difference for Asthma Group • No differences in GM-CSF and extracellular Superoxide Dismutase. **Bronchoalveolar Lavage** For diesel exhaust exposure compared to air for Control and Asthma Groups: • Significant higher relative lymphocyte count in Control Group. No difference in relative lymphocyte count in Asthma Group; • Lower relative macrophage count for Control Group. No difference in relative macrophage count in Asthma Group • No significant differences in relative cell counts of neutrophils, eosinophils and Mast Cells; • No significant differences in total protein or albumin concentrations. **Endobronchial biopsy** **Control Group** For diesel exhaust exposure compared to air: • Significant higher VCAM-1 and P-Selectin expressed on

			6 hours after exposure with biopsies taken from the anterior aspect of the main carina and the subcarninea of the third and fourth generation airways on the right side or from the posterior aspect of the main carina and corresponding subcarinae on the left side. The biopsies were immunostained for quantification of: • Counts of neutrophils, mast cells, lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$), eosinophils and $CD14^+$ cells. Cells counted separately for epithelium and submucosa; • Proportion of blood vessels stained for ICAM-1, VCAM-1, E-selectin, P-selectin. • GRO-α, IL-4, IL-8, IL-10, TNF-α, GM-CSF, NF-κB and RANTES in bronchial epithelium. Total RNA extracted from tissue samples and RT-PCR ELISA used to quantify relative changes in mRNA for IL-1β, IL-5, IL-8, TNF-α, and IFN-γ synthesis.	endothelium; • Significant increase in IL-8 mRNA; • No significant difference differences in inflammatory cell count in bronchial submucosa; • Significantly lower $CD3^+$ cells in bronchial epithelium **Asthmatic Group** Comparison of paired biopsy samples taken after diesel exhaust exposure and air exposure from the same subject found no significant difference in any end point measured except: • submucosa eosinophil count which was lower for diesel exhaust exposure; • IL-10 levels significantly higher in bronchial epithelium.
To test the hypothesis that low-level, purified zinc oxide fume exposure would reproduce the dose-dependent inflammatory cellular responses observed in earlier galvanised steel welding studies. (Kuschner et al 1995)	6 female, 8 male healthy people 3 never smoked, 2 former smokers (quit more than 5 years before study, 9 current smokers. mean age 35.6 years with standard deviation 7.9 years.	[1] Mouth breathing facemask. [2] Purified zinc oxide fume with median primary particle diameter ranging between 0.008 and 0.04 µm and a mass mean diameter of 0.17 µm OR medical grade air (control). [3] Range of particle concentrations 2.76 - 37.0 mg/m^3, mean 16.4 mg/m^3. [4] 15 to 120 minutes of exposure assumed resting. [5] Single blind randomised cross over design	**Self Reported Symptoms** Each subject was asked to record any symptoms and record his/her body temperature during the evening following the afternoon exposure. **Plethysmography** Thoracic gas volume, Airway Resistance (Raw) and methacholine provocative dose before exposure to zinc oxide fume (or air for control) and 18 hours post exposure. **Spirometry** FEV_1 before exposure to zinc oxide fume (or air control) and 18 hours post exposure. **Total Lung Capacity** Single breath helium dilution method **Carbon Monoxide Diffusing Capacity** Single breath method	**Self Reported Symptoms** No subject reported any symptoms indicative of metal fume fever or body temperature elevation. **Plethysmography** No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure. **Spirometry** FEV_1 was minimally lower from baseline post exposure but the reduction was consistent with diurnal fluctuations. **Total Lung Capacity** No statistically significant difference for lung function parameters between before exposure (baseline) and post exposure. **Carbon Monoxide Diffusing Capacity** No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure.

			Peripheral Blood Polymorphonuclear leukocyte (neutrophil) concentration determined prior to lung function testing, that is before exposure to zinc oxide fume (or air for control) and 18 hours post exposure **Bronchoalveolar lavage** Lavage fluid instilled to a segmental bronchus of the right middle lobe 20 hours after the air (control) or zinc oxide fume exposures. BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes and proportions of T Cell, $CD4^+$, $CD8^+$ and B Cell phenotypes. BAL samples were analysed for concentrations of TNF-α, IL-1β, IL-6, IL-8, IL-10, MIP1-α and total protein.	**Peripheral Blood** Polymorphonuclear leukocyte (Neutrophil) concentration was not significantly different between the before and after exposure blood samples. **Bronchoalveolar lavage** Grouped data showed a significant higher count of Polymorphonuclear leukocyte (neutrophil) cells following the zinc oxide fume exposures relative to the air (control) exposure. When the data were stratified according to cumulative zinc oxide exposure (a proxy for dose) and dose-response relationship was apparent Linear regression of the data found that cumulative zinc exposure was a statistically significant predictor of Polymorphonuclear leukocyte (neutrophil) increase. Compared with the air exposure, the lymphocyte count was significantly higher for the post zinc oxide exposure samples but there was no significant difference in the ratios of the various lymphocyte phenotypes. TNF-α and IL-8 were significantly higher following zinc oxide exposure compared with air exposure. Linear regression found that cumulative zinc exposure was a statistically significant predictor of an increase in TNF-α, and IL-8 concentrations. The linear regression also indicated a threshold of about 500 $mg.min/m^3$ cumulative zinc exposure.
To determine whether magnesium oxide inhalation provokes a similar response to the inflammatory cellular influx observed following zinc oxide fume exposure. (Kuschner et al 1997)	2 female, 4 male. Non-smokers, 3 former smokers. 21-43 years age range	[1] Mouth breathing facemask. [2] Purified magnesium oxide fume, 98.6% of particles by weight below 1.8 µm in diameter. [3] Range of particle concentrations 5.8-230 mg/m^3, median 133.0 mg/m^3 [4] Subjects were their own controls. Single blind design.	**Self Reported Symptoms** Each subject was asked to record any flu-like symptoms, eg myalgias, fatigue, rigors and his/her body temperature during the evening on day of exposure. **Spirometry** FEV_1 before exposure to magnesium oxide fume (or air for control) and 18 hours post exposure. **Total Lung Capacity** Single breath helium dilution method **Carbon Monoxide Diffusing Capacity** Single breath method **Peripheral Blood** Complete blood counts and differentials were obtained pre-exposure and 18 hour post exposure to determine	**Self Reported Symptoms** None of the subjects reported symptoms post exposure for either air or magnesium oxide fume. **Spirometry, Total Lung Capacity, Carbon Monoxide Diffusing Capacity, Peripheral Blood Bronchoalveolar lavage** No significant differences in any of the end points measured between air and magnesium oxide exposure

			neutrophil concentrations. **Bronchoalveolar lavage** Lavage fluid instilled to a segmental bronchus of the right middle lobe 20 hours after the air (control) or magnesium oxide fume exposures. BAL counts of macrophage, Polymorphonuclear leukocyte (neutrophil) and Lymphocytes. BAL samples were analysed for concentrations of TNF-α, IL-1, IL-6, IL-8.	
To investigate the effect of diesel particles on clinical measures and airway inflammation, using inflammatory markers in induced sputum and exhaled carbon monoxide as an index of oxidative stress. (Nightingale et al 2000)	Healthy non-smoking people. 7 Female and 3 male. Mean age 28 year, ± 3 year SEM.	[1] Exposure chamber [2] Clean air (control) or diesel exhaust particles 200 μg/m^3 PM_{10} (DEP). [3] 2 hours at rest. Random sequence 3 weeks or more apart. [4] Randomised, double blind cross over design.	**Spirometry** FEV_1 and FVC before exposure and repeated every 30 minutes for 4 hours post exposure. FEV_1 and FVC measurements repeated 24 hours after exposure. **Bronchial Reactivity** Methacholine PC_{20} for FEV_1 determined 4 hours after exposure. **Exhaled Carbon Monoxide** Exhaled carbon monoxide measured before and after exposure and repeated every 30 minutes for 4 hours post exposure. Exhaled carbon monoxide measurements were repeated 24 hours after exposure. **Peripheral Blood** Blood samples taken before and immediately and 24 hours after exposure. Plasma concentrations of IL-6, TNF-α and P-Selectin determined. **Induced Sputum** Induced sputum sample collected 4 hours after exposure. Sputum supernatant assayed for TNF-α, IL-8 and MPO concentrations. Cell pellet resuspended for differential cell counts.	**Spirometry** No differences in FEV_1 or FVC. **Bronchial Reactivity** No differences in methacholine PC_{20} for FEV_1. **Exhaled Carbon Monoxide** Exhaled carbon monoxide was significantly higher for DEP exposure reaching a maximum 1 hour after exposure. **Peripheral Blood** No difference between DEP exposure and control plasma concentrations of IL-6, TNF-α and P-Selectin concentrations. **Induced Sputum** No significant difference in induced sputum concentrations of TNF-α or IL-8 concentrations. MPO concentration was significantly higher for DEP exposure. Differential neutrophil count was significantly higher for DEP exposure and macrophage differential count significantly lower. No differences in the differential counts of lymphocytes, eosinophils or epithelial cells.

To test the hypothesis that exposures to diesel exhaust might induce inflammatory and mediator responses in the airway and peripheral blood. (Salvi et al 1999)	Healthy non-smoker. 4 female, 11 male. 21-28 year age range. Normal lung function. Negative skin prick tests to common airborne allergens	[1] Exposure chamber [2] Air (control) or diluted fresh diesel exhaust PM_{10} 300 $\mu g/m^3$ [3] 1 hour of a cycle of 15 min moderate exercise followed by 15 min of rest. Random sequence 3 weeks or more apart. [4] Randomised, single blind (or possibly double blind) cross over design.	**Spirometry** (PEFR, FVC, FEV_1, FEF_{25-75}) immediately before and after each exposure. **Peripheral blood** collected 6 hours after each exposure. Total cells, differential counts and platelet count determined. **Bronchial wash and bronchoalveolar lavage** Bronchial wash (BL) and bronchoalveolar lavage (BAL) performed for bronchus of middle lobe or lingua 6 hours after exposure. Samples analysed for: • cell type/counts • albumin, total protein, LDH, IL-8, ICAM-1, methylhistamine and fibronectin. **Endobronchial biopsy** 6 hours after exposure with biopsies taken from the anterior portion of the main carina and the subcarninea of the third and fourth generation airways on the right side or from the posterior part of the main carina and corresponding subcarinae on the left side. The biopsies were immunostained for quantification of: • counts of neutrophils, lymphocytes ($CD3^+$, $CD4^+$, $CD8^+$ cells), macrophages, eosinophils inflammatory cell counted separately for epithelium and submucosa; • proportions of blood vessels stained for ICAM-1, VCAM-1, E-selectin, P-selectin, LFA-1 ligand and VLA-4 ligand.	**Spirometry** No difference in spirometry measurements made before and after exposures. **Peripheral Blood** Differences in diesel exhaust exposure compared to air: • Neutrophil and platelet count higher • $HLA\text{-}DR^+$ cell count lower **Bronchial Wash** Differences in diesel exhaust exposure compared to air: • significantly higher neutrophil count. • macrophage count and lactic dehydrogenase activity showed a tendency to be greater though not statistically significant. **Bronchoalveolar Lavage** Differences in diesel exhaust exposure compared to air: • higher B Cell proportion amongst total cells • no significant difference in proportions of $HLA\text{-}DR^+$, $CD3^+$, $CD8^+$, or $CD25^+$ cells • methyl histamine and fibronectin concentrations significantly higher • no difference in concentrations of total protein, albumin, IL-8, C3a, C5a and soluble ICAM-1. **Bronchial Biopsies** Differences in diesel exhaust exposure compared to air: • neutrophil count in epithelium and submucosa higher • mast cell count in submucosa higher; • total T Cell count higher in epithelium and submucosa comprising greater numbers of $CD4^+$ cells in the epithelium and submucosa and $CD8^+$ cells in the epithelium. • no differences in the number of activated T Cells ($CD25^+$), macrophages, eosinophils or B Cells. • markedly higher proportion of blood vessels staining for ICAM-1 or VCAM-1 • No difference in data for E-selectin and P-selectin • Cells expressing the LFA-1 ligand were higher in the epithelium and submucosa • Cells expressing the VLA-4 ligand were higher in the submucosa though not significantly so.
To test the hypothesis that the leukocyte	See Salvi et al 1999	See Salvi et al 1999	**Bronchial wash** Performed 6 hours after exposure and total RNA extracted	**Bronchial Wash** Differences in cytokine/chemokine mRNA in bronchial wash cells for

infiltration and the various inflammatory responses induced by diesel exhaust exposure in healthy human airways were mediated by enhanced chemokine and cytokine production by resident cells of the airway tissue and lumen. (Salvi et al 2000)			from cells and RT-PCR ELISA used to quantify relative changes in mRNA for IL-1β, IL-4, IL-5, IL-8, TNF-α, IFN-γ and GM-CSF synthesis. **Endobronchial biopsy** 6 hours after exposure • immunostained for quantification of, GRO-α, IL-4, IL-5, IL-6, IL-8, TNF-α,GM-CSF and ENA-78; quantification was performed separately for the epithelium and submucosa. • total RNA extracted from tissue samples and RT-PCR ELISA used to quantify relative changes in mRNA for IL-1β, IL-4, IL-5, IL-8, TNF-α, IFN-γ and GM-CSF.	diesel exhaust exposure compared to air: • Significantly higher proportions of IL-8 mRNA • No difference on the mRNA levels of IL-1β, IL-4, IL-5, TNF-α, IFN-γ or GM-CSF. • **Endobronchial Biopsy** Differences in cytokine/chemokine mRNA in bronchial tissue for diesel exhaust exposure compared to air: • Significantly higher proportions of IL-8 mRNA • IL-5 mRNA higher but just short of statistical significance; • No difference in the mRNA levels of IL-1β, IL-4, TNF-α, IFN-γ or GM-CSF. • Higher levels of the chemokines IL-8 and GRO-α in the bronchial epithelium.

Table 5.3.3.3 Comparison across studies of airway and lung fluid samples

Symbols: **x** no difference between exposure and control; ↑ elevated in exposure; ↓ lower in exposure; blank either not measured or measured and not reported.

	Frampton et al (1992)	Ghio et al (2000)	Ghio et al (2000)	Holgate et al (2002)	Holgate et al (2002)	Kuschner et al (1995)	Kuschner et al (1997)	Nightingale et al (2000)	Salvi et al (1999)	Salvi et al (1999)
	H_2SO_4 / NaCl	Concentrated Ambient Air Particles / Air	Concentrated Ambient Air Particles / Air	Diesel Exhaust / Air Healthy Group	Diesel Exhaust / Air Healthy Group	Zinc Oxide / Air	Magnesium Oxide / Air	Diesel Exhaust Particles / Air	Diesel Exhaust / Air	Diesel Exhaust / Air
Sample	Bronchoalve-olar Lavage	BL Fluid	BAL Fluid	BW Fluid	BAL Fluid	Bronchoalveolar Lavage	Bronchoalveolar Lavage	Induced Sputum	Bronchial Wash	Bronchoalveolar Lavage
Macrophage	x	↓	x	↓	↓	↓	x	↓		x
Monocytes		↑	x							
Lymphocytes	x	x		x	↑	↑	x	x		
$CD3^+$	x					x				x
$CD4^+$	x					x				x
$CD8^+$	x					x				x
Epithelial		x	x					x		
Neutrophil	x	↑	↑	↑	x	↑	x	↑	↑	
Eosinophil				x	x			x		
Mast Cell	x			x	x					
B Cell						x				↑
Methylhistamine				x						↑
MPO			x	x				↑		
Extracellular Superoxide Dismutase				x						
PGE_2		x								
Total Protein	x	↓	x	x	x	x	x			x
Albumin	x			x	x					x
IL-1β						x	x			
IL-5										
IL-6		x	x	↑		x	x			
IL-8		x	x	↑		↑	x	x		x
IL-10						**not detected**				
IL-13										
MIP1-α						**not detected**				
IFN-γ										
TFN-α						↑	x	x		
GM-CSF				x						
α_1-antitrypsin		x								

Fibrinogen			↓							
Fibronectin		x	x							↑
Soluble ICAM-1				x						x
C3a										x
C5a										x

Table 5.3.3.4 Comparison across relevant studies of bronchial tissue samples			
Symbols: **x** no difference between exposure and control; ↑ elevated in exposure; ↓ lower in exposure; blank either not measured or measured and not reported.			
		Holgate et al (2002)	Salvi et al (1999 and 2000)
		Diesel Exhaust / Air	Diesel Exhaust / Air
Cell adhesion molecules in Endothelium	ICAM-1	**x**	↑
	VCAM-1	↑	↑
	P-Selectin	**x**	**x**
	E-Selectin	↑	**x**
Cytokine or Chemokine mRNA expression in bronchial biopsy samples	IL-1β	**x**	**x**
	IL-4		**x**
	IL-5	**x**	**x**
	IL-6		
	IL-8	↑	↑
	IL-10		
	IL-13	**x**	
	TNF-α	**x**	**x**
	IFN-γ	**x**	**x**
	GM-CSF		**x**
	NF-κB		
	RANTES		
	GRO-α		
Cytokines or Chemokines in bronchial biopsy Epithelium	IL-6	**x**	
	IL-8	**x**	↑
	IL-10	**x**	
	IL-13		
	TNF-α	**x**	
	IFN-γ		
	GM-CSF	**x**	
	NF-κB	**x**	
	RANTES	**x**	
	GRO-α	**x**	↑
Cytokines or Chemokines in bronchial biopsy Submucosa	IL-4		**x**
	IL-5		**x**
	IL-6		**x**
	IL-8		**x**
	TNF-α		**x**
	GM-CSF		**x**
	GRO-α		**x**
Inflammatory Cells in Bronchial Epithelium	Neutrophil	**x**	↑
	Mast	**x**	**x**
	Eosinophil	**x**	
	CD3+	↓	↑
	CD4+	**x**	↑
	CD8+	**x**	↑
	Macrophage	**x**	
Inflammatory Cells in Bronchial Submucosa	Neutrophil	**x**	↑
	Mast	**x**	↑
	Eosinophil	**x**	
	CD3+	**x**	↑
	CD4+	**x**	↑
	CD8+	**x**	**x**
	Macrophage	**x**	

Table 5.3.3.5 Comparison across relevant studies of peripheral blood samples

Symbols: **x** no difference between exposure and control; ↑ elevated in exposure; ↓ lower in exposure; blank either not measured or measured and not reported.

	Fine et al 1997	Ghio et al (2000)	Holgate et al (2002)	Kuschner et al (1995)	Kuschner et al (1995)	Nightingale et al (2000)	Salvi et al (1999)
	Zinc Oxide / Air	Concentrated Ambient Air Particles / Air	Diesel Exhaust / Air	Zinc Oxide / Air	Magnesium Oxide / Air	Diesel Exhaust Particles / Air	Diesel Exhaust / Air
IL-6	↑					**x**	
TNF-α	**x**					**x**	
P-Selectin						**x**	
RBC		**x**					
Neutrophils		**x**	**x**	**x**	**x**		↑
Lymphocytes		**x**	**x**				↓ **(HLA-DR$^+$)**
Monocytes		**x**	**x**				
Platelets		**x**					↑
Haemoglobin		**x**	**x**				
Haematocrit		**x**					
Ferritin		**x**					
Fibrinogen		↑					
Blood Viscosity		**x**					

*↓HLA-DR$^+$ means the number of lymphocytes expressing the HLA-DR surface molecule was significantly reduced for DE exposure compared with the control exposure day, which the investigators interpreted as selective lymphopenia.

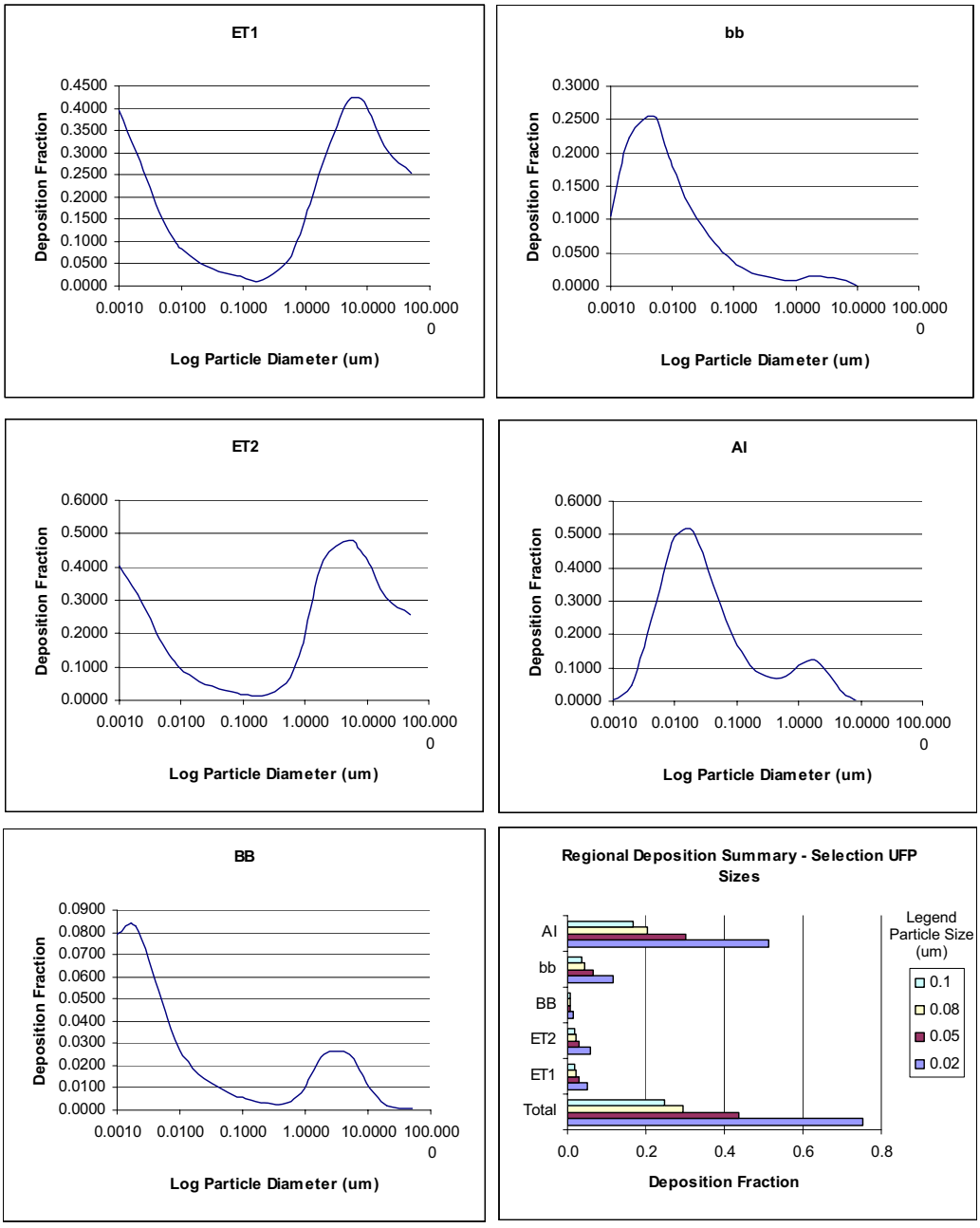

Figure 5.3.1 Plot of Regional Deposition Fractions calculated by LUDEP Computer Program for Adult Males and a Light Level of Exertion. **ET1** Anterior Nasal Passage; **ET2** Mouth, Posterior Nasal Passage, Pharynx and Larynx; **BB** Trachea and Bronchi; **bb** Bronchiole including Terminal Bronchiole, **AI** - Alveolar Interstitial (Respiratory Bronchiole, Alveolar Ducts, Sacs and Alveoli). Data from Bloch et al (2001)

ONGOING STUDIES

Study on a difference in the uptake of ultrafine particles in healthy subjects, asthmatics and smokers (http://www.snap.se/Projekt/upptag.htm).

Study location: Sweden, Swedish National Air Pollution and Health Effect Program

Principal investigators: Gerd Sällsten, Occupational and Environmental Medicine, Göteborg University; Hans Welinder, Occupational and Environmental Medicine, Lund Universitet; Tom Bellander, Occupational and Environmental Medicine, Stockholm County Council

The objectives: To modify a clinically used method to produce ultrafine radiolabelled particles, and to use these particles to determine whether there is a difference in lung retention between healthy subjects, asthmatics, and smokers. Further, to determine the uptake of ultrafine particles into the systemic circulation. The hypothesis is that due to various degrees of respiratory epithelial inflammation, a greater part of the ultrafine particles can pass the lung barrier of asthmatics and even a greater extent among smokers.

The design: The measurement of lung clearance of inhaled 99mTc-labelled carbon particles, Technegas, will be used to assess the uptake. Technegas is an ultrafine radioactive aerosol normally used for lung scintigraphy. The particle size distributions will be measured by use of the particles electrical mobility. The amount ultrafine particles entering the blood system will be determined by radioactivity in urine. The working plan includes generation of a stable aerosol of ^{99}mTc-labelled ultrafine particles <100 nm by dilution of the aerosol produced by a Technegas generator. Ln a study on voluntary humans the uptake of inhaled ultrafine particles will be measured in healthy subjects, asthmatics, and smokers. The leakage, i.e. the activity not bound to particles in the aerosol, will be estimated in vitro in order to measure the correct uptake of particles. By ultra filtration it is possible to separate particles from the fraction of activity that is not bound to particles.

Some tests will be conducted with the particles labelled with isotopes other than ^{99}mTc, i.e. ^{111}In with a half-life of 2.8 days, which will enable to follow clearance up to about 30 days in a study on human volunteers. It is also intended to generate ^{99}mTc-labelled ultrafine particles of different sizes by alteration of temperature, aerosol concentration, and time factor. A study on healthy volunteers will reveal if the size of the ultrafine particles have any influence on the uptake.

WOODPART - Acute effects of wood smoke particles - an experimental study of real life exposures. (http://www.snap.se/Projekt/woodpart.htm)

Study location: Sweden, Swedish National Air Pollution, and Health Effect Program

Principal investigators: Gerd Sällsten, Occupational and Environmental Medicine, Göteborg University; Hans Welinder, Occupational and Environmental Medicine, Lund Universitet; Tom Bellander, Occupational and Environmental Medicine, Stockholm County Council

The objectives: To determine whether moderate exposure to particles (fresh or somewhat aged) from wood smoke in a real life situation causes an inflammatory response in airways or peripheral blood of healthy subjects.

The design: 15 healthy subjects will be examined before and on repeated occasions after: (1) 4 hours of exposure to fresh wood smoke ($PM_{2,5}$ about 150 mg/m^3), (2) 4 hours of exposure to aged wood smoke ($PM_{2,5}$ about 150 mg/m^3), (3) 4 hours of breathing normal indoor air.

Exposure will take place in a normal residential setting using wood burning in an appropriate wood stove/open fireplace. Particle mass ($PM_{2.5}$, PM_1), elemental characterisation (XRF), number concentrations (SMPS), and 'black smoke' will be measured, as well as gaseous compounds (NO_2, aldehydes). Established (eNO) and novel (condensate markers) methods will be used for assessing airway inflammation using breath analysis. In addition, inflammatory markers in peripheral blood (CRP, fibrinogen, neutrophils, erythrocytes, platelets, factor VII, D-dimer, CC16) will be quantified. Subjective symptoms (airways, eyes, and general) will be recorded using VAS scales. Results will be presented on the regional, national, and international levels, to authorities, stakeholders, in journals, and at workshops and conferences.

Respiratory and cardiovascular effects of diesel exhaust in COPD.

Study location: Sweden, Swedish National Air Pollution, and Health Effect Program

Principal investigators: Gerd Sällsten, Occupational and Environmental Medicine, Göteborg University; Hans Welinder, Occupational and Environmental Medicine, Lund Universitet; Tom Bellander, Occupational and Environmental Medicine, Stockholm County Council

The objectives: The overall objective is to characterize the respiratory and cardiovascular effects of diesel exhaust in elderly and individuals with COPD, groups which have been suggested to be sensitive to air pollution effects. It is hypothesised that diesel exhaust would induce a worsening of the pre-existing inflammation in the airways of individuals with COPD, which may lead to an exacerbation of the disease. It is also postulated that this could be accompanied by changes in heart rate variability and also changes in blood coagulation, as compared with a group of age matched healthy individuals.

The specific scientific objectives are to study diesel exhaust effects in COPD and elderly healthy subjects, in terms of: (1) pulmonary function changes, (2) cardiovascular effects, (3) inflammatory respiratory and systemic effects, (4) differences and similarities in responses between subjects with copd and age matched elderly healthy control subjects and (5) determine mechanistic pathways whereby elderly susceptible individuals experience adverse health effects identified in epidemiological studies.

The design: The study will use diesel engine exhaust exposure chamber together with state of the art techniques developed by the laboratory. This includes induced

sputum and measurements of free radical activity, oxidative stress, proinflammatory and apoptosis markers in airways and blood, together with coagulation factors, long term ECG and lung function tests. The study design includes 15 COPD and 15 elderly subjects being exposed to DE 100 $\mu g/m^3$ PM_{10} and filtered air in a randomised sequence and monitored up to 48 hours after exposures.

5.4 RECOMMENDATIONS FOR PRIORITIES FOR FUTURE AUSTRALIAN STUDIES TO ADDRESS GAPS IN KNOWLEDGE IN THE AREA OF HEALTH EFFECTS OF ULTRAFINE PARTICLES IN GENERAL AND IN THE AUSTRALIAN CONTEXT.

Based on the experience gained from the epidemiological studies reported to date and taking the improved scientific understanding of the characteristics and dynamics of ultrafine particles in atmospheric systems compared to a few years ago, future exposure/epidemiological studies are likely to provide much clear answers on the associations between ultrafine particles and health outcomes. The designs of such studies can now target the specifics of ultrafine particles, which differ from other size fractions and characteristics of ambient particulate matter. The recently published World Health Organization "Guidelines for Concentration and Exposure-Response Measurement of Fine and Ultrafine Particulate Matter for Use in Epidemiological Studies" (WHO 2002), is an example of the progress in understanding of how ultrafine particle specifics should be dealt with in study designs to provide the desirable study outcomes. Specific recommendations for future health outcome studies include:

- Studies should be conducted over longer periods of observation. This relates to studies with the focus both on acute and short-term effects. Such studies would enable comparisons to be made between periods likely to have high exposure and periods likely to have low exposure. Longer periods of observation would also allow an evaluation of the lag phase between exposure and effect.

- Study designs and statistical approaches used should be such that the effects related to particle size of interest (<0.1 micrometres) could be decoupled from other characteristics of the particles or complex pollutant mixtures.

- Studies should be conducted with larger sample sizes. Larger samples would enable better modelling of the role of age, sex, and other demographic and clinical variables in the effect of ultrafine particles on the outcome of interest. In addition, studies should specifically target potentially susceptible subgroups and provide information on the susceptibility on relevant groups of the population.

- Taking the differences in ultrafine particle concentrations and other characteristics between different geographical locations (resulting from the differences in the local sources, their strength and characteristics, meteorology, topography, etc), as well as the differences in demographic, socio-economic and urban use factors, etc, it is expected that the type and the magnitude of the responses will differ between different locations. Therefore, it is recommended that health outcome studies would be conducted in selected places in Australia to quantify the relationship between exposure to ultrafine particles and health outcomes in an Australian setting. The outcomes of such studies would provide an adequate guidance to the

decision makers on the most desirable steps in controlling exposure to ultrafine particles in Australia.

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6. RELATIONSHIP BETWEEN THE SULFUR CONTENT OF DIESEL FUELS AND THE NUMBER OF ULTRAFINE PARTICLES IN DIESEL EMISSIONS.

This part of the literature review establishes the current knowledge on the relationship between fuel sulfur content and the number of ultrafine particles in diesel emissions. Before tackling the question of the influence of the fuel sulfur level on particle emission it is necessary to first develop an understanding as to what constitutes diesel exhaust, the complex issues of nanoparticles in diesel exhaust, their physical properties, chemical composition and formation. The first section of this chapter explains the general characteristics of emissions from diesel vehicles without a special focus on any of the pollutants. In the second part, an attempt is made to address the questions of why is it important to know the size distribution of diesel particulate matter (DPM) and what is the role of nanoparticles as part of the DPM. The current state of knowledge on the mechanisms governing nanoparticle formation, physical and chemical properties is presented. It is important to stress that the mechanisms governing nanoparticle formation are still not fully understood and the theories presented here still have to be confirmed by more hard scientific data. A short description of what constitutes diesel fuel is given with a focus on sulfur content. It is worth mentioning that one of the most useful, and up-to-date source of data on this topic was the Dieselnet Technology Guide (Majewski, 2003) from which larger parts were taken in these first few general sections.

The fourth section focuses on the main topic of our literature review, the influence of the fuel sulfur level on diesel emissions with a special attention to nanoparticle emissions. It is surprising how little information is available on this topic. The initial search has led to more than 150 references (journal articles, conference presentations, reports, etc.) on the fuel sulfur level and diesel emissions, but only a small number of them covered the topic of nanoparticle emissions. From those only the relevant references were chosen that lead to some definite conclusions.

Finally, in the last section, the main conclusions are presented regarding the current state of knowledge and the scale of the problem. Recommendations are provided for the appropriate way of addressing the gaps in knowledge as well as for adequate management responses.

6.1 GENERAL CHARACTERISTICS OF EMISSIONS FROM DIESEL ENGINES

Diesel engines, like other internal combustion engines, convert chemical energy contained in the fuel to mechanical power. Diesel fuel is a mixture of hydrocarbons, which theoretically produce only carbon dioxide (CO_2) and water vapour (H_2O) during combustion. Indeed, diesel exhaust gases are primarily composed of CO_2, H_2O and the unused portion of engine charge air. Concentrations of these gases in diesel exhaust vary depending on the engine and its load and speed conditions, typically in the following ranges:

CO_2 - 2 ... 12%
H_2O - 2 ... 12%
O_2 - 3 ... 17%
N_2 - balance.

None of these diesel combustion products, with the exception of CO_2 for its greenhouse gas properties, have adverse health or environmental effects. The same cannot be said of other products such as sulfur oxides and unburned hydrocarbons (see sections 6.1.1 to 6.1.4).

Diesel emissions include also pollutants, which are toxic to humans and/or cause other detrimental environmental effects. The diesel pollutants, which are by-products of diesel combustion, originate in several non-ideal processes occurring during real combustion. These processes include incomplete combustion of fuel, reactions between mixture components under high temperature and pressure, combustion of engine lubricating oil and oil additives. They also include combustion of non-hydrocarbon components of diesel fuel, such as *sulfur compounds* and various fuel additives. The total concentration of pollutants in exhaust gases from today's new diesel engines, however, amounts to a fraction of a percent.

Some diesel emissions are regulated in the U.S., Europe, and several other countries. Regulated emissions include the following compounds:

- Diesel Particulate Matter (DPM) also referred to as PM (particulate matter) or TPM (total particulate matter), regulated by the mass of emitted particles
- Nitrogen Oxides (NO_x)
- Hydrocarbons (HC) including either the total hydrocarbons (THC) or only the non-methane hydrocarbons (NMHC)
- Carbon Monoxide (CO).

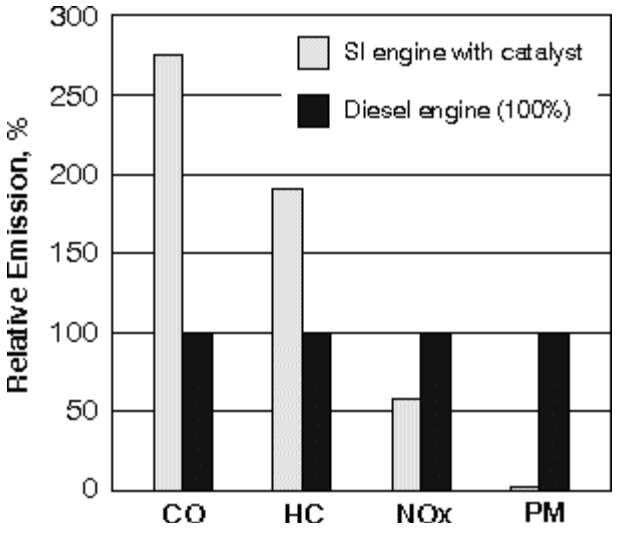

Figure 6.1 Comparison of Regulated Emissions - Spark Ignited and Diesel Engines

Because diesel engines operate with "internal" mixture formation and with compression ignition, where combustion takes place during and after fuel injection, diesel emissions are somewhat different from those observed in spark-ignited engines. An example comparison of regulated emissions from a light-duty diesel engine with those from a gasoline engine is shown in Figure 6.1 (Bosch, 1994). Both engines were tested on the European test cycle (4-cylinder MY1992 engines, 1.7L displacement). Emissions of carbon monoxide and hydrocarbons from diesel engines are significantly lower than those from gasoline engines. Diesel NO_x emissions are also usually lower than those

from gasoline engines. However, if a gasoline engine is equipped with a three-way catalyst, then its emissions could be lower than those for diesel engines. A real drawback of the diesel engine is its high particulate matter mass emissions that are practically absent from gasoline exhaust gases. Reduction in the emissions of particulate matter and NO_x are the focus of today's diesel emission control technologies.

Several non-regulated pollutants or suspected pollutants can be found in diesel exhaust, usually at concentration levels much lower than the regulated emissions. Some of them are part of the complex diesel particulate matter emission; others are totally separate species in the gas phase. The list of unregulated diesel emissions includes the following compounds:

- PAH – poly-nuclear aromatic hydrocarbons, heavy organic compounds found mostly in the DPM but some of PAHs are also present in the gas phase.
- SOF - "soluble organic fraction" constituting part of DPM.
- Aldehydes, R-CHO, derived from hydrocarbons (formaldehyde, HCHO, is regulated in some applications).
- Sulfur dioxide, SO_2, from sulfur present in the fuel.
- Nitrous oxide, N_2O (nitrous oxide is not included in NO_x).
- Dioxins - diesel emissions are suspected to be a source of dioxin emissions.
- Metal oxides - several engine lubricating oil additives include organo-metal-compounds resulting in some metal oxide emissions including such metals as copper zinc, and calcium and non metals like phosphorus. Fuel additives as a means of diesel emission control may result in emissions of iron, copper, cerium, or other metals.

6.1.1 Nitrogen Oxides – NO_x

Nitrogen oxides as defined by emission regulations include nitric oxide NO and nitrogen dioxide NO_2. Concentrations of NO_x in diesel exhaust are typically between 50 and 1000 ppm. If concentrations are given in mass units, NO_x is usually expressed as NO_2 equivalent. Nitric oxide is a colourless and odourless gas that may be synthesised directly from nitrogen and oxygen under high temperature and pressure:

$$(1)\quad N_2 + O_2 = 2NO - 182.4 \text{ kJ/mole}$$

The negative heat effect represents an endothermic reaction. NO is produced according to reaction (1) in the engine cylinder, where temperature and pressure are high. At low temperature and pressure, the chemical equilibrium moves to the left side of equation (1). Thermodynamically, nitric oxide has a tendency to decompose to nitrogen and oxygen under conditions in diesel exhaust. The rate of decomposition, however, equals practically zero and NO_x control from diesel engines remains an unsolved problem.

In older technology engines, approximately 95% of nitrogen oxides were composed of NO and only 5% of NO_2. The proportion of NO_2 in total NO_x in newer, turbocharged diesel engines can be as high as 15% and more. NO can be easily oxidised by oxygen into nitrogen dioxide at ambient conditions:

$$(2)\quad 2NO + O_2 = 2NO_2 + 113.8 \text{ kJ/mole}$$

The above reaction occurs spontaneously (but not instantaneously) in the NO - air mixture after exhaust gases are discharged into the atmosphere. NO_2 is a very toxic red-brown gas with an unpleasant irritating odour. NO_2 is extremely reactive and exhibits strong oxidation properties. NO_2 reactions, which occur in various types of emission control catalysts, may include oxidation of hydrocarbons, carbon monoxide as well as diesel particles.

Nitrogen oxides are highly active ozone-generating precursors playing an important role in the smog chemistry. Besides diesel particles, NO_x is one of the most critical pollutants found in diesel exhaust.

6.1.2 Hydrocarbons – HC

Hydrocarbons found in the gas phase of diesel exhaust are a mixture of many hydrocarbon species derived from diesel fuel and from lubricating oil. Shorter carbon chains characterize fuel hydrocarbons while lube oil hydrocarbons contain the heavier material. C_nH_m, the generic chemical formula for hydrocarbons, represents a molecule with n atoms of carbon and m atoms of hydrogen.

In engine emission standards, hydrocarbons are commonly regulated as either total hydrocarbons (THC), or as non-methane hydrocarbons (NMHC): the latter category excluding the simplest hydrocarbon methane (CH_4) due to its different atmospheric reactivity to longer chain hydrocarbons. Even though the concentration of methane in diesel exhaust is very low, certain HC emission standards from diesel engines are expressed as NMHC for the sake of compatibility with other emission regulations.
Hydrocarbons, especially those with longer carbon chains, may have a characteristic or irritating odour. Some of them, such as benzene, are toxic and/or carcinogenic. Various hydrocarbon derivatives, e.g. aldehydes, are also present in diesel exhaust gases. Many aldehydes have an irritating odour and/or are toxic. Some of them, such as formaldehyde, are classified as carcinogens. Concentration of gaseous hydrocarbons in diesel exhaust ranges from approximately 20 to 300 ppm.

Diesel exhaust hydrocarbons are divided between the gas phase and the particle (liquid or adsorbed) phase. There is no clear distinction between volatile and non-volatile hydrocarbon species. As a guideline, compounds with vapour pressure of above 0.1 mmHg in standard conditions (20°C, 760 mmHg) can be considered volatile. Volatile diesel hydrocarbons contain aliphatic and aromatic species with up to approximately 24 carbon atoms in their molecule. *Hydrocarbon emission regulations refer to the volatile gas phase HCs.* The particle phase hydrocarbons are referred to as SOF.

Hydrocarbons may be oxidised by oxygen to produce carbon dioxide and water (3), which is one of the fundamental reactions occurring in emission control catalysts. Under mild oxidizing conditions hydrocarbons form aldehydes or ketones (4).

(3) $C_nH_m + (2n + m/4)O_2 = nCO_2 + (m/2)H_2O$
(4) $R\text{-}CH_3 + O_2 = R\text{-}CHO + H_2O$

In the atmosphere, hydrocarbons undergo photochemical reactions with NO_x leading to formation of smog and ground level ozone. Different hydrocarbons exhibit different

activity as ozone precursors. Methane (CH_4), the simplest hydrocarbon, does not exhibit any activity and is not considered an ozone precursor. This is the reason why many regulations exclude methane from regulated hydrocarbon emission by setting standards for non-methane hydrocarbons (NMHC).

6.1.3 Carbon Monoxide – CO

Carbon monoxide (CO) is an odourless, colourless, and a very toxic gas of about the same density as that of air. At high concentrations CO is very flammable, burning in air with a bright blue flame.

CO emissions from today's new diesel engines are relatively low. Carbon monoxide concentrations in diesel exhaust range from approximately 10 to 500 ppm.
At elevated temperatures, or over an oxidation catalyst, carbon monoxide can be oxidised by oxygen to form carbon dioxide, as follows:

$$2CO + O_2 = 2CO_2 + 565.6 \text{ kJ/mole}$$

The reaction produces a high heat effect, which, in the case of CO-rich exhaust gases, can cause a significant increase of the gas temperature in catalytic reactors designed to oxidize CO. Adiabatic oxidation of 1% of CO in the exhaust stream raises the gas temperature by approximately 100°C.

6.1.4 Sulfur Dioxide - SO_2

Although sulfur dioxide is an unregulated diesel emission it plays a critical role in nanoparticle formation and therefore will be discussed in this review. Sulfur dioxide originates from the sulfur in fuel and in engine lubricating oil. SO_2 is a colourless gas with a characteristic, irritating odour. Sulfur dioxide can be oxidised to sulfur trioxide (SO_3), which is the precursor of the sulfuric acid responsible for sulfate particle emissions. The majority of sulfur in raw diesel exhaust exists as SO_2. Only approximately 2 - 4% of fuel sulfur is emitted as SO_3 from the engine.

The exhaust concentration of sulfur dioxide is in direct proportion to the fuel sulfur level. In fact, SO_2 concentrations can be calculated from the fuel consumption and its sulfur content with good accuracy. Such calculations are based on an air to fuel ratio of 20:1, which is typical for diesel engines operating at full load conditions. Diesel fuels of 500 ppm S produce exhaust SO_2 levels of about 20 ppm.

As the levels of sulfur in fuels decrease, engine lubricating oils become an important source of SO_2 in diesel exhaust. Diesel lube oils typically contain 4,000 - 10,000 ppm sulfur, primarily as part of their additive package. Anti-wear additives typically contain zinc, sulfur and phosphorus in the form of zinc dithiophosphates. Many detergent additives also contain alkyl sulfonates (DECSE, 1999).

6.1.5 Diesel Particulate Matter

Particulate matter—perhaps the most characteristic of diesel emissions—is responsible for the black smoke traditionally associated with diesel-powered vehicles. The diesel particulate matter emission is usually abbreviated as PM or DPM, the latter acronym being more common in occupational health applications. Diesel particles form a very complex aerosol system. *Despite a considerable amount of basic research, neither the formation of DPM in the engine cylinder, nor its physical and chemical properties or human health effects are fully understood.* Nevertheless, on the basis of what is already known, DPM is perceived as one of the major harmful emissions produced by diesel engines. Diesel particles are subject to diesel emission regulations worldwide and, along with NO_x, have become the focus in diesel emission control technology.

Contrary to gaseous diesel emissions, DPM is not a well-defined chemical species. The definition of particulate matter is in fact determined by its sampling method, the detailed specification of which is an important part of all diesel emission regulations. DPM sampling involves drawing an exhaust gas sample from the vehicle's exhaust system, diluting it with air, and filtering through sampling filters. The mass of particle emissions is determined based on the weight of DPM collected on the sampling filter. It is quite obvious that any changes in the procedure, for example using a different type of sampling filter or different dilution parameters, may produce different results. Standardization of sampling methods is of the utmost importance if results from different laboratories are to be comparable. Such standards have been developed for the measurement of *PM mass* in the area of public health regulations (i.e., emission standards for diesel engines and vehicles) worldwide. Ongoing research in Europe is aimed at developing standardised measuring methods based on particle number emissions, rather than mass, for the inclusion in future emission standards in addition to the PM mass metric (Andersson, 2002). So far no common standard has been reached in the area of diesel occupational health regulations, where a number of different measuring methods and corresponding DPM definitions exist in parallel.

Diesel particles are composed of elemental carbon particles, which agglomerate and adsorb other species to form structures of complex physical and chemical properties. Diesel particles have a bimodal size distribution. They are a mixture of *nucleaction mode* and *accumulation mode* particles, schematically shown in Figure 6.2. Nucleaction mode particles are very small: according to most authors, their diameters are between approximately 0.007 and 0.04 μm. More recent studies redefined the nucleaction mode particle size range to be even smaller, from 0.003 to 0.03 μm (Kittelson, 2002), thus making them comparable to certain large molecules. Nucleaction mode particles are often referred to as *nanoparticles*, although these two terms are not the same. Nanoparticles are usually defined as particles below 50 nm in diameter (0.05 μm). This is an arbitrary definition, not related to the physical properties of diesel exhaust; nanoparticles include practically all nucleaction mode particles, but may also contain a certain fraction of the accumulation mode particles.

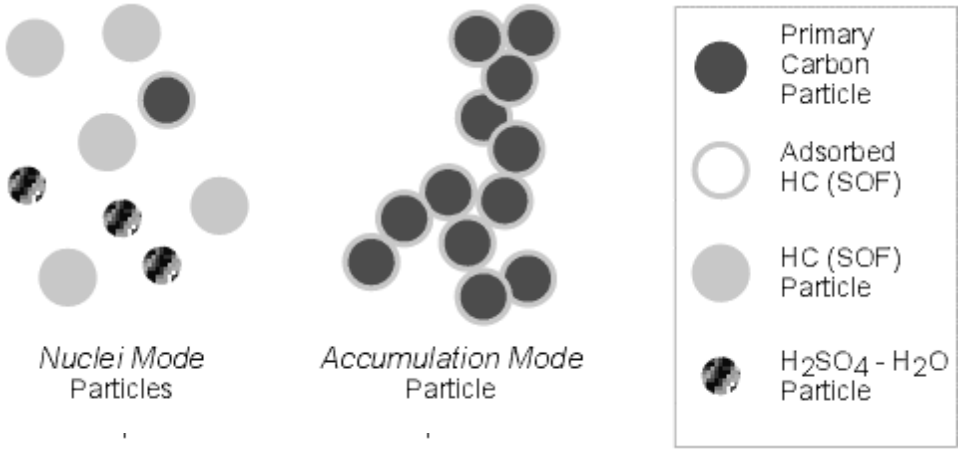

Figure 6.2 Schematic of Diesel Particulate Matter

The nature of nucleaction mode particles is still studied in research laboratories. It is believed that nucleaction mode particles are primarily volatile: they consist mainly of hydrocarbon and hydrated sulfuric acid condensates. These are formed from gaseous precursors as temperature decreases in the exhaust system. After mixing with cold air, be it in the laboratory dilution tunnel or in the ambient air, these volatile particles are very unstable: their concentrations strongly depending on dilution conditions such as dilution ratio and residence time. A small amount of nucleaction mode particles may consist of solid material, such as carbon or metallic ash from lube oil additives (Tobias, 2001; Kittelson, 1998). Nucleaction mode particles constitute the majority of particle number – of the order of 90% - but only a few percent of the PM mass.

Agglomeration of primary carbon particles and other solid materials, accompanied by adsorption of gases and condensation of vapours, form accumulation mode particles. They are composed mainly of solid carbon mixed with condensed heavy hydrocarbons (Figure 6.2), but may also include sulfur compounds, metallic ash, cylinder wear metals, etc. Diameters of the accumulation mode particles are between approximately 0.04 and 1 μm with a maximum concentration between 0.1 and 0.2 μm (Brown, 2000; Kittelson, 1998). Most PM mass emission (but only a small proportion of the total particle number) is composed of agglomerated particles.

Particles leaving the engine are composed primarily of solid phase, carbon material (SOL). Both individual (nucleaction mode) and agglomerated carbon particles are formed in the combustion chamber. In the exhaust system, depending on the temperature, the particles undergo limited oxidation and further agglomeration. Some particles are deposited on the exhaust pipe walls due to thermophoretic forces (i.e., mass transfer driven by temperature gradient). Other PM precursors including hydrocarbons, sulfur oxides, and water are present in the hot diesel exhaust as gases or vapours.

Another source of solid material in diesel exhaust is metal ash compounds derived from lubricating oil additives as well as from engine wear. Nucleation of volatile ash constituents is believed to take place during expansion stroke in the engine cylinder. The ash nucleaction can then agglomerate to form accumulation mode particles. The relative proportion of ash generally increases in new engines, due to less carbon particles and lower total PM mass.

Physical and chemical properties of DPM change once the exhaust gases enter the dilution tunnel, are mixed with air and cooled to below 52°C. Heavy hydrocarbons, which are derived from lubricating oil and unburned fuel, condense or adsorb onto the surface of carbon particles forming the organic portion of DPM (SOF). If the amount of carbon particles that can act as a "sponge" is insufficient, hydrocarbons will nucleate, forming increased numbers of volatile (liquid) nucleaction mode particles. In the dilution tunnel, the total hydrocarbon material from the combustion chamber becomes finally proportioned between particle (SOF) and gas phase hydrocarbons (at least in theory; in practice a portion of diesel hydrocarbon material may be measured and accounted for twice: in the particle phase and in the gaseous phase).

Sulfate particles are formed in the dilution tunnel through a hetero-nucleation process from the molecules of H_2SO_4 and water. During PM measurements, sulfate particles are deposited on the filters together with the carbonaceous material. It was once believed that sulfuric acid is attached to or associated with carbon particles. Later research found that sulfate particles may also be separate from carbon particles (Walters, 1988). It is now envisioned that sulfate particles, while existing in the accumulation mode, mixed with carbon and organic SOF material, are also an important source of volatile (H_2SO_4 - H_2O) nucleaction mode particles (Kittelson, 2002). Depending on the availability of metal-based compounds, sulfuric acid may also form solid (non-volatile) sulfate salts.

6.2 PARTICLE SIZE DISTRIBUTIONS AND NANOPARTICLE EMISSIONS

Interest in particle size distributions and nanoparticle emissions from diesel engines was sparked by reports that newer technology engines - designed for low PM mass emissions - may still generate high particle numbers. The most significant study indicating such possibility (involving measurement of particle size distributions from an older and a newer generation heavy-duty diesel engine) was published by the Health Effects Institute (HEI) (Bagley, 1996). As indicated by later research which was based on more comprehensive data, from several engine models, old and new technology engines produce, in general, nucleaction modes of similar magnitude; the PM mass reductions in new engines are due to a smaller number of particles in the accumulation mode (Kittelson, 2002; Ristovski, 2002; Andersson, 2002). It is believed that emissions from the new engine in the HEI study - an experimental 1991 design - have not reflected general emission trends in new technology engines. That study, however, must be credited with prompting quality research by the diesel industry, academia, and governments, leading to a greatly increased understanding of particle emissions from internal combustion engines. In a later chapter, we will discuss the results of this study in more detail.

6.2.1 Diesel Particle Size Distribution

Since the mid-1990's, particle size distributions from internal combustion engines have been receiving increased attention due to the possible adverse health effects of fine and ultrafine particles. Diesel emission control strategies, based on both engine design and aftertreatment, are being examined and re-evaluated for their effectiveness in the control of the finest fractions of diesel particles and particle number emissions. However, a fair performance assessment of various control technologies can be possible only if the research community reaches a consensus on the definition and the measurement techniques of the smallest fractions of diesel particles. The determination

of particle sizes and numbers is much more sensitive to the measuring techniques and parameters than the quantification of particle mass emissions. Dilution and sampling methods are key variables that must be taken into consideration to ensure accurate and repeatable results. On the other hand, particle-sizing instruments exist that have significantly better sensitivities than the gravimetric measurement: these present an attractive alternative for the PM emission measurement in future engines, provided standardised measuring methods are developed.

A typical size distribution of diesel exhaust particles is shown in Figure 6.3 (note that a logarithmic scale is used for particle aerodynamic diameter). Nearly all diesel particles have sizes of significantly less than 1μm. As such, they represent a mixture of fine, ultrafine, and nanoparticles. Due to the current PM sampling techniques (diluted exhaust, temperature <52°C), diesel particulate matter includes both solids (such as elemental carbon and ash) and liquids (such as condensed hydrocarbons, water, and sulfuric acid). Formation of particles starts with nucleation, which is followed by subsequent agglomeration of the nuclei particles. The nucleation occurs in both the engine cylinder (carbon, ash) and the dilution tunnel (hydrocarbons, sulfuric acid, water), through homogeneous and heterogeneous nucleation mechanisms.

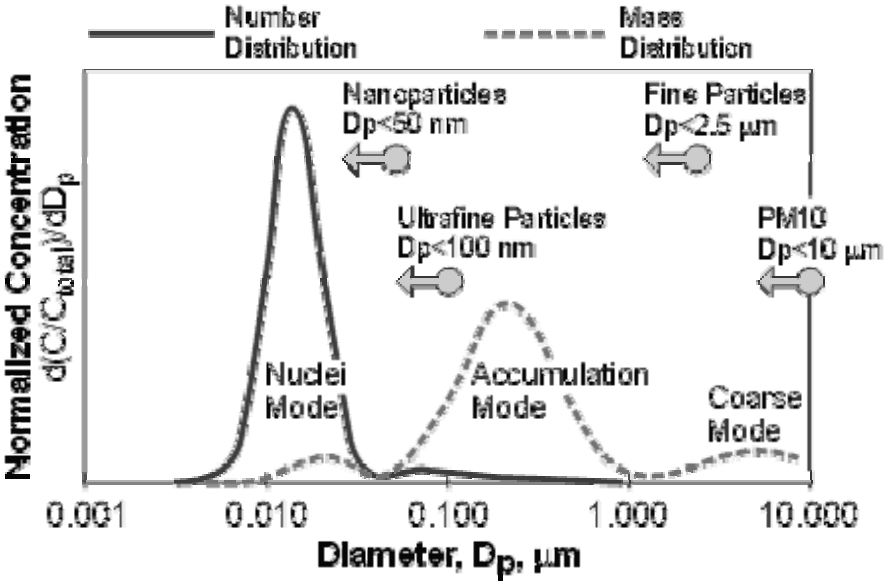

Figure 6.3 Diesel Particle Size Distribution (Kittelson, 2002)

Size distributions of diesel particles have a well-established bimodal character, which corresponds to the particle nucleation and agglomeration mechanisms, with the corresponding particle types referred to as the *nucleaction mode* and the *accumulation mode*. Size distributions are usually presented using either particle mass or particle number weighting. In each representation normal-logarithmic distribution curves are produced, as shown in Figure 6.3. Both the maximum particle concentration and the position of the nucleaction and accumulation mode peaks, however, depend on which representation is chosen. In mass distributions, the majority of the particles (i.e., the particle mass) are found in the accumulation mode. In number distributions, on the other hand, most particles are found in the nucleaction mode. In other words, diesel particulate matter is composed of numerous small particles holding very little mass, mixed with relatively few larger particles, which contain most of the total mass. A

small fraction of diesel particles resides in a third, coarse mode (Figure 6.3). These three particle modes can be characterised as follows:

Nucleaction mode: The diameter of the original nucleus, such as formed during sulfuric acid nucleation, is about 1 nm (Abdul-Khalek, 1999). Today's measuring techniques are capable of detecting a minimum particle size of approximately 3 nm. According to various definitions, the diameters of nucleaction mode particles were generally less than 40-50 nm (0.04-0.05 μm). Based on particle size research in the 1990's technology heavy-duty diesel engines, it has been postulated that the nucleaction mode extends through sizes from 3 to 30 nm (0.003-0.03 μm) (Kittelson, 2002; Hall, 2001). All of the above size ranges place nucleaction mode particles entirely within the nanoparticle range. The maximum concentration of nucleaction mode particles occurs at 10-20 nm. The nucleaction mode, depending on the engine technology and particle sampling technique, typically contains only 0.1-10% of the total PM mass; however it often includes more than 90% of the total particle count. Sometimes the nucleaction mode particles present as much as 99% of the total particle number. Nucleaction mode particles are composed mostly of volatile condensates (hydrocarbons, sulfuric acid) and contain little solid material.

Accumulation mode: The accumulation mode is made of submicrometre particles of diameters ranging most often from 30 to 500 nm (0.03-0.5 μm), with a maximum concentration between some 100-200 nm (0.1-0.2 μm). As shown in Figure 6.3, the accumulation mode extends from the upper end of the nanoparticle range through to the ultrafine and fine particle range. Accumulation mode particles are made of solids (carbon, metallic ash) intermixed with condensates and adsorbed material (heavy hydrocarbons, sulfur species).

Coarse mode: These particles with aerodynamic diameters above 1 μm (1000 nm) contain 5-20% of the total PM mass and practically no contribution to particle numbers (Kittelson, 2002). The coarse particles are not generated in the diesel combustion process. Rather, they are formed through deposition and subsequent re-entrainment of particle material from walls of the engine cylinder, exhaust system, or the particle sampling system.

6.2.2 Current Theories on Nanoparticles: Composition and Formation

Generally, diesel nanoparticles include the same constituents that are found in the total particulate matter emissions including elemental carbon, ash, hydrocarbons, sulfuric acid, and water. Contrary to the total PM emission, diesel nanoparticles cannot be chemically analysed to precisely determine their composition. There is no sampling/analytical procedure that would allow for separation of a sufficient mass of the nanoparticle material for such an analysis. As a result, the exact formulation of diesel nanoparticles has to be studied through indirect experiments. A consensus has been forming that diesel nanoparticles are mostly volatile. In most cases, they are composed primarily of hydrocarbon and sulfuric acid condensates with small contribution of solid material, such as ash and carbon.

The nanoparticle composition can be dramatically changed by exhaust emission control measures, such as diesel particle filters or fuel additives. Very high numbers of nanoparticles were measured downstream of particle filters in the VERT study

(Verminderung der Emissionen von Realmaschinen im Tunnelbau), a study set up by the Swiss EPA to evaluate the possibility of curtailing emissions with after-treatment of exhaust-gases from existing engines (Mayer, 1997). A special sampling technique was developed to distinguish between solid particles and volatile condensates. The diluted sample passed through a heated vapour trap (thermodenuder), filled with active carbon, before entering the particle size analyser. Depending on the vapour trap temperature, different fractions of particle condensates were driven off the sample. An example of such condensate analysis is presented in Figure 6.4. The data were generated on a 100 kW DI diesel engine equipped with a particle filter at full load with low sulfur fuel (< 0.04% S). The exhaust gas temperature in the particle trap was 460°C. The temperature in the active carbon vapour trap was changed between 120 and 250°C, as shown in the graph.

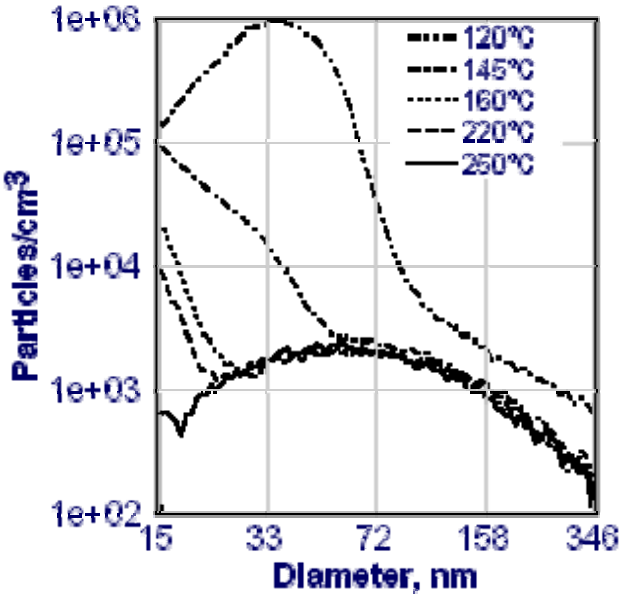

Figure 6.4 Solid Particles and Condensates in Diluted Diesel Exhaust

The high peak of 30 - 40 nm particles disappears from the graph when the sample is heated to 145°C. It is an indication that these particles included volatile materials, presumably hydrocarbons and hydrated sulfuric acid. As the temperature was increased to 250°C, the high nanoparticle emissions disappeared almost completely (note the logarithmic scale in the graph). It can be concluded that high nanoparticle emissions observed downstream of particle filters were composed primarily of condensates (strictly speaking, this conclusion was valid only within the measuring system detection limit of 15 nm, below which solid particles still could be found).

In the same study, particle distributions with iron-based fuel additive were analysed from an engine without a particle filter. Distributions were found with two particle concentration peaks: (1) the additive ash particle peak at about 20 nm and (2) the carbon particle peak at about 90 - 100 nm. As shown in Figure 6.5, the additive caused substantial increase of nanoparticle emissions. Analysis with the heated vapour trap did not significantly change these results, confirming the mostly solid character of nanoparticles in that case. These results indicate that metallic fuel additives, if used, may be a significant contribution to solid ash nanoparticle emissions.

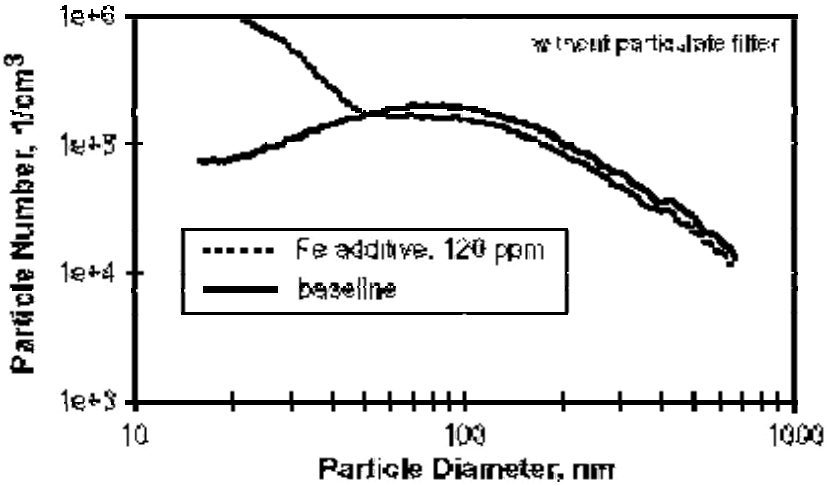

Figure 6.5 Nanoparticle Emissions with Fuel Additive

Nanoparticle volatility experiments were also conducted in the CRC E-34 study (Kittelson, 2002). The set-up involved a Cummins ISM engine and a nano-tandem differential mobility analyser (nano TDMA). The instrument consisted of two differential mobility analysers (DMA) with a heating section between the two DMAs. It allowed a single particle size to be selected with the first DMA, which was then heated, and the resulting size change determined with the second DMA.

Several particle sizes representing the bottom size end of the accumulation mode (50 nm) and the nucleaction mode (down to 7 nm) were subjected to the experiment. It was expected that in this size range, representing the overlap between accumulation and nucleaction modes, a mix of solid and liquid particles would be found. Upon heating, solid particles consisting primarily of carbon and volatile particles consisting primarily of hydrocarbons would be differentiated. Volatile particles would shrink until they would totally disappeared. Solid particles, on the other hand, would shrink only a little as any volatile material on their surface would be removed and then their sizes would stabilize. The results of the experiment, for initial sizes of 50, 30, 12 and 7 nm, are shown as a function of temperature in Figure 6.6. The 50 nm particles split into a mainly solid mode that shrinks to a diameter of 43 nm, and a volatile mode that rapidly shrinks to 15 nm. The 30 nm and 12 nm particles show a similar behaviour, being differentiated into solid and volatile fractions. However, the 7 nm particles continued shrinking upon heating, not showing evidence of containing any solid material. (Volatile particles do not disappear due to a possible instrument artefact around its detection limit.)

The volume fraction that remained in the 12-30 nm particles after heating was also determined in the study. It was found that the volatile fraction amounted to 97-98% of the particle volume, leaving only 2-3% for possible non-volatile, solid cores in the tested particles. Similar heating experiments, as well as evaporation calculations, were conducted for particles made of pure alkanes of different carbon chain length. It was determined that the shrinkage of particles from diesel engines was in the same range as for C28-36 normal alkanes, thus corresponding to the hydrocarbons in the engine lube oil. These results suggest that hydrocarbons that make up diesel nanoparticles are derived mostly of the engine lube oil. It should be mentioned, however, that some fuel effect studies found increased nanoparticle numbers with more volatile fuels, indicating that fuel derived HCs may also present a noticeable contribution (Wedekind, 2000).

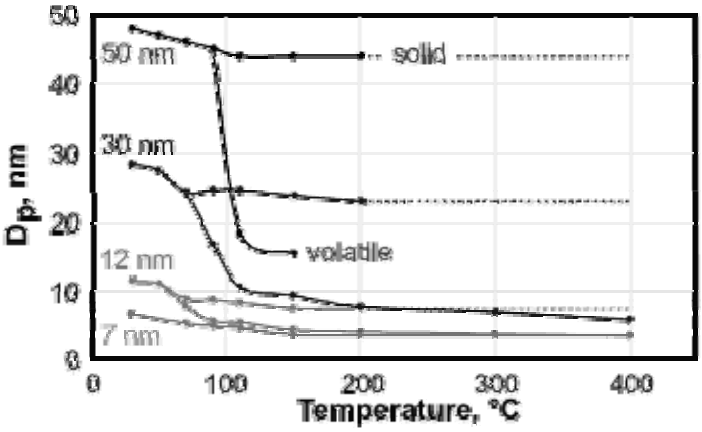

Figure 6.6. Volatility of Diesel Nanoparticles

Various hypotheses have been formulated to explain the increased particle numbers that were seen in certain studies with new diesel engines. It was once suspected that the high particle numbers were related to high injection pressures, which are used by engine manufacturers as a strategy for meeting emission targets. That theory, however, has not been confirmed. On the contrary, a continuous decrease in mass and number emissions was shown when injection pressure increased from 400 to 1000 bar. In another study, the injection pressure had only a small influence, and only under some engine operating conditions, on the particle size distribution (Pagan, 1999).

It is believed that increased number emissions are a simple consequence of:

(1) high concentration of nanoparticle precursors (HC, SO_4), combined with
(2) decreased mass of accumulation mode particles.

Normally, the accumulation particles act as a "sponge" for the condensation and/or adsorption of volatile materials. In the absence of that sponge, gas species that are to become liquid (or solid) will nucleate to form large numbers of small particles. The driving force for the gas to particle conversion is the saturation ratio, defined as the ratio of the partial pressure of a species to its saturated vapour pressure. For the constituents of the SOF or sulfuric acid, the maximum saturation ratios occur during dilution and cooling of the exhaust and are typically achieved at dilution ratios between 5 and 30 (Abdul-Khalek, 1998).

The above nucleation theory explains the high number emissions seen downstream of particle traps (which remove solid accumulation mode particles, but not necessarily nanoparticle precursors), as well as high number emissions from engines of high SOF fraction, such as the '91 Cummins engine in the HEI study (Bagley, 1996). It is also consistent with the observation of high particle numbers from gasoline engines, which are believed to be composed primarily of liquid condensates (Graskow, 1998). In all of these cases, the low quantities of agglomerated particles present in the gas cannot provide enough surface area for the condensation/adsorption of volatile material. As the species approach saturation, high numbers of small particles are produced through nucleation.

A similar nucleation mechanism may apply to the formation of ash particles. In that case, the ash nucleation takes place inside the engine during the expansion stroke rather

than in the dilution tunnel. Once these ash nuclei are formed, they may serve as heterogeneous nucleation sites for SOF and other species during dilution and cooling in the exhaust. It was suggested that some particles in the high SOF nucleaction mode might include ash cores (Abdul-Khalek, 1998).

6.3 AFTERTREATMENT TECHNOLOGIES

The two main particulate matter control technologies are Diesel Oxidation Catalyst and Diesel Particle Trap. Although there are a variety of other techniques that are used in reducing PM emissions, such as SCR (selective catalytic reduction with ammonia/urea) catalyst systems, chemical aftertreatment (with cyanuric acid), Plasma Exhaust Treatment, the majority of them are still under development and not in extensive use.

6.3.1 Diesel Oxidation Catalyst

In general, the overall effect of the DOC on the total PM emission could be a decrease as well as an increase. The total diesel particulate matter (TPM) emission is composed of three major fractions including the carbonaceous particles, the organic particles (SOF), and sulfates (SO_4). Each of these fractions behaves differently over the diesel oxidation catalyst. Oxidation catalysts reduce the SOF fraction and have little effect on the carbonaceous portion of PM in diesel exhaust. This limits the reduction in PM emissions that an oxidation catalyst can achieve. Typical transformations of the three fractions and the resulting total PM emissions are schematically illustrated in Figure 6.7. As apparent from the graph, PM emissions can be reduced in the DOC through the removal of their organic fraction (SOF). The maximum total particle matter reduction is dependent on the magnitude of the SOF (compared to the carbonaceous portion) in the engine-out exhaust, and is usually between 20 and 30% (Harayama 1992). The sulfate fraction of diesel particles (SO_4) is increased in the DOC due to the oxidation of SO_2 with subsequent formation of sulfuric acid. Under certain conditions, however, the SOF decrease can be more than off-set by an increase of sulfate PM, leading to an overall increase in TPM emission (if high sulfur fuels are used, sulfate particle emissions may be much higher than shown in Figure 6.7). Therefore low sulfur fuels and special catalyst formulations are required to limit the catalytic generation of sulfate particles from sulfur dioxide present in the exhaust gas.

Reports on changes in PM carbon fraction over DOCs (Lylykangas 2002, Brueck 2001) must be always approached with careful scrutiny. The magnitude of change in many of such reports is on the threshold of detection. Measurement error can be magnified due to the analytical methods that determine insolubles only indirectly, by subtracting sulfate and SOF from total PM. If sulfuric acid reacts with metals, such as with calcium from lube oil additives, the resulting sulfate salts may be accounted for as insoluble material.

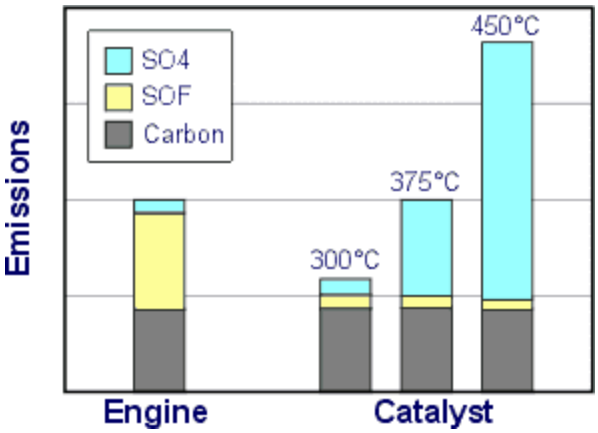

Figure 6.7 Impact of Diesel Oxidation Catalyst on PM Emission

Impact on Particle Number Emission

Contradictory published reports exist on the effect of diesel oxidation catalysts on particle number emissions. Since diesel particle number emissions can be attributed primarily to nucleaction mode particles which are composed mostly of hydrocarbon and sulfuric acid condensates, one can easily explain the performance of the DOC by analysing its effect on nucleaction mode particle precursors. If the catalyst removes hydrocarbons (gas phase and SOF), it prevents their subsequent nucleation, thus reducing the particle number emission. If, however, the catalyst produces sulfates, an effect more prominent with high sulfur fuels and more active, noble metal catalysts, the particle numbers may be increased due to sulfuric acid nucleation.

Experiments which attempt to quantify the impact of DOCs on particle numbers must be very carefully designed. Catalysts can be a source of additional error, such as sample loss due to thermophoretic forces or sample additions due to solid particle blow-off and/or release of condensates from the washcoat.

6.3.2 Diesel Particle Trap

Diesel particle traps are devices that physically capture diesel particles to prevent their release to the atmosphere. Some of the diesel filter materials which have been developed show quite impressive filtration efficiencies, frequently in excess of 90%, as well as acceptable mechanical and thermal durability. In fact, diesel traps are the most effective control technology for the reduction of particle emissions with high efficiencies. More precisely, due to the particle deposition mechanisms utilised in these devices, traps are effective in controlling the solid fraction of diesel particles, including elemental carbon (soot) and the related black smoke emission. It must be remembered that traps may have limited effectiveness, or be completely ineffective, in controlling the non-solid fractions of PM, such as the SOF or sulfate particles. For this reason, trap systems designed to control the total PM emission are likely to incorporate additional functional components targeting the SOF emission (e.g., oxidation catalysts) and sulfate particles (e.g., ultra low sulfur fuels).

Currently available filters for diesel engines are either ceramic wall-flow monolith filters or filter tubes covered with multiple layers of a yarn-like ceramic fibre material.

The filter material contains many pores, or small holes, that allow the exhaust gases to pass through while collecting the particles from the raw exhaust.

The particulate matter that is collected by the filter eventually needs to be removed. This process is called regeneration. Two general approaches for regeneration of the trap have been investigated. One approach employed, called a passive system, is the use of catalytic material on the filter, which causes regeneration, in a continuous or periodic manner, during the regular operation of the system. The other approach, known as an active system, includes an electric heater or fuel burner to periodically raise the filter temperature, oxidise the particle and regenerate the trap, as dictated by an electronic control unit.

Diesel traps are very effective in reducing PM emissions. Their drawbacks are durability/reliability problems and a decrease in fuel economy due to high exhaust gas pressure drop and, in the case of active systems, due to the operation of the heater or burner.

All diesel particle traps of practical importance are diesel particle filters (DPF). Even though the term “trap” covers a wider range of devices, it is often used as a synonym for “diesel filter”. Such use of the term “diesel trap” in reference to filter devices was more common in older literature. In recent years, there is a trend to replace it with the more precise term “diesel particle filter”.

The impact of particle filters on PM composition is illustrated in Figure 6.8 (Andersson and Wedekind, 2001). The filter, a two-stage system incorporating an oxidation catalyst and a wall-flow DPF, was installed on an Euro 1 engine and tested on the R-49 schedule, which is dominated by the full speed - full load mode, resulting in high average exhaust gas temperature. It is clear from the graph that the DPF is extremely effective in removing elemental carbon particles (black, bottom portion of each bar). SOF fractions from fuel and oil are somewhat reduced by the catalyst portion of the filter system. Despite the use of ultra low sulfur fuels, sulfate particles are increased (especially so in the test with UKULSD fuel of higher sulfur content). The increase in the sulfate particles could be due to the catalyst portion of the filter system, which is known to produce sulfates by oxidation of SO_2 with subsequent formation of sulfuric acid. This effect is more prominent if a fuel has higher sulfur content, such as the UKULSD, which has higher sulfur content than the SWCL1.

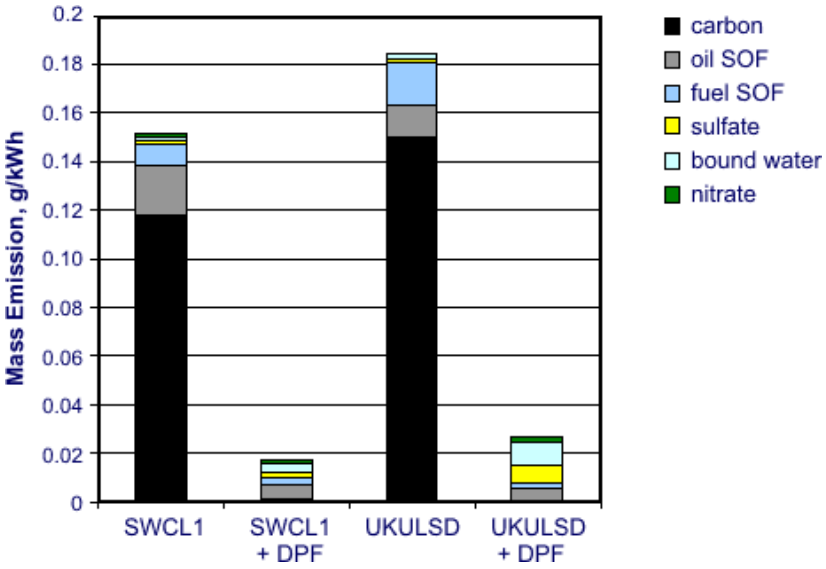

Figure 6.8 Effect of Particle Filter on PM Composition Euro 1 engine on R-49 cycle; SWCL1 (Swedish Class 1) fuel <10 ppm S; UKULSD (UK ultra low sulfur diesel) fuel - 50 ppm S

Impact on Particle Number Emission

While most PM mass emissions are composed of solid matter (or solid particles with adsorbed vapours), liquid material constitutes a very significant part of diesel nanoparticles which are the main contributor to particle number emissions. If the liquid material, including sulfates and SOF, is formed in the PM sampling system, i.e., downstream of the filter, the DPF will be ineffective in reducing nanoparticle and particle number emissions. Even worse, by retaining carbon particles, the DPF removes the material, which otherwise acts as a "sponge" for condensates formed in the sampling system. Therefore, particle filters tend to increase the formation of nanoparticles through nucleation. In effect, DPFs reduce the numbers of mostly solid agglomeration mode particles, replacing them by mostly liquid nucleaction mode nanoparticles, as shown in Figure 6.9 (Burtscher, 2001).

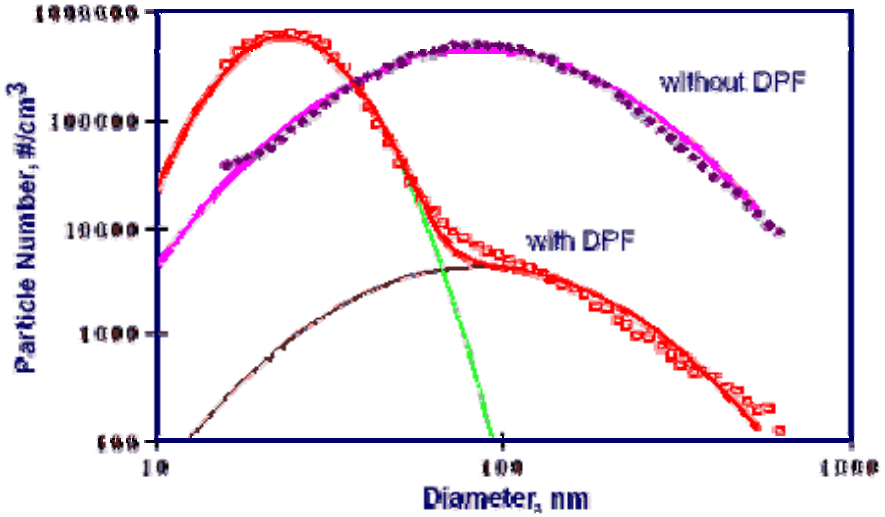

Figure 6.9 Typical Particle Size Distributions with and without DPF

As a result, several studies measured increased particle numbers with particle filters, as illustrated in Figure 6.10 (Andersson, 2001). The data were generated on the hot ECE R-49 test cycle and with a catalytic filter system. High particle number emissions are clearly related to sulfates, which are generated at high exhaust temperatures in the catalyst, as indicated by the higher particle numbers in tests with higher sulfur content in the fuel.

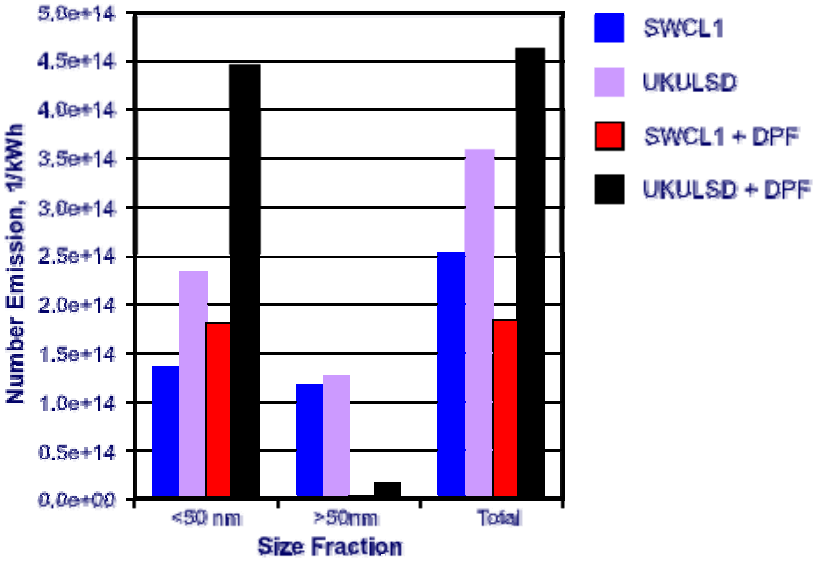

Figure 6.10 Effect of Particle Filter on Particle Number Emissions. Euro 1 engine on R-49 cycle; SWCL1 fuel <10 ppm S; UKULSD fuel - 50 ppm S

In the general case, however, the impact of DPFs on particle number emissions has to be described as inconclusive; the particle numbers may be either increased or decreased. The main parameters influencing the DPF performance or the performance assessment can be listed as follows:

- *Presence of nanoparticle precursors*. This includes SO_3/sulfuric acid which may be catalytically generated from sulfur precursors in the fuel. Hence, lower fuel sulfur content will result in less nanoparticle emission. Hydrocarbons are another nanoparticle precursor. If HCs are removed from the system, e.g., by using a catalyst, lower nanoparticle emissions may be expected. Such phenomena as adsorption/desorption of HCs on accumulated soot in the exhaust system may also contribute to the overall particle number emission.
- *Exhaust gas temperature*. Sulfate particles are formed at high temperature conditions, such as at full engine load. Therefore, in catalytic systems, higher particle numbers will be measured over hot engine test cycles.
- *Particle sampling parameters.* In the absence of standard measuring methods of particle number emissions, laboratories are free to choose any measuring set-up and parameters. As discussed previously, the choice of parameters, such as dilution ratios or dilution tunnel residence times, can critically influence the measurement. Measurements are particularly unstable and irreproducible in systems with high rates of particle nucleation and condensation (as opposed to testing configurations that attempt to measure exclusively solid nanoparticles).

The above discussion was based on an assumption that liquid condensates are counted as particles. As standards are being developed to quantify diesel particle number emissions, a controversy exists as to the inclusion of liquid condensates in particle number measurements (Burtscher 2001). Several laboratories use sample-conditioning techniques that "dry" the gas sample by removing volatile nanoparticle precursors before particle number measurements are taken. If particle filters are evaluated using this technique, they show consistently good particle number reduction performance, as shown in Figure 6.11 (Mayer 2000). This is an indication that solid nanoparticles are retained with high efficiency in a variety of DPF media.

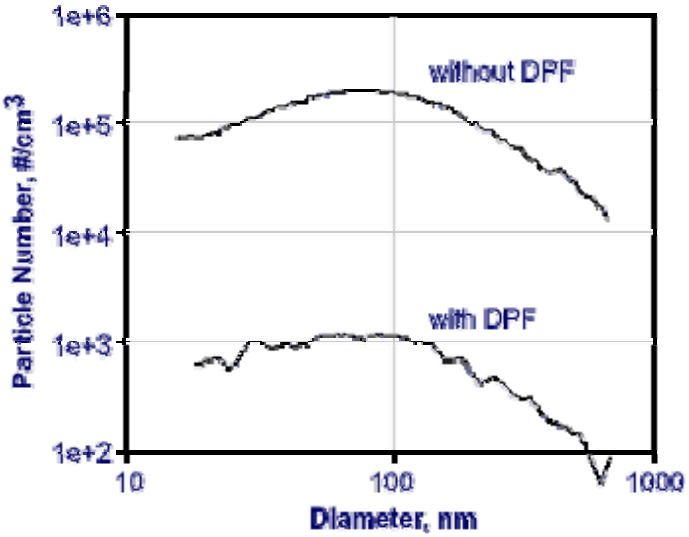

Figure 6.11 Particle Size Distribution with and without DPF - Solid Particles. Ceramic monolith filter on Caterpillar construction engine

6.4 DIESEL FUELS

Diesel fuels are mixtures of hydrocarbons with boiling points in the range of 150 to 380°C. They are obtained from the distillation of crude oil. Refineries are increasingly blending conversion products (cracked components) with the primary distillation streams in order to meet the appropriate product specifications as well as to meet the market demands for different proportions of gasoline, diesel fuel, and other petroleum products.

6.4.1 Diesel Fuel Properties

The requirements placed on diesel fuels are specified in standards, such as the ASTM D975 in the US and EN 590 in the European Union. The most important parameters specified by the standards include: Cetane number, Viscosity, Cold behaviour, Flash point, Volatility, Lubricity, Sulfur, and Additives. As this review will concentrate only on the effects of sulfur we shall not discuss other fuel properties.

Sulfur in diesel fuel and its impacts

Diesel fuels contain chemically bound sulfur. The amount of sulfur depends on the crude oil quality and the components used in blending the fuel. In particular, some crack components have high sulfur content. Refineries can reduce the sulfur content of diesel fuel by treatment with hydrogen. Sulfur increases the lubricating properties of diesel fuels. Therefore, fuels of low sulfur levels typically require lubrication additives to avoid potential damage to fuel injection equipment (Batt, 1996).

There are a number of negative effects of sulfur in diesel fuel, which can be categorised as follows (Ruzicka, 1999):

Emissions

- Sulfur dioxide emissions — most of the sulfur is converted in the engine into sulfur dioxide, a substance of a negative environmental impact.
- Sulfate particle emissions — a fraction of the sulfur is oxidised to sulfur trioxide. The SO_3 binds with water, forms sulfuric acid, and contribute to the total PM emission.

Corrosion and wear

- Corrosion of exhaust system components by sulfur condensates — especially troublesome in exhaust gas recirculation coolers (McKinley, 1997; Kreso, 1998).
- Increasing wear of engine parts through the corrosion of internally formed acid.

Exhaust aftertreatment

- The SO_2/SO_3 shift is increased very significantly if oxidation catalysts are used in the emission control system, resulting in increased PM emissions.
- Sulfate particles are also generated in catalytic particle filters, both the CRT and catalysed traps. Under certain conditions, the benefit of reducing the carbon fraction of diesel PM can be more than offset by the generated sulfates.
- Deactivation of NO_x adsorbers by sulfur is one of the most important obstacles in implementing this technology.
- Many catalysts are poisoned by sulfur (reversibly or otherwise). There may be interferences with future emission control systems that are currently unknown.

In order to minimise these adverse effects, there are strong pressures to reduce the level of sulfur in diesel fuels worldwide. Until early 1990's, the sulfur level in diesel fuel was not subject to environmental regulations. The maximum sulfur level in good quality fuels, as listed by fuel specifications, was at about 0.5% (5000 ppm = 5000 mg/kg). In the 1990's, environmental regulations limited the maximum sulfur level to about 500 ppm; this type of diesel fuel was typically referred to as "low sulfur diesel". Further pressures from the increasingly stringent diesel emission standards, such as the Euro 4 and US2007, will require the use of aftertreatment devices such as DPF. In order to implement these technologies the maximum sulfur levels have to be limited to 50 or 10 ppm. Diesel fuels of typically 15 ppm S (but not more than 50 ppm) are commonly referred to as "ultra low sulfur diesel" (ULSD). In Europe, diesel (and gasoline) fuels of maximum sulfur content of 10 ppm are termed "sulfur-free" fuels.

More recently, fuel quality has been further influenced by national and local regulations aimed at reducing emissions. Many countries introduce tax incentives for cleaner burning, better quality fuels (primarily lower sulfur, but also lower density, aromatics,

higher cetane) to offset their higher production costs. Examples of fuel specifications and tax incentives in the 1990's are listed in Table 6.1 (Lee, 1998).

Table 6.1 Diesel Fuel Specifications

	Sulfur	Cetane No.	Total Aromatics	Density	T90/95	Tax Incentive
	max ppm	Min	max % vol.	g/cm3	max °C	$/ton
US No.2 (ASTM D975)	500	40	-	-	338	-
CARB[a]	500	40	10	-	338	-
EU 1996 (EN 590)	500	49	-	0.82-0.86	370	-
Japan No.2	500	45[b]	-	-	350	-
Japan No.3	500	45[b]	-	-	330	-
Sweden Class I	10	50[b]	5	0.80-0.82	285	97[c]
Sweden Class II	50	47[b]	20	0.80-0.82	295	54[c]
Finland Sw II	50	47[b]	20	0.80-0.82	295	34
Denmark Sw I	10	50/47[b]	5	0.80-0.82	285	85
Denmark City Bus	500	50	-	0.82-0.855	325	50
UK City Diesel	10	49[b]	-	0.80-0.83	-	37.5

a - or fuel must show emissions equivalent or better compared to CARB reference fuel of 500 ppm S, 48 cetane number, 10% aromatics, 1.4% polyaromatics, 0.83-0.86 g/cm3 density, max T90 of 321°C.
b - cetane index
c - initial tax incentive at introduction (1991), current tax incentive lower (e.g. $76/ton Swedish Class I in 1998)

Sulfur content in Australian diesel fuels

The level of sulfur both in diesel and petrol fuels is regulated by the Fuel Quality Standards Act 2000. The Act provides a legislative framework for setting national fuel standards for Australia. The main object of the Act is to regulate the quality of fuel supplied in Australia in order to:

(a) reduce the level of pollutants and emissions arising from the use of fuel that may cause environmental and health problems;
(b) facilitate the adoption of better engine technology and emission control technology; and
(c) allow the more effective operation of engines.

More details of the Act can be found on the following website:
http://www.ea.gov.au/atmosphere/transport/fuel/index.html

The first standards made under the Fuel Quality Standards Act 2000 were environmental standards for petrol and diesel fuels. The first suite of national fuel

standards, which came into force on 1 January 2002, regulates petrol and diesel parameters that have a direct impact on the environment ('environmental' standards). A second suite of national fuel standards came into force on 16 October 2002. These standards address those parameters of petrol and diesel that do not have a direct impact on emissions but, if not controlled, can have adverse impacts on the efficient operation of the engine ('operability' standards). Both environmental and operability standards are summarised in Table 6.2. More details including the Petrol Standards can be found on the Australian Department of Environment and Heritage web site at:
http://www.ea.gov.au/atmosphere/transport/fuel/standardstable.html

The level of sulfur in Australian diesel fuel was set to 500ppm effective from 31st December 2002. Future standards to be implemented from 1st January 2006 will further reduce the fuels sulfur level below 50 ppm.

Table 6.2 Diesel Standard

Parameter	**Proposed standard**	**Date of effect**
Sulfur	500 ppm 50 ppm	31 Dec 2002 1 Jan 2006
Cetane Index	46 (min) index	1 Jan 2002
Density	820 (min) to 860 (max) kg/m^3 820 (min) to 850 (max) kg/m^3	1 Jan 2002 1 Jan 2006
Distillation T95	370°C (max) 360°C (max)	1 Jan 2002 1 Jan 2006
Polyaromatic hydrocarbons (PAHs)	11% m/m (max)	1 Jan 2006
Ash and suspended solids	100 ppm (max)	1 Jan 2002
Viscosity	2.0 to 4.5 cSt @ 40°C	1 Jan 2002
Carbon Residue (10% distillation residue)	0.2 mass % max	16 Oct 2002
Water and sediment	0.05 vol % max	16 Oct 2002
Conductivity @ambient temp	50 pS/m (Min) @ambient temp (all diesel held by a terminal or refinery for sale or distribution)	16 Oct 2002
Oxidation Stability	25 mg/L max	16 Oct 2002
Colour	2 max	16 Oct 2002
Copper Corrosion (3 hrs @50°C)	Class 1 max	16 Oct 2002
Flash point	61.5°C min	16 Oct 2002
Filter blocking tendency	2.0 max	16 Oct 2002
Lubricity	0.460 mm (max) (all diesel containing less than 500ppm sulfur)	16 Oct 2002

6.5 INFLUENCE OF THE FUEL SULFUR LEVEL ON DIESEL EMISSIONS

6.5.1 Regulated Emissions

During combustion, the organic sulfur compounds in diesel fuel are first degraded to molecules or radicals such as H_2S, HS, S, or S_2 and then oxidised via SO radical to SO_2. The reactions are very fast, resulting in practically complete conversion of sulfur to SO_2.

Other than the changes in the levels of exhaust SO_2, changing fuel sulfur content does not cause any observable effects on gaseous engine-out emissions. The emissions of HC, CO, and NO_x are practically independent from the fuel sulfur level. This can be illustrated by the data shown in Figure 6.12, where emissions of CO were constant at all sulfur levels, HC increased slightly (3-8%) with increased sulfur level, and NO_x slightly decreased (DECSE, 2000). Average fuel consumption (BSFC) increased with increased sulfur, but the data showed considerable scatter. Similar results were obtained in a local study conducted by QUT in collaboration with BP and BCC (Ristovski, 2002).

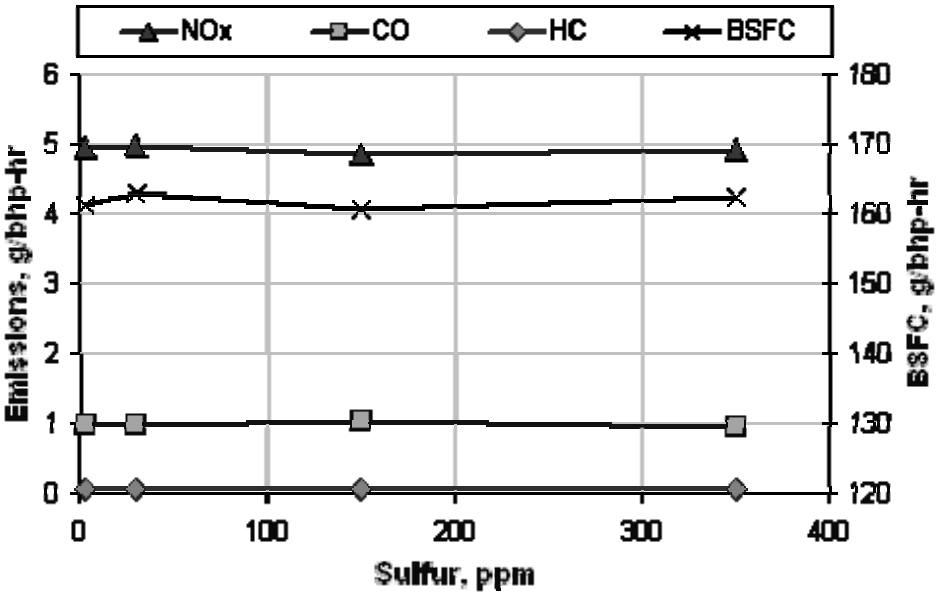

Figure 6.12 Fuel Sulfur Effects on Gaseous Emissions and BSFC

Direct impact of fuel sulfur on emissions is limited to emissions of particulate matter. A fraction of the fuel sulfur is converted to sulfur trioxide and sulfuric acid that, due to the current definition of diesel particulate matter by regulatory authorities, is accounted for as PM emission. That emission is known as “sulfate particles”. Studies have shown that the amount of sulfur converted to sulfate is usually about 2% of the fuel sulfur content (Cowley, 1993), and rarely exceeds 4%. This conversion rate depends only slightly on the engine technology. It should be emphasised that the impact of sulfur is limited to sulfur particles. There is no evidence that reduced sulfur levels have any influence on the carbonaceous portion of PM and on the black smoke.

Since sulfates are just one of several components of the DPM emissions, lowering fuel sulfur levels has only limited potential as a means of PM control. The reduction of diesel fuel sulfur levels from 0.30% to 0.05%, as legislated in the U.S. in 1994, yielded

relatively large benefits of about 0.04-0.08 g/bhp-hr PM reduction. However, further reductions of fuel sulfur from the current 0.05% to lower levels has only a small incremental PM reduction benefit of about 0.008-0.016 g/bhp-hr.

6.5.2 Nanoparticle Emissions

Of the150 publications, which refer to the influence of fuel sulfur level on nanoparticle emissions, only a few are directly focused on this topic. Most of the studies concentrate on particle mass and not number emissions. Overall, these studies have limitations, which makes it difficult to provide robust conclusions on the influence of sulfur levels on nanoparticle emissions. Most studies do not utilise a large sample size of vehicles and usually investigate only one or a few engine types. Furthermore, some of the studies were conducted on engine dynamometers while others investigated vehicles on chassis dynamometers. This added additional uncertainties when comparing different studies. It should also be noted that there is still not a clear consensus in the scientific community on the procedures for particle number size distribution measurement during vehicle emission testing. Different studies have used different sampling methods, which are often not described in full detail. Since sampling method itself affects particle formation processes, a meaningful comparison of the results of different studies is not always possible.

One of the largest studies conducted so far (final report submitted in June 2001) has been the Diesel Emission Control – Sulfur Effects (DECSE) program (DECSE, 2001). This program was a joint effort of the U.S. Department of Energy (DOE), two national laboratories, manufacturers of heavy-duty compression ignition (CI) engines, and manufacturers of emission control systems. The objective of DECSE was to conduct tests to determine the effects of various levels of sulfur in fuel on the emission exhaust control systems that could be used to lower nitrogen oxides and particulate matter from diesel vehicles in the years 2002 to 2004. As the sulfur content in diesel fuel is known to adversely affect the operation of diesel exhaust emission control systems, DECSE had also documented the need for low-sulfur fuel. The tests were conducted on a Caterpillar 3126 engine, Cummins ISM370 and Navistar T444E with nominal fuel sulfur levels of 3 ppm, 30 ppm, 150 ppm and 350 ppm. Four emission control technologies were tested:

1. NO_x adsorbers,
2. diesel particle filters (DPF)
3. lean NO_x catalyst, and
4. diesel oxidation catalyst.

Tests were conducted for NO_x concentrations, hydrocarbons, carbon monoxide, and particle mass. Although no work has been done on particle number emissions, the authors recommended that future work should be conducted on investigation of the influence of fuel sulfur level on particle number emissions with various emission control technologies.

Historically, the first and largest program was conducted by the Health Effects Institute (HEI) (Bagley, 1996). They analysed the influence of fuel sulfur content on the emissions from two heavy-duty diesel engines with two types of fuel with sulfur level of 0.32% High Sulfur (CS), and 0.01% (100ppm) or Low Sulfur (LS). The two analysed engines were a 1988 LTA 10-300 (L10) equipped with a ceramic particle trap

and a 1991 LTA 10-310 equipped with an OCC. Cummins Engine Co. manufactured both engines. The tests were conducted on an engine dynamometer. The engines were analysed for modes 9, 10 and 11 corresponding to 25%, 50% and 75% load at rated speed. The measurements were conduct without the aftertreatment devices, in baseline mode, and with the aftertreatment devices, trap mode. Although the investigators observed a reduction in SO_4 below the detection limits, a significant difference in TPM levels between the CS and LS fuels was found only at mode 9 base-line. For mode 11 and 9 trap, there was little difference in TPM levels even when the SO_4 component decreased.

They have conducted a limited number of measurements of particle size distributions. Their main finding was a significant reduction of the number of smaller (nuclei-mode) particles when the sulfur levels were reduced from 3200ppm to 100ppm. The study recommended further investigation of the influence of the fuel sulfur level and aftertreatment devices on particle number and size distribution.

Only recently several reports have been published on the influence of fuel specification on particle number, mass and size of emitted particles (Andersson, 2001; Andersson, 2001; Kittelson, 2002; Wedekind, 2000; Ristovski, 2002).

A recent European study conducted within the DETR/CONCAWE/SMMT Particle Research Programme has concentrated on the influence of the sulfur level on nanoparticles emissions (Andersson, 2001; Andersson, 2001). The Anderson and Wedekind (2001) study investigated only 3 heavy duty diesel vehicles (EURO I, EURO II and EURO III) for 3 different sulfur fuel levels: 340-ppm, 53-ppm, and less than 10-ppm. Measurements were conducted for both steady state (R49) and transient cycles (ETC).

For the steady state R49 cycle they found that the changes in accumulation mode particles could be attributed to changes in engine technology. However, the variation in nanoparticles might be influenced by fuel properties. The effects of engine technology proved larger on regulated particle mass emissions than those of fuel specification. With both engines, fuel with highest sulfur content (340 ppm) emitted highest and fuel with lowest sulfur content (<10ppm) lowest weighted cycle nanoparticle emissions. In chemical terms although hydrocarbon and sulfate masses were small, the influences on nanoparticle formation can be significant.

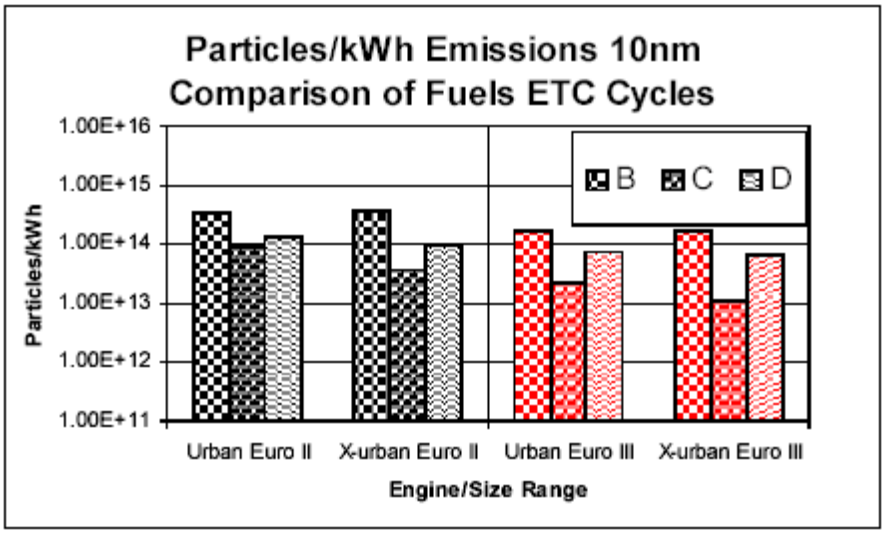

Figure 6.13 Emissions of 10nm Particles During the Urban and Extra-urban Phases of the ETC (Wedekind, 2000)

In Figure 6.13, the emissions of 10nm particles during the Urban and Extra-urban phases of the ETC as a function of fuels sulfur level are presented for EURO II and EURO III type engines. Fuels B, C, and D correspond to 300, <10 and 53 ppm sulfur levels respectively. Although 10nm particles may be more sensitive to transient events with the <10ppm fuel, absolute production is still higher for fuels with higher sulfur level content, e.g., 300 and 53 ppm fuels. It is possible that this effect is related to the differences in nucleation sites provided by fuel specific sulfation. However, as an influence, the sulfur content of the fuel cannot be fully decoupled from other chemical and physical effects within the tested fuels, such as the total aromatic content, which was 21.2, 4.4, and 16.4 % for fuels B, C, and D, respectively.

The light duty part of their program (Andersson, 2001) studied three different vehicles (EURO II and EURO III class equipped with a DPF) over four different sulfur fuel levels (500, 300, 50 and <10 ppm sulfur content) and transient and steady state conditions. They found that the lowest numbers of nucleation mode particles were emitted by the fuel with less than 10ppm sulfur, which resulted in the lowest total number of particles. They concluded that the engine technology effects dominated the accumulation mode (particles larger than 50nm), while the fuel dominated the nucleation mode particles.

Main findings and recommendation from these 2 studies were:

- The changes in accumulation mode particles could be attributed to changes in engine technology. However, the variation in nanoparticles might be influenced by fuel properties.
- The effects of engine technology proved larger on regulated particle mass emissions than those of fuel specification.
- The lowest numbers of nucleation mode particles were emitted by the fuel with less than 10ppm sulfur, which resulted in the lowest total number of particles.
- This study also showed that regulated PM and particle number emissions do not necessary correlate, therefore the focus of the future work should be upon particle number and particle number weighted size distributions.

The recommendations for future work from this study can be summarised:

1. As this program examined only several vehicles from a limited fleet an investigation should be extended to better represent the current and future fleets.
2. New engines and aftertreatment technologies may present new particle production challenges and solutions. These should be investigated.
3. The specific influence of fuel and lubricants should be studied by testing matrices where key parameters of interest, such as sulfur, volatility, and aromatic content are decoupled.
4. The effect of fuel and lubricant sulfur should be studied to determine the influence of this parameter as a source of the condensation sites when nucleation modes form.
5. Moves toward cycles that more closely represent real world driving and measurements methods including dilution parameters should be investigated.
6. Further work is required to develop sampling and measurement standards for particle size and number so that comparable data sets can be produced. Within this new instrumentations should be tested.

A recent study (Bertola, 2001), from collaboration between two Federal Laboratories in Switzerland, analysed the influence of fuel properties and injection parameters on the particle number size distribution. For the fuel composition, five different fuels including low sulfur diesel, zero-sulfur and zero aromatics diesel, two blending portions of oxygenated diesel additive and rapeseed-methylester (biodiesel produced locally in Switzerland) were used. Measurements were carried out on a single-cylinder research engine focusing on exhaust particulate matter emissions. Unfortunately, the levels of sulfur in all tested fuels were not presented and there is a significant difference in the composition of the fuels used, so any changes in emissions could not be attributed only to the sulfur level content. The interesting result from this study is that a nucleation mode is present with all fuels tested when the engine is operated at extremely high injection pressures. At higher injection pressures, a dependency seems to be present between oxygen content in the fuel and formation of nanoparticles. Compared to the reference fuel with 50 ppm sulfur, the blends containing Butylal and the zero aromatics zero sulfur diesel showed lower particle concentrations.

The main findings of this study are:

- A nucleation mode is present with all fuels tested, independent of the sulfur content, when the engine is operated at extremely high injection pressures.
- At higher injection pressures, a dependency seems to be present between oxygen content in the fuel and formation of nanoparticles.
- Compared to the reference fuel with 50 ppm sulfur, the blends containing Butylal and the zero aromatics zero sulfur diesel showed lower particulate concentrations.

Another study concentrating only on a single engine and on an engine dynamometer was conducted by Wei et al (2001). Wei et al (2001), who studied the emissions from a 1995 model medium-range diesel engine operating at 50% load. Two fuel sulfur contents were used: 440 ppm (low sulfur) and 10 ppm (ultra low sulfur). They found that increasing the fuel sulfur content increased the formation of nucleation mode particles, but did not significantly influence the accumulation mode. The 10 ppm sulfur fuel gave smaller concentrations of nucleation mode particles than the 440 ppm sulfur

fuel. The peak particle number concentration in the nucleation mode was much higher with the low sulfur fuel than with the ultra low sulfur fuel. The number of particles in the nucleation mode was also strongly influenced by temperature, with larger concentrations formed at lower dilution temperatures. For both types of fuel, many more nanoparticles were formed during dilution of engine exhaust in the atmosphere at an ambient temperature of 10°C than at 20°C. The total number concentration produced by the two fuels was quite similar at temperatures above 30°C, but, as the temperature was reduced further, the total number produced by the higher sulfur fuel increased much more rapidly. At 15°C, the total number concentration produced by the low sulfur fuel was nearly 7 times higher than that produced by the ultra low sulfur fuel.

The main findings of the study are:

- Increasing the fuel sulfur content increased the formation of nucleation mode particles but did not significantly influence the accumulation mode.
- The number of nanoparticles produced depended strongly on the temperature of the dilution air with the highest number produced for both types of fuel during dilution of engine exhaust in the atmosphere at an ambient temperature of 10°C than at 20°C.

Although the CRC-43 Diesel Aerosol Sampling Methodology project (Kittelson, 2002) did not directly concentrate on the influence of the sulfur level on nanoparticles emissions, the influence of specially formulated fuel and lube oil was studied. Measurements were conducted only on one engine on the engine dynamometer and a CVS facility. Nanoparticle emissions for fuels with 3 different levels of sulfur (1, 49 and 325 ppm) and 2 different lubricating oils (4000 ppm and 385 ppm sulfur) were analysed. They observed that for conventional lube oil (385 ppm) and both 1 ppm and 49 ppm sulfur fuel, there is no significant formation of a nucleaction mode. When present, most of the nucleaction mode was removed when the TD was connected to the SMPS, suggesting that the nucleaction mode is composed of volatile particles. But the most surprising result was the large influence of specially formulated lube oil. Contrary to expectations low sulfur oil led to an increase in nanoparticles formation in nearly all cases. It is possible that the increase in nanoparticles formation by low sulfur oil was related to the formulation of the oil necessary to compensate for the removal of sulfur. It could also be due, in part, to the release of volatile components from the oil, related to the lack of oil break- in. Increasing fuel sulfur also increased nanoparticle emissions, especially at high load. They point out the importance of lube oil on nanoparticles formation a result previously observed by others (Sakurai, 2001).

Recent local studies conducted by Ristovski et al. (Ristovski, 2002; Ristovski, 2002) examined particle emissions from a fleet of twelve in-service buses fuelled by low (500 ppm) and ultra low (50 ppm) sulfur diesel at four driving modes on a chassis dynamometer. The examined busses were between 1 and 12 years old (pre EURO I, EURO I and EURO II). Both size and number as well as particle mass were measured. They found that the particle mass emission rates were not significantly different for the two fuel types. However, the particle number emission rates were 30-60% higher with the LS fuel over the ULS fuel. Most of the excess particles were smaller than 50 nm (nanoparticles) and resided in the nucleation mode.

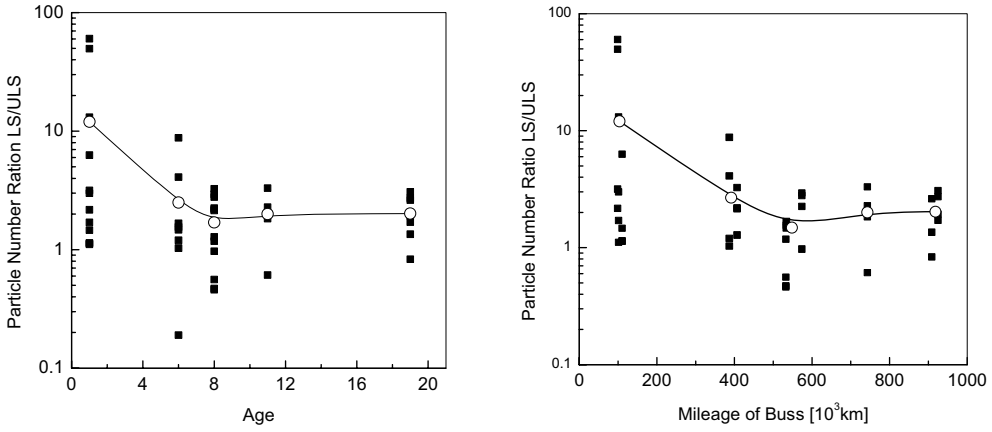

Figure 6.14 Ratios of particle number emission rates with LS and ULS diesel fuel in all four modes as a function of the a) age and b) mileage of the buses (Ristovski, 2002)

The study further investigated whether the age or mileage of a bus had an influence on the particle emissions and how, if at all, they are affected by the sulfur content of the fuel. Figure 6.14 presents the ratio of the particle number emission rates with LS fuel to ULS fuel for each bus and mode as a function of the age/mileage of the busses. The buses were classed into five groups according to their age/mileage. The interesting observation is that the ratio was highest for the more modern engines and decreased with age and mileage until the age of 8 years, and with mileage until about 500,000 km, after which remained constant. In other words, the reduction of particle number emissions with reduced fuel sulfur content was highest for the modern buses. It decreased with age and mileage but did not show any difference between the emissions with the two types of fuel after they passed a certain age and mileage.

In the engines of newer design, there is a decrease of particle mass emission and therefore a decrease in the number and the surface of particles in the accumulation mode. The accumulation particles act as a "sponge" for the condensation and/or adsorption of volatile materials. In the absence of that sponge, gas species, which are to become liquid or solid, will nucleate to form large numbers of small particles. The driving force for the gas to particle conversion is the saturation ratio, defined as the ratio of the partial pressure of a species to its saturated vapour pressure, in this case sulfuric acid. By reducing the sulfur level the partial pressure is reduced and nucleation prevented. If there are more particles in the accumulation mode, as in the case of older type engines, the available surface area will be larger and the process of adsorption will dominate over the process of nucleation. When the process of adsorption of volatile components onto accumulation mode particles becomes dominant, the formation of nucleation mode particles will be partially suppressed. For newer types of engines the number of particles, and therefore the available surface, in the accumulation mode is lower, and the process of nucleation becomes dominant. Reducing the available surface in the accumulation mode will lead to the increase in the number of particles in the nucleation mode. In these types of engines the number fraction in nucleaction mode is 99%, that is, the majority of particles are in the nucleation mode.

As in newer types of engines, the majority of particles are in the nucleation mode: preventing the formation of this mode will result in a significant decrease of the total number of particles, in some cases up to two orders of magnitude. Therefore, the

reduction of the particle number would be much more prominent in the engines that have lower particle mass emissions, such as Euro 2 type engines.

In the instances where the formation of the nucleation mode was already suppressed with 500ppm (LS) fuel (i.e. possibly due to a greater particle surface area being available in the accumulation mode) there was only a small reduction, if any, in the total particle number emission with 50 ppm (ULS) fuel.

It is interesting to note that the nucleation mode in this study was also not totally suppressed with the ULS fuel and in a small number of cases (only in 3 cases out of around 50) the nucleation mode was observed with ULS fuel but not with LS fuel. This indicates that the sulfuric acid inhibits the formation of the nucleation mode, but is not the only component responsible for the formation of this mode.

A similar finding that lubricating oil, unburned hydrocarbons from the fuel as well as PAH could also play a critical role in the formation of the nucleation mode was confirmed by Kittelson et al 2002. (Kittelson, 2002)

The main findings of this study are:

- The reduction of particle number emissions (mainly in the nucleation mode) with reduced fuel sulfur content, from 500 ppm to 50 ppm, was highest for the newer type of engines (Euro 2) that have smaller particle mass emissions than the other tested engines (Euro I and pre Euro I).
- The reduction of particle number emission decreased with the age and mileage of the tested vehicles, but did not show any statistically significant difference between the emissions with the two types of fuel for vehicles older than 8 years, and with mileage above 500,000 km.
- In the instances where the formation of the nucleation mode was already suppressed with 500 ppm (LS) fuel, there was only a small reduction, if any, in the total particle number emission with 50 ppm (ULS) fuel.
- The formation of the nucleation mode was also not totally suppressed with the 50 ppm (ULS) fuel and in a small number of cases the nucleation mode was observed with ULS fuel but not with LS fuel. This indicates that the sulfuric acid inhibits the formation of the nucleation mode but is not the only component responsible for the formation of this mode.

6.6 SUMMARIES AND RECOMMENDATIONS FOR FUTURE WORK

6.6.1 Summary: Nanoparticle Formation and Emissions

1. Size and concentration of nucleaction mode particles

    a. The nucleaction mode extends through sizes from 3 to 30 nm (0.003-0.03 μm). All of the above size ranges place nucleaction mode particles entirely within the nanoparticle range.
    b. The maximum concentration of nucleaction mode particles occurs at 10-20 nm.
    c. The nucleaction mode, depending on the engine technology and particle sampling technique, typically contains only 0.1-10% of the total PM mass, but it often includes more than 90% of the total particle count. Sometimes the nucleaction mode particles present as much as 99% of the total number of particles.

2. Chemical Properties:

    a. The nature of nucleaction mode particles is still being studied in laboratories.
    b. Nucleaction mode particles and accumulation- mode particles are externally mixed across a wide size range, with the chemical components being distributed between two particle types: (a) “less volatile” particles, probably comprised of an elemental carbon core with a small organic component; and (b) “more volatile” particles.
    c. The volatility of the Diesel nanoparticles was found to resemble that of C24-C32 normal alkanes, which implies a significant contribution of lubricating oil to these particles.
    d. The organic component of total Diesel particles and nucleaction mode particles appears to be comprised predominantly of unburned lubricating oil, whereas the contribution of fuel to the total organic component appears to be relatively small, no more than 20 % and probably much less.

3. What influences the nucleaction mode particles:

    a. The nucleaction mode is much more sensitive to engine operation, dilution and sampling conditions than is the accumulation mode.
    b. Cold temperatures favored nucleaction mode formation.
    c. The formation of nanoparticles from particle precursors is influenced by the residence time in the dilution tunnel or exhaust system. Short residence time in the exhaust and sampling system prior to dilution favor nanoparticle formation, while short residence time in the dilution system suppresses nanoparticle growth.

d. Storage and release of volatile material in the exhaust system, and prior engine operating history influence the formation of nucleaction mode particles.

4. Control and mitigation:

a. Engine technology effects were observed to be larger than fuel effects for accumulation mode particles, which reflected the observations for particle mass. Fuel effects were observed to be larger than engine technology effects for nucleation mode particles, which reflected the observation for particle number.
b. Diesel particle filters can effectively remove accumulation mode (solid) particles from the exhaust, but can emit volatile precursors that lead to nanoparticle formation and a large nucleaction mode under high load conditions.

6.6.2 Summary: Influence of the fuel sulfur level on nanoparticle formation

1. Sulfuric acid nanoparticles form as a result of condensation of hydrated sulfuric acid. They are formed from gaseous precursors as temperature decreases in the exhaust system, and after mixing with cold air, be it in the laboratory dilution tunnel or in the ambient air. The diameter of the original nucleus is believed to be about 1 nm.

2. Fuel sulfur enhances nucleation but is not the major component of the nucleaction mode. The C24-C32 normal alkanes, from the lubricating oil, have a more significant contribution to these particles (see 6.6.1 point 2).

3. Nanoparticles are more easily formed when fuels with high sulfur content (500ppm and above) are used, but under some engine conditions, such as light load, nucleaction mode formation is independent of fuel sulfur content and heavy hydrocarbons like those in lubricating oil could play a major role.

4. It has been observed that in some engines particle number emissions with low sulfur fuels (below 50ppm) can be up to 100 times lower than with higher sulfur fuels (500ppm). For these engines the reduction in particle mass emission was negligible.

5. The reduction of particle number emissions with reduced fuel sulfur content is greater in engines that emit a smaller concentration of accumulation mode particles, smaller mass emissions (new technology vehicles or vehicles with DPFs).

6. The reduction in particle number emission with the reduction of sulfur level will not show any statistically significant change as the vehicles reach an age of 8 years.

6.6.3 Recommendations for Future Investigations

All of the studies except one (Ristovski, 2002) examined only a few vehicles/engines from a limited fleet with most of the engines of a newer design. In order to assess the magnitude of the problem, a more extensive investigation should be designed to better represent the current and future fleets.

New engine designs and aftertreatment technologies may present new particle production challenges and solutions. These should be investigated.

The reduction of fuel sulfur level is very often accompanied by a significant change in other fuel properties such as aromatic content and volatility. In many of the studies so far these parameters were not decoupled. The specific influence of fuel and lubricants should be studied by testing matrices where key parameters of interest, such as sulfur, volatility and aromatic content are decoupled.

The effect of not only fuel sulfur content but also lubricant sulfur content should be studied to determine the influence of this parameter on the formation and emissions of nanoparticles.

Further work is required to develop sampling and measurement standards for particle size and number so that comparable data sets can be produced. For this purpose assessment and adoption of the existing instruments and techniques should be conducted.

6.6.4 Recommendations on Management Response

Since sulfates are just one of several components of the particle mass (PM) emissions, lowering fuel sulfur levels has only limited potential as a means of PM control. The reduction of diesel fuel sulfur levels from 3000 ppm to 500 ppm, as legislated in the U.S. in 1994, yielded relatively large benefits of about 0.04-0.08 g/bhp-hr PM reduction. However, a further reduction of fuel sulfur from the 500 ppm to lower levels has only small incremental PM reduction benefit of about 0.008-0.016 g/bhp-hr. The main benefit in reducing sulfur levels further below 500 ppm towards 50 ppm and lower will be in the reduction in particle number emissions. This reduction will be in the number of particles emitted in the nanoparticle range. Further to achieve EUROIV and even EUROIII standards of emissions new diesel emission control technologies have to be implemented (aftertreatment devices such as DOC, DPF, etc.). The influence of the sulfur level on the emission of nanoparticles with aftertreatment devices is still unknown.

Previous studies have shown that the reduction of nanoparticle emission with the reduction of fuel sulfur level below 500 ppm depends on the age/mileage of the vehicle. In order to assess the scale of the problem on the whole Australian diesel fleet, more data are needed on the dependence of the reduction of nanoparticle emission as a function of age/mileage of the vehicles. The available scientific data, which is from a single study, cannot give us this information as that study has been conducted on only one type of vehicle present in the diesel fleet (buses).

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APPENDIX A. PREPARATION AND ORGANISATION OF THE REPORT

A.1 THE CONSULTANCY TEAM

Leader of the Consultancy Team

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The Team

The consultancy team, which undertook the project consists of two Principals both experts in this area, Professor Lidia Morawska and Professor Michael R Moore, of the National Research Centre for Environmental Toxicology (EnTox) with Associates Dr Zoran Ristovski and Dr Cheryl Swanson and Research Associates Victoria Agranovski and David Hughes, as well as International Expert Advisers, Dr Annette Peters, Germany and Professor C Arden Pope, USA. Dr Peters and Professor Pope are the principal international experts who significantly contributed towards progress in understanding particle effects on health. Dr Peters was one of the chief investigators in the first major epidemiological study on ultrafine particles in Erfurt, Germany. The affiliations and skills of the team members are delineated in Table A-1. They brought together proficiency in Air Science, Toxicology, Epidemiology, Information Science and Risk Assessment. In addition, each represents a group with broad knowledge of the problems associated with definition of air-based and health research, and each brings their individual strengths to the team.

Table A-1 Team Members

Name	Affiliation	Skills
Principals		
Professor Lidia Morawska	Queensland University of Technology, ILAQH	Physico-chemistry of particles, ultrafine particles, human exposure, risk assessment
Professor Michael R. Moore	National Research Centre for Environmental Toxicology, University of Queensland.	Toxicology, metabolic medicine, human health risk assessment
Associates		
Dr Zoran Ristovski	Queensland University of Technology, ILAQH	Ultrafine particle motor vehicle emissions (diesel and spark ignition), transport and measurements, aerosol instrumentation

Table A-1. Team Members (Continued)

Name	Affiliation	Skills
Dr Cheryl Swanson	Queensland University of Technology, ILAQH	Epidemiology, clinical research, clinical trails, biostatistics
Research Associates		
Victoria Agranovski, MSc	Queensland University of Technology, ILAQH	Air quality, ultrafine particles in atmospheric systems, measurement methods
David Hughes	University of Queensland, National Research Centre for Environmental Toxicology, University of Queensland	Air quality, modelling, biostatistics, toxicology
International Expert Advisers		
Dr Annette Peters	GSF National Research Centre for Environment and Health Institute of Epidemiology, Neuherberg, Germany	Environmental epidemiology, top international expert in particle epidemiology, specifically, ultrafine particles
Professor C Arden Pope	Brigham Young University, Utah, USA	Environmental epidemiology, top international expert in particle epidemiology, author of major reviews in this area.

A.2 LITERATURE SEARCH

Literature search for the project consisted of the following elements:

1. Identification of key databases and internet search engines

   Two types of published information on ultrafine particles in the context of their potential to induce adverse health effects in exposed humans and animals were considered for the review: peer review international journals and reports published by major national and international organisations' databases.

   Literature search of journal publications has been conducted using the following databases: Blackwell Synergy, Ebsco, IEL Informit Online, ProQuest, ScienceDirect, SwetsWise, Web of Science, Wiley Inter Science, Academic Search Elite, Biological Abstracts, CINAHL; ERIC; INSPEC; MEDLINE; SAE MOBILITY DATABASE.

2. Contacting international organisations, who have conducted studies and reviews in areas relevant to the topic:

   First a comprehensive review was conducted of the material published and available on the websites of the following organisations:

   - Environment Canada
   - Health Effect Institute
   - HMSO

- US Environmental Protection Agency
- World Health Organization
- The National Institute of Environmental Health Sciences (USA)
- Gesondheidsraad: Health Council of the Netherlands
- Committee on the Medical Effects of Air Pollutants (UK)
- The Centre for Science and Environment (India)
- BUWAL (Swiss EPA),
- Dieselnet
- Society of Automotive Engineers
- US Department of Energy/Office of Transportation Technologies
- US Department of Energy/National Renewable Energy Laboratory (DOE/NREL)
- Coordinating Research Council (CRC)
- CONCAWE
- RICARDO

In addition, senior officers from HEI, US EPA and WHO were contacted to ensure that all relevant material published by these organizations is available to the Team.

A.3 LITERATURE SCREENING AND ORGANISATION

Literature search, which focused on the general topic of ultrafine particles and health resulted in the generation of lists of:

- 658 journal publications (1970-2003)
- 72 reports and other documents

In regard to the studies published in peer review journals, the first stage of literature screening focused on the identification of those publications, which did not fully describe the studies conducted, or which partly duplicated work presented by the same authors in other publications (for example, conference papers, if full journal papers of the same work were published). Although full details of these publications have been included in the bibliography section of this report, these documents were not given further consideration.

The materials found on the websites of the international organisations typically contained bibliographic citations for journal articles, conference papers, and technical or government documents relating to: health effects of air pollutants, general information on particulate matter, a listing of issues currently under discussion as well as a listing of health effect related research reports. Full-text reports or executive summaries on the progress of ongoing studies were typically available. Those ongoing studies, which were directly relevant to the issue of the health effect of ultrafine particles were included in the current review.

All the publications selected for the review were divided into two broad discipline categories: (i) epidemiology (population based and observational in concept), and (ii) toxicology (laboratory based and experimental in concept). All papers in these two groups were tabulated together with a short summary of the topic and outcomes of the each paper. These summary tables are presented as appendices to this report.

Members of the consultancy team are actively involved in the research on ultrafine particle emissions from internal combustion engines and have developed an up to date large database of relevant publications over several years on the topic. The literature search on the link between the sulfur content of diesel fuels and the number of ultrafine particles in diesel emissions resulted in over 150 publications. The majority of these publications, although investigating different aspects of the influence of fuel sulfur level on diesel vehicle emissions, were not directly concerned with ultrafine particle emissions. Only a small number of these were included in the literature review and have not tabulated in the same way as the health related papers.

In addition to these two major groups of papers, relating to the two main objectives of the report is the introductory part of the report. This provides general background on airborne particle matter, ultrafine particles, their sources, characteristics and behaviour. It is based on selected papers and examples from the published literature to highlight the points necessary for understanding and interpretation of the material reviewed in the two main parts of the report (chapters 5 and 6)

A. 4 CONSULTATION WITH THE STAKEHOLDERS AND REVIEW

An invitation was sent to a list of 57 stakeholders including professionals from the government, academia and industry. They were asked to voice any comments or suggestions they may have in relation to the review as well as to review the first draft of the document. Out of these, 19 expressed interest in reviewing the first draft of the report and 5 provided comments for the document. Three of the stakeholders provided commnts on the first draft of the report.

As explained above, the draft report has been reviewed by two external reviewers: Dr Annette Peters and Professor C Arden Pope.

APPENDIX B. STATISTICAL METHODS

The contents of this appendix are intended to provide a quick reference and overview of some of the analytical techniques referred to in this report. A more detailed presentation is beyond the scope of this report, however, relevant references are provided as a starting point for exploring the topic further.

Time Series Analysis and Regression

A time series is an ordered sequence of values of a variable at equally spaced time intervals. Data points taken over time may have an internal structure such as autocorrelation, trend or seasonal variation, that should be accounted for and time series analysis provides the means to determine that structure. Analysis of such series enables the investigator to obtain an understanding of the underlying forces and structure that produced the observed data. Analysis can also enable the fitting of a model to the data for forecasting and monitoring purposes.

Briefly, the analytical strategy is to identify a model and to estimate and diagnose the fit of the parameters. Analysis follows an iterative procedure to determine the most parsimonious or economical set of parameters and uses the autocorrelation function, the partial autocorrelation function and the Q-statistic in this procedure. Modelling is helped by inspection of various graphical displays, eg. time series plots of the dependent variable, predicted time series plots, residual time series plots, periodograms, residual-residual plots, cross-correlation plots. Various terms are identified to include in the model, eg. autoregressive, linear, trend, second and third order polynomials, as well as dummy variables representing categorical data.

Generalised Linear Models

Generalised linear Models or GLMs are a unifying framework that includes classical regression models with a normally distributed dependent variable and categorial regression models like logistic regression or Poisson regression. Various other nonstandard regression type models are also included. Generalised linear models are used for regression modelling with non-normal data and involve a minimum of extra complication compared with normal linear regression. GLMs allow most of the familiar ideas of normal linear regression to apply while covering a wide range of common situations.

A main feature of GLMs is the presence of a linear predictor, which is built from explanatory variables. This linear predictor is linked to the mean response by a so-called link function, which may take various forms. Many ideas of linear regression carry over to this wider class of models. An important extension of GLMs is the incorporation of nonparametric parts in the predictor. The parametric model assumes that variables enter the model in the form of a linear predictor in non- and semiparametric regression techniques, however, this assumption is weakened when the the covariates are allowed to have unspecified functional form.

An important consideration is that (generalised) linear models are easily understood and can be summarised and communicated to others in a straightforward manner. In addition, parameter estimates from these models can be used to predict or classify new cases simply and readily.

Generalised additive models

The generalised additive model can be considered as an alternative to the common linear model. Generalised additive models are flexible in that they allow the effect of each independent variable to be modelled non-parametrically while requiring that the effect of all the independent variables is additive. The purpose of generalised additive models is to maximize the quality of prediction of a dependent variable Y from various distributions, by estimating unspecific (non-parametric) functions of the predictor variables, which are "connected" to the dependent variable via a link function. It should be noted that generalised additive models can be difficult to interpret, particularly when complex nonlinear effects of some or all of the predictor variables are involved. The generality of generalised additive models through the use of regression smoothers to obtain a satisfactory fit to the data results in added complexity. When the fit of GAM and GLM models are comparable, the simpler generalised linear model is preferable to the more complex generalised additive model.

Generalised estimating equations (GEE)

Generalised Estimation Equations, or GEEs, are methods of parameter estimation for correlated data. When data are collected on the same units across successive points in time the observations are repeated and these repeated observations are correlated over time. The standard errors of the parameter estimates will not be valid and hypothesis testing results will be non-replicable if this correlation is not taken into account.

Comparing utilization rates across quintile groups or regions is traditionally done using the direct standardization approach that adjusts for confounding discrete factors such as age and sex. A model-based approach, on the other hand, can adjust for continuous and well as discrete factors and the GEE method of parameter estimation specifically is more efficient for statistical hypothesis testing with correlated longitudinal data.

GEE was introduced by Liang and Zeger in 1986, as a method of estimation of regression model parameters for dealing with correlated data. Regression analysis with GEE is a useful choice when the outcome measure of interest is discrete, such as binary or count data which might be from a binomial or Poisson distribution, rather than continuous.

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APPENDIX C. TOXICOLOGICAL STUDIES

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Al-Humadi et al., (2002a)	Rats	Inflammation	Brown Norway rats were exposed by intratracheal instillation of saline, carbon black (CB), or diesel exhaust particles (DEP) (5 mg/kg) on day 1, followed by exposure to ovalbumin (OVA, 90 mg/m^3) or saline for 30 minutes on days 1, 8, 15, and 29. Animals were sacrificed on day 30.	The results show that both DEP and CB augmented OVA-induced allergic sensitization, and that particle composition of DEP may not be a critical factor for the adjuvant effect. OVA exposure causes significant depletion of intracellular GSH in lymphocytes, which may play a key role in OVA-mediated immune responses.
Al-Humadi et al., (2002b)	Rats	Inflammation	Study characterised the effects of diesel exhaust particles (DEP) on thiol regulation in alveolar macrophages (AM) and lymphocytes. AM and lymph node (thymic and tracheal) cells (LNC) (at different time points) were obtained from rats exposed intratracheally to DEP (5 mg/kg) or saline, and measured inflammatory markers, thiol levels, and glutathione reductase (GSH-R) activity.	The results indicate that DEP exposure caused lung inflammation and affected thiol levels in both AM and LNC.
Baeza-Squiban et al. (1999)	In vitro (human bronchial epithelial cells)	Lung inflammation	DEP were tested on a human bronchial epithelial cell line (16HBE) in comparison with carbon black particles (CB) devoid of PAH.	The data suggest that the activation of NF-kappa B and the expression of c-fos could contribute to the proliferation and chronic inflammation processes induced in lungs after DEP exposure.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Baggs et al., (1997).	Rats	pulmonary inflammatory	Male Fisher 344 rats were exposed for 6 hours a day, 5 days a week, for 3 months to 1) filtered air (control); 2) TiO_2-D, 20 nm particle size, 23.5 mg/m^3) TiO_2-F, 250 nm, 22.3 mg/m^3; or 4) crystalline SiO_2, a positive control particle (similar to 800 nm particle size, 1.3 mg/m^3). Groups of 3-4 animals were sacrificed at 6 and 12 months following the completion of exposure. Pulmonary effects of exposure were evaluated using standard hematoxylin and eosin-stain sections, histochemical stains for collagen, and immunohistochemical assays for cell turnover.	Six months after animals were exposed to SiO_2, they had moderate focal interstitial fibrosis and moderately severe focal alveolitis. Animals exposed to TiO_2-D had slightly less fibrosis. The least fibrosis was seen in the TiO_2-F group. At 1 year after exposure, fibrosis was still present but decreased in the SiO_2 group. The amount of interstitial fibrosis in the TiO_2-D- and TiO_2-F-treated animals had largely returned to untreated. Although initially irritant, TiO_2-induced lesions regressed during a 1-year period following cessation of exposure. Inhaled ultrafine particles of TiO_2 (TiO_2-D, 20 nm particle size) lead to a greater pulmonary inflammatory response than larger pigment-grade particles (TiO_2-F, 250 nm).
Bai et al. (2001)	In vitro (human pulmonary artery endothelial cells)	cytotoxic	Investigated the cytotoxic mechanism of DEP on human pulmonary artery endothelial cells focusing on the role of active oxygen species. Organic compounds in DEP were extracted by dichloromethane and methanol.	DEP-extracts damaged endothelial cells under both subconfluent and confluent conditions. Superoxide, hydrogen peroxide, and other oxygen-derived free radicals are likely to be implicated in DEP-extract-induced endothelial cell damage. Conclusions: NO is also involved in DEP-extract- mediated cytotoxicity, which was confirmed by direct measurement of NO production. These active oxygen species, including peroxynitrite, may explain the mechanism of endothelial cell damage upon DEP exposure during the early stage.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Barrett et al., (2003).	Dogs: 6 allergic and 6 non-allergic	Immune and airway responses	Studied the effects of ultrafine particles on immune and airway responses in a beagle dog model of allergic asthma. Six allergic (ragweed sensitive) and six nonallergic dogs were exposed to ultrafine carbon particles (232.3 +/- 2.5 $\mu g/m^3$), 35.2 +/- 0.3 nm) for 1 h, followed by a challenge with vehicle (water) as a negative control. Immune responses 3 days before and after particle exposure were assessed by measuring total immunoglobulin E (IgE) and ragweed-specific IgE and IgG in serum and bronchoalveolar lavage fluid (BALF), and cell differentials in BALF. Each dog was exposed a second time to ultrafine carbon particles (251.4 +/- 5.3 $\mu g/m^3$, 34.9 +/- 0.5 nm) for 1 h followed by a challenge with ragweed and the same measurements.	Airway resistance did not change during particle exposure in any of the dogs, and ragweed-induced airway reactivity was not altered by particle exposure. Total and ragweed-specific serum IgE and total IgE in BALF were higher in allergic dogs at all time points. Particle exposure did not affect antibody levels in serum or BALF in allergic dogs. Nonallergic dogs developed specific IgG in response to multiple inhalation exposures to ragweed, but this was not associated with particle exposure. Neutrophils were elevated in BALF for all groups 1 day after particle exposure. In conclusion, despite the induction of low level inflammation in the lungs of allergic and nonallergic dogs, exposure to ultrafine carbon particles did not alter airway reactivity or immune responses.
Beck-Speier et al., (2001).	In vitro (immune cells)	Physiologic responses of immune cells	Evaluated physiologic responses of immune cells on exposure to the agglomerates of 77 nm elemental carbon [(EC); specific surface area 750 m^2/g] and 21 nm titanium dioxide (TiO_2) particles (specific surface area 50 m^2/g) by the release of lipid mediators by alveolar macrophages (AMs). .	The results indicate that surface area rather than mass concentration determines the effect of AUFPs, and that activation of phospholipase A(2) and COX pathway occurs at a lower particle surface area than that of 5- LO-pathway.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Bion et al., (2002).	In vitro (cultures of rat lung slices)	Inflammation	Exposed biphasic air/liquid organotypic cultures of rat lung slices to continuous flows of diluted exhausts from diesel engines. The size distribution of the particulate matter and the bioavailability of pollutants were preserved, thus allowing to mimic in vitro the in vivo atmosphere/tissue interactions that occur mainly through diffusion mechanisms. The toxicity response profile has been assessed in terms of tissue viability, oxidative stress, DNA injury, and the early phase of inflammatory reaction.	Exhaust filtration, addition to fuel of rapeseed methyl ester, and preincubation of lung tissue with soy isoflavones modulated the toxicity response profile of exhausts.
Boland et al. (1999)	in vitro models of human airway epithelial cells	Lung inflammation, immune responses	The involvement of diesel exhaust particles (DEPs) in respiratory diseases was evaluated by studying their effects on two in vitro models of human airway epithelial cells. The cytotoxicity of DEPs, their phagocytosis, and the resulting immune response were investigated in a human bronchial epithelial cell line (16HBE14o-) as well as in human nasal epithelial cells in primary culture.	DEP exposure induced a time- and dose-dependent membrane damage. DEPs underwent endocytosis by epithelial cells and translocated through the epithelial cell sheet. DEPs also induced a time-dependent increase in interleukin-8, granulocyte-macrophage colony-stimulating factor, and interleukin-1 beta release. This inflammatory response occurred later than phagocytosis, and its extent seems to depend on the content of adsorbed organic compounds because carbon black had no effect on cytokine release. Conclusions: Exposure to diesel exhaust particles (DEPs) stimulates human airway epithelial cells to secrete the inflammatory cytokines interleukin-8, interleukin-1 beta, and granulocyte-macrophage colony-stimulating factor

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
				(GM-CSF) involved in allergic diseases.
Boland et al. (2000)	In vitro (human bronchial epithelial cells)	Lung inflammation	Studied the mechanisms underlying the increase in GM-CSF release elicited by DEPs using the human bronchial epithelial cell line	DEP treatments increased GM-CSF mRNA levels. Comparison of the effects of DEPs, extracted DEPs, or extracts of DEPs reveiled that the increase in GM-CSF release is mainly due to the adsorbed organic compounds and not to the metals present on the DEP surface. Conclusions-the increase in GM-CSF release is mainly due to the adsorbed organic compounds and that the effect of native DEPs requires endocytosis of the particles. Reactive oxygen species and tyrosine kinase(s) may be involved in the DEP-triggered signaling of the GM-CSF response.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Bommel et al. (2000)	In vitro (human cells)	Allergic responses	Investigated whether pyrene, a major compound of diesel exhaust particles, can affect the production of IL-4.	Pyrene induced transcription of IL-4 messenger RNA and expression of IL-4 protein in primary human T cells. Pyrene, but not related polyaromatic hydrocarbons, enhanced basal transcription of the human and mouse IL-4 promoter. Conclusions- pyrene may promote allergic diseases by inducing the production of IL-4.
Bonvallot et al. (2001)	In vitro (airway epithelial cells)	Inflammation	Compared the effects of native DEP (nDEP), organic extracts of DEP (OE-DEP), and carbonaceous particles, represented by stripped DEP (sDEP) and carbon black particles (CB), in order to clarify their respective roles.	Demonstrated, for the first time, in airway epithelial cells in vitro that nDEP induce the expression of the CYP1A1, a cytochrome P450 specifically involved in polycyclic aromatic hydrocarbons metabolism, thereby demonstrating the critical role of organic compounds in the DEP-induced proinflammatory response.
Brown et al., (2001).	Rats, In vitro	Respiratory	Investigated proinflammatory responses to various sizes of polystyrene particles as a simple model of particles of varying size including ultrafine.	There was a significantly greater neutrophil influx into the rat lung after instillation of 64-nm polystyrene particles compared with 202 nm and 535 nm particles and this was mirrored in other parameters of lung inflammation, such as increased protein and lactate dehydrogenase in bronchoalveolar lavage. Conclusions - the results suggest that ultrafine particles composed of low-toxicity material such as polystyrene have proinflammatory activity as a consequence of their large surface area.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Bunger et al. (2000)	In vitro (human cells)	mutagenic effects	DEPs from four different fuels were studied for content of polynuclear aromatic compounds and mutagenic effects. Two biodiesel fuels, rapeseed oil methylesters (RME) and soybean oil methylesters (SME), were compared directly with two fossil diesel fuels with the normal (DF) and a low sulfur content (LS-DF).	The results indicate that diesel exhaust particles from RME, SME and LS-DF contain less black carbon and total polynuclear aromatic compounds and are significantly less mutagenic in comparison with DF. A high sulfur content of the fuel and high engine speeds (rated power) and loads are associated with an increase in mutagenicity of diesel exhaust particles.
Bunger et al., (2000).	In vitro	Cytotoxic and mutagenic effects	Studied cytotoxic and mutagenic effects due to exposure to DEP: biodiesel (rapeseed oil methyl ester, RME) and common fossil diesel fuel (DF). A test tractor was fuelled with RME and DF and driven in a European standard test cycle (ECE R49) on an engine dynamometer. Particle numbers and size distributions of the exhausts were determined at the load modes "idling" and "rated power". Filter-sampled particles were extracted and their cytotoxic properties tested using the neutral red assay, Mutagenicity was tested using the Salmonella typhimurium/microsome assay.	While the size distributions and the numbers of emitted particles at "rated power" were nearly identical for the two fuels, at "idling" DF emitted substantially higher numbers of smaller particles than RME. The RME extracts caused fourfold stronger toxic effects on mouse fibroblasts at "idling' but not at "rated power" than DF extracts. The extracts at both load modes were significantly mutagenic in TA98 and TA100. However, extracts of DF showed a fourfold higher mutagenic effect in TA98 land twofold in TA100) than extracts of RME. The lower mutagenic potency of DEP from RME compared to DEP from DF is probably due to lower emissions of polycyclic aromatic compounds. The higher toxicity is probably caused by carbonyl compounds and unburned fuel, and reduces the benefits of the lower emissions of solid particulate matter and mutagens from RME.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Bunn et al., (2001).	In vitro (alveolar macrophages)	Capacity of alveolar macrophages to phagocytose inhaled UFP	The capacity of alveolar macrophages (AM) to phagocytose inhaled material was used to directly assess exposure of normal children to particles. AM from 22 children aged 3 months to 16 years with no respiratory symptoms were obtained by nonbronchoscopic bronchoalveolar lavage prior to elective surgery. In each child the size and composition of environmental particles within single sections from 100 separate AM was determined by electron microscopy and microanalysis.	Single and clusters of particles were seen in AM from all children. The percentage of particle-containing AM ranged from 1% to 16% per child. Particles consisted of a carbonaceous core and all were ultrafine (<0.1 μm). Other elements such as metals and silicon were not detected. The percentage of particle-containing AM did not change with age, but was increased in children whose parents lived on a main road compared with those living on a quiet residential road (median 10% v 3%, p = 0.014). Conclusions: All children had AM containing ultrafine carbonaceous particles. The predominant source of these particles is most likely to be from the combustion of fossil fuels.
Carero et al. (2001)	In vitro (human cells)	cytotoxic and genotoxic potency	Studied cytotoxic and genotoxic potency of DEP, urban particulate matter (UPM), and Carbon black (CB) by exposing human cells (A549 and THP-1 cell lines) in vitro to CB, DEP (SRM 1650, NIST), and UPM (SRM 1648, NIST) for 48 hr. Cytotoxicity was assessed using the AlamarBlue assay, whereas genotoxicity was assessed using the single-cell gel electrophoresis (comet assay).	The CB, DEP, and UPM particles showed no significant cytotoxicity. However, all three particles were able to cause significant DNA damage, although to a different extent in the two cell lines. The genotoxicity of washed particles and dichloromethane extracts was also investigated. In THP-1 cells CB washed particles and DEP extracts caused significant DNA damage. This difference in effect may be related to differences in size, structure, and composition of the particles. These results suggest that CB, DEP, and UPM are able to cause DNA damage and, therefore, may contribute to the causation of

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
				lung cancer.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Carero et al., (2001).	In vitro (human cells)	Cytotoxicity	In this study the cytotoxic and genotoxic potency of these three particle types was investigated by exposing human cells (A549 and THP-1 cell lines) in vitro to CB, DEP (SRM 1650, NIST), and UPM (SRM 1648, NIST) for 48 hr. Cytotoxicity was assessed using the AlamarBlue assay, whereas genotoxicity was assessed using the single-cell gel electrophoresis (comet assay). The particles were characterised with regard to their mean diameter in tissue culture medium (CB 100 nm, DEP 400 nm, UPM 2 μm), their total carbon content (CB 99%, DEP 85%, UPM 15%), and their acid-soluble metal composition (UPM much greater than CB similar to DEP). The concentrations ranged from 16 ng/ml to 16 μg/ml for cytotoxicity tests and from 16 ng/ml to 1.6 μg/ml for genotoxicity tests.	The CB, DEP, and UPM particles showed no significant cytotoxicity. However, all three particles were able to cause significant DNA damage, although to a different extent in the two cell lines. The genotoxicity of washed particles and dichloromethane extracts was also investigated. In THP-1 cells CB washed particles and DEP extracts caused significant DNA damage. This difference in effect may be related to differences in size, structure, and composition of the particles. These results suggest that CB, DEP, and UPM are able to cause DNA damage and, therefore, may contribute to the causation of lung cancer.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Carero et al., (2002).	In vitro (human cells)	Allergic sensitization	Investigated the potential of particle types (carbon black, diesel exhaust particles and urban air particles (0.1-1000 ng/cm^2), to induce the expression of HLA-DR (the expression of HLA-DR on the cell membrane of antigen-presenting cells is of major importance for the induction of an allergic response in the airways) on differentiated THP-1 cells, taken as a model for alveolar macrophages. Assessed the 'adjuvant' potential of the particles on interferon (IFN)-gamma, a known enhancer of HLA-DR.	By themselves, the particles (0.1-1000 ng/cm^2) were not able to induce HLA-DR on the THP-1 cells after an incubation of 48 h. However, even at very low concentrations, carbon black (from 1 ng/cm^2 on) and diesel exhaust particles (from 0.1 ng/cm^2 on), interacted with IFN-gamma (100 U/mL) to enhance HLA-DR expression (up to 2.5-fold increase). Conclusions: Results may reflect in vitro one of the mechanisms by which pollutant particles exert an 'adjuvant' activity and may partially explain how exposure to particles can be related to the enhancement of allergic sensitization.
Casillas et al. (1999)	In vitro (human cells)	Immune responses	Studied the mechanisms of allergic inflammation due to DEP exposure	An important primary effect that can explain the DEP-associated humoral and cellular immune responses is the induction of macrophage responses by DEP chemicals. This includes effects on macrophage production of cytokines and chemokines, which may play a role in enhancing allergic inflammation. A potent mechanism in macrophages exposed to DEP chemicals involves the generation of reactive oxygen species (ROS), leading to cellular activation or apoptosis which can be abrogated by antioxidants.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Cassee et al., (2002a)	Rats: healthy & with pulmonary hypertension	Pulmonary toxicity	Tested the hypothesis that secondary model aerosols exert acute pulmonary adverse effects in rats, and that rats with pulmonary hypertension (PH), induced by monocrotaline (MCT), are more sensitive to these components than normal healthy animals. In addition, tested the hypothesis that fine particles exert more effects than ultrafines. Healthy and PH rats were exposed to ultrafine (0.07-0.10 µm; 4 x 10^5 particles/cm^3) and fine (0.57-0.64 µm; 9 x 10^3 particles/cm^3) ammonium aerosols during 4 h/day for 3 consecutive days. The mean mass concentrations ranged from 70 to 420 µg/m^3, respectively, for ultrafine ammonium bisulfate, nitrate, and ferrosulfate and from 275 to 410 µg/$m^3$for fine-mode aerosols. Bronchoalveolar lavage fluid (BALF) analysis and histopathological examination were performed on animals sacrificed 1 day after the last exposure.	Histopathology of the lungs did not reveal test atmosphere-related abnormalities in either healthy or PH rats exposed to the ammonium salts, or to a combination of CB + nitrate. Alveolar macrophages in rats exposed to CB only revealed the presence of black material in their cytoplasm. There were no signs of cytotoxicity due to the aerosol exposures (as measured with lactate dehydrogenase [LDH], protein, and albumin contents in BALF). Macrophages were not activated after MCT treatment or the test atmospheres, since no changes were observed in N-acetyl glucosaminidase (NAG). Cell differentiation profiles were inconsistent, partly caused by an already present infection with Haemophilus sp. The results show that at exposure levels of ammonium salts at least one order of magnitude higher than ambient levels, marked adverse health effects were absent in both healthy and PH rats.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Cassee et al., (2002c)	Rats	Pulmonary toxicity	Cadmium chloride ($CdCl_2$) was used as a model for toxic aerosol particles to (1) investigate the role of particle size in the development of pulmonary effects, and (2) evaluate the MPPDep model, by comparing predicted deposition with measured deposition of $CdCl_2$ in the respiratory tract. Rats (ten per group) were exposed for a single 4-h period to $CdCl_2$ particles at various sizes, i.e. 33, 170, 637 and 1495 nm, all at a target concentration of 1 mg/m^3. Immediately after exposure, four of ten rats per group were killed and trachea, lung lobes, heart, liver and kidneys were collected and preserved to determine the amount of $CdCl_2$ present in each of these organs. $CdCl_2$-induced toxicity, as measured by lactate dehydrogenase (LDH), N-acetyl glucosaminidase (NAG) and protein levels in bronchoalveolar lavage fluid, was determined in the remaining six rats per group the day after exposure.	Animals exposed to 33 nm particles showed the highest level of respiratory toxicity, followed by animals exposed to 637 nm particles, then to 170 nm particles and finally by those exposed to 1495 nm particles. Pulmonary cadmium levels showed a similar relationship. The results suggest that the induction of pulmonary toxicity following inhalation exposure to soluble CdCl(2)particles in the range 30-1500 nm depends on the amount of deposited material, which in its turn depends on the initial (aerodynamic) particle size. Conclusions: For soluble particles the deposited pulmonary mass (dose) of particles is important for toxicity and is dependent of particle size.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Castranova et al. (2001)	Rats and mice	inflammatory	Investigated the effects of exposute to DEP on the susceptibility of the lung to infections. Summarised data concerning the effect of exposure to diesel exhaust particles on these alveolar macrophage functions and the role of adsorbed organic chemicals compared to the carbonaceous core in the toxicity of diesel particles.	The results support the hypothesis that exposure to diesel exhaust particles increases the susceptibility of the lung to infection by depressing the antimicrobial potential of alveolar macrophages. This inhibitory effect appears to be due to adsorbed organic chemicals rather than the carbonaceous core of the diesel particles.
Churg et al., (1998a)	In vitro (rat tracheal explants)	Inflammation	Examined the relationship between particle uptake by pulmonary epithelial cells and particle size. Exposed rat tracheal explants to fine particles (0.12 μm) or ultrafine particles (0.021 μm) of titanium dioxide for 3 or 7 days.	The results suggest that the behavior of particles of different size is complex: UFPs persist in the tissues as relatively large aggregates, whereas the size of FP aggregates becomes smaller over time. UFPs appear to enter the epithelium faster, and once in the epithelium, a greater proportion of them is translocated to the subepithelial space compared with FPs. However, if it is assumed that the volume proportion is representative of particle number, the number of particles reaching the interstitial space is directly proportional to the number applied; i.e., overall, there is no preferential transport from lumen to interstitium by size.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Churg et al., (1999).	In vitro (rat tracheal explants)	Inflammation	Examined whether particle size affects mediator generation. Exposed rat tracheal explants, an inflammatory cell-free model of the airway wall, to various concentrations up to 500 μg/cm^3 of fine (0.12 μm) or ultrafine (0.021 μm) titanium dioxide (anatase), maintained the explants in an organ culture in air for 1-7 days, and used RT-PCR to examine the expression of fibrogenic mediators and procollagen.	The results suggest that ultrafine particles are intrinsically able to induce procollagen expression even in the absence of inflammatory cells; that chronic exposure to PM_{10} may result in chronic airflow obstruction, in part because of ultrafine particle-mediated increases in airway wall fibrosis; and that chemically identical dusts of differing size can produce quite different patterns of gene expression in the airway wall.
Devalia et al. (1999)	In vitro (human cells)	Inflammation	Investigated constitutive and diesel exhaust particles (DEP)- induced release of several pro-inflammatory mediators and the differences between cytokine release from bronchial epithelial cells (HBEC) of asthmatic patients and non-asthmatic subjects.	The results suggest that the increased sensitivity of the airways of asthmatics to air pollutants such as DEP may, at least in part, be a consequence of greater constitutive and pollutant-induced release of specific pro-inflammatory mediators from their bronchial epithelial cells.
Devalia et al (1999)	In vitro (bronchial epithelial cells)	Inflammation, Asthma	Cultured bronchial epithelial cells (HBEC) from biopsies of atopic mild asthmatic patients and nonatopic non-asthmatic subjects, and investigated constitutive and diesel exhaust particles (DEP)-induced release of several pro-inflammatory mediators.	The results suggest that the increased sensitivity of the airways of asthmatics to air pollutants such as DEP may be a consequence of greater constitutive and pollutant-induced release of specific pro-inflammatory mediators from their bronchial epithelial cells.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Dick et al., (2003).	Rats , In vitro	Toxicity, Inflammation	By using four types of UFP (carbon black (UFCB), cobalt (UFCo), nickel (UFNi), and titanium dioxide (UFTi), determined the attributes of the UFP (surface area, chemical composition, particle number, or surface reactivity) that contribute most to its toxicity and proinflammatory effects both in vivo and in vitro.	The results suggest that UFP in PM_{10} may cause adverse effects via oxidative stress, and this could have implications for susceptible individuals. Susceptible individuals, such as those with COPD or asthma, already exhibit preexisting oxidative stress and hence are in a primed state for further oxidative stress induced by PM.
Doornaert et al., (2003).	In vitro	Inflammation	Investigated the effects of DEPs on the interaction of 1-HBE cells (16HBE14o-) with the cell and matrix microenvironment based on evaluation of integrin-type cell/ matrix ligand expression, cytoskeleton (CSK) stiffness, and matrix remodelling via matrix metalloproteinase (MMP)-1, MMP-2, and MMP-9 expression.	Showed that, in addition to their ability to increase the production of inflammatory cytokines, DEPs could also alter the links between actin CSK and the extracellular matrix, suggesting that they might facilitate HBE cell detachment in vivo.
Elder et al., (2000).	Rats, Mice	Inflammation	Evaluated hypothesis that carbonaceous ambient ultrafine particles and ozone can act together to induce greater oxidative stress and inflammation in the lung than when administered alone and that these effects would be amplified in the compromised, aging lung.	Found significant effects of carbon particles as well as a consistent interaction between carbon and ozone as determined by analysis of variance (ANOVA). However this interaction was in the opposite direction in young rats versus old rats and old T-SK mice: Carbon and ozone interacted such that ROS activity was depressed in young rats, whereas it was enhanced in old rats and old T-SK mice, indicating age-dependent functional differences in elicited pulmonary inflammatory cells. Conclusions: Ultrafine carbonaceous particles inhaled for short periods of time can

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
				induce significant pulmonary inflammation and oxidative stress that are modified by age, co pollutants, and a compromised respiratory tract.
Fahy et al. (1999)	In vitro (human cells)	Inflammation	Investigated the effects of diesel organic extracts on chemokine production by peripheral blood mononuclear cells	The results suggest that the chemokine pathways are modulated by DEP-PAHs at the transcriptional level, reinforcing the idea that the development of inflammatory reactions might be affected by diesel exhaust emission.
Fahy et al. (2000)	In vitro (human cells)	Allergic responses	Investigated synergistic effect of diesel organic extracts and allergen Der p 1 on the release of chemokines by peripheral blood mononuclear cells from allergic subjects	The results suggest that simultaneous exposure of allergic patients to DEPs and allergens could result in high local chemokine levels via MAP kinase pathways activation, increasing the likelihood of reaching a critical threshold leading to the initiation of respiratory allergic symptoms.
Fujimaki et al. (2001)	Mice	Lung inflammation	Investigated the roles of CD4+and CD8+T cells in adjuvant activity of diesel exhaust particles in mice	The results suggest that DEP injection may affect not only the function of CD4+ cells but also that of CD8+ T-cell subsets to modulate the synthesis of proinflammatory cytokine in PEC and type-1 and type-2 cytokine production in spleens.
Fujimaki et al. (2001a)	guinea-pigs	Lung inflammation	Investigated induction of the imbalance of helper T-cell functions in mice exposed to diesel exhaust	Low dose DE inhalation is shown to adversely affect the cytokine and antibody production in mice by altering CD4(+) and CD8(+) T-cell functions.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Granum et al. (2000)	Mice	Allergic responses	Investigate which physical properties (weight, size, number, and surface area) of particles might be important for the allergic effects. NIH/Ola mice were given 2 intraperitoneal injections with PSP plus OVA or OVA alone, over a 16-day period. The mice were exsanguinated at the end of each experiment, and the serum concentration of IgE anti-OVA was measured.	The results indicate that the total number and total surface area of UFP, rather than the dose weight, are important parameters for the IgE adjuvant activity.
Granum et al. (2001)	Mice	Lung inflammation	Investigated immediate and delayed IgE adjuvant effects caused by particles in a mouse model.	The results indicate that individuals exposed to particulate ail pollution at one point of time may develop an increased reaction towards allergens inhaled later that day or even several days after the particle exposure.
Greenwell et al., (2002).	In vitro	Bioreactivity	Carbon Black M120 and Diesel Exhaust Particles (DEP) were tested as $PM_{2.5-10}$ surrogates, DEP displaying the greatest oxidative bioreactivity.	Both urban $PM_{2.5}$ (fine fraction) and $PM_{2.5-10}$ (coarse fraction) (Cardiff, S. Wales, UK) caused significant damage, the coarse fraction displaying higher oxidative capacity. The soluble components were found to be responsible for most of the bioreactivity in both PM sizes. Low molecular components of fresh lung lavage were found to offer most antioxidant protection, and surrogate Epithelial Lining Fluid (sELF) showed significant amelioration of DNA damage by the coarse fraction but less effect against the fine.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Han et al. (2001)	Mice	Lung inflammation	Investigated the involvement of reactive oxygen species (ROS) in the lung injuries (pulmonary tumors, asthma- like symptoms) induced by DEP.	The results provided the first direct evidence that the intratracheal exposure to DEP in mice produced (OH)-O in the lung through an iron-catalysed reaction of superoxide/H_2O_2.
Hashimoto et al. (2000)		Lung inflammation	Investigated the intracellular signal transduction pathway and the involvement of reduction and oxidation (redox) control in DEP-activated signalling.	Found that p38 MAP kinase plays an important role in the DEP-activated signalling pathway that regulates IL-8 and RANTES production by BECs and that the cellular redox state is critical for DEP-induced p38 MAP kinase activation reading to IL-8 and RANTES production.
Heo et al. (2001)	Mice	Allergic responses	Investigated the mechanisms of allergic responses due to exposure to DEP	Co- injection of mice with DEP and ovalbumin three times over a 2 week period lead to a rapid elevation of ovalbumin-specific IgE, IgG1 and also IgG1a, compared with ovalbumin alone. When DEP were injected 1 day before or after ovalbumin on each occasion, their adjuvant effect was considerably muted, suggesting that the adjuvant effect of DEP is short-lived, or that a physical interaction between ovalbumin and DEP is required. Both the core carbon particles and the organic extract enhanced ovalbumin specific IgE and IgG 1 levels. Thus the adjuvant effect of DEP in this model is due both to the physical and the chemical attributes of the particles. The tricyclic hydrocarbons phenanthene (the most prevalent polycyclic aromatic hydrocarbon in DEP) and anthracene were both capable of enhancing

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
				antigen-specific IgE and IgG1 production. The phenolic antioxidant, butylated hydroxyanisole, which can affect gene expression via the antioxidant responsive element (ARE), had a lesser effect.
Hiura et al. (1999)	In vitro (human cells)	Lung inflammation	Investigated the mechanism of inflammatory processes in the respiratory tract as well as the cellular targets for DEP.	Found that the phagocytosis of DEP by primary alveolar macrophages or macrophage cell lines, RAW 264.7 and THP-1, leads to the induction of apoptosis through generation of reactive oxygen radicals (ROR). The apoptotic effect on macrophages is cell specific, because DEP did not induce similar effects in nonphagocytic cells. DEP that had their organic constituents extracted were no longer able to induce apoptosis or generate ROR. The organic extracts were, however, able to induce apoptosis. DEP chemicals also induced the activation of stress-activated protein kinases, which play a role in cellular apoptotic pathways. Conclusions: Organic compounds contained in DEP may exert acute toxic effects via the generation of ROR in macrophages.
Hiura et al. (2000)	In vitro (human cells)	cytotoxic, inflammatory effects	Investigated the cytotoxic and proinflammatory effects of DEP in the respiratory tract.	Found that DEP chemicals induce apoptosis in macrophages via a toxic effect on mitochondria.

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Ito et al. (2000)	Rats	Lung inflammation	Investigated proinflammatory effects of DEP in the respiratory tract. Tested the hypothesis that instillation of DEP induces formation of peroxynitrite in cells migrated in lung. Rats were intratracheally instilled with DEP suspension (2 mg/0.5 ml/kg) and killed 24 h later. Alveolar cells were collected by broncho-alveolar lavage.	The results indicate that DEP exposure results in peroxynitrite formation in migrated cells, which leads to pulmonary inflammation.
Johnston et al., (2000).	Rats	Inflammation	Used UF Teflon (PTFE) fumes (count median particle size ~ 16 nm) to test three hypotheses: (i) uf PTFE (polytetrafluoroethylene) fume particles are causally involved in the induction of acute lung injury, (ii) uf PTFE elicit greater pulmonary effects than larger sised PTFE accumulation mode particles, and (iii) preexposure to the UF PTFE fume particles will induce tolerance.	Teflon fumes at ultrafine particle concentrations of 50 mg/m^3 were extremely toxic to rats when inhaled for only 15 min. When generated in argon, the ultrafine Teflon particles alone are not toxic at these exposure conditions; neither were Teflon fume gas-phase constituents when generated in air. Only the combination of both phases when generated in air caused high toxicity, suggesting either the existence of radicals on the surface or a carrier mechanism of the ultrafine particles for adsorbed gas compounds. Aging of the fresh Teflon fumes for 3.5 min led to a predicted coagulation to >100 nm particles which no longer caused toxicity in exposed animals. This study shows the importance of preexposure history for the susceptibility to acute ultrafine particle effects.

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Juvin et al., (2002).	In vitro (human lung epithelial cells)	Inflammation	Investigated whether diesel exhaust particles lead to an internalisation process and alter the production of proinflammatory cytokines, such as interleukin-8 and granulocyte macrophage-colony-stimulating factor by human alveolar type II cells. Cells from the human lung epithelial cell line A-549 were incubated with diesel exhaust particles or with inert particles for different periods of time. Phagocytosis was studied with electron microscopic analysis and flow cytometry. Cytokines were quantified in supernatants with enzyme-linked immunosorbent assay.	Both diesel exhaust particles and inert particles were similarly engulfed by alveolar type II cells. Diesel exhaust particles induced a dose- and a time-dependent increase in granulocyte macrophage-colony-stimulating factor release and a transient inhibition of interleukin-8 release, but inert particles did not. Diesel exhaust particles were taken up by alveolar type II cells, and they altered cytokine production. Alveolar type cells, therefore, may represent a target site for the deleterious effects of diesel exhaust particles.
Juvin et al., (2002).	In vitro (rat alveolar cells)	Immune response to infection and allergens	Investigated the effect of DEPs on the production of phosphatidylcholine (PC), a major constituent of surfactant, by rat alveolar type II (ATII) primary cells in vitro.	The results demonstrate that incubation of ATII cells with DEPs lead to a time- and dose-dependent increase in labelled PC release. This effect was mimicked by nitric oxide (NO) donors and cGMP and was abolished by inhibitors of NO synthase (NOS). In addition, a NOS inhibitor inhibits by itself the basal secretion of PC. Examination of the effects of DEPs on NOS gene expression showed that DEPs increase NO production and upregulate both protein content and mRNA levels of the inducible NOS (NOS II). Conclusions: DEPs alter the production of surfactant by ATII cells through a NO-dependent signalling pathway.

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Kawasaki et al. (2001)	In vitro (human cells)	Lung inflammation	Investigated proinflammatory effects of DEP in the respiratory tract. Studied the effects of several components extracted from DEPs on interleukin (IL)-8 expression in human bronchial epithelial cell line BEAS-2B and normal human airway epithelial cells obtained from very peripheral airways by an ultrathin bronchoscope. Used several agents active on signal transduction pathways in cytokine expression, such as the protein kinase C inhibitor staurosporin, antioxidant agents including N-acetyl cysteine (NAC) and pyrrolidine dithiocarbamate (PDTC), and p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580.	Found that DEPs augmented the production of inflammatory cytokines by human airway epithelial cells in vitro. Benzene-extracted components showed effects mimicking DEPs on IL-8 gene expression, release of several cytokines (IL-8; granulocyte macrophage colony-stimulating factor and regulated on activation, normal T cells expressed and secreted) and nuclear factor (NF)-kappaB activation. Also found that NAG, PDTC, and SB203580 suppressed the activities of DEPs and their benzene extracts, suggesting the roles of oxidants-mediated NF-KB activation and p38MAPK pathways. Finally, benzo[a]pyrene, one of the important compounds included in the benzene component, replicated the activities shown by DEPs.
Kim et al., (2003).	In vitro (collagen gel model)	Inflammation	The three-dimensional collagen gel contraction model was used to assess that ultrafine carbon particles UFC) could affect tissue repair.	The results demonstrate the ability of ultrafine particles to contribute to altered tissue repair and extend the known mechanisms by which these biologically active particles exert their effects.

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Koike et al., (2002).	In vitro (human epithelial cells)	Inflammation	To characterize the effect of the DEP extract on AM systematically, analysed the gene expression in AM exposed to DEP extract using the Atlas Rat Toxicology Array II. The finding in cDNA microarray was further confirmed by Northern blot analysis. AM were exposed to 10 mg/ml of DEP extract for 6 h in order to elucidate early response to DEP extract in AM.	The transcription of 6 genes in the cDNA microarray was significantly elevated by exposure of the AM to DEP extract. These genes were haem oxygenase (HO)-1 and -2, thioredoxin peroxidase 2 (TDPX-2), glutathione S- transferase P subunit (GST-P), NAD(P)H dehydrogenase, and proliferating cell nuclear antigen (PCNA). The antioxidative enzymes such as HO, TDPX-2, GST-P, and NAD(P)H dehydrogenase may play a role in the pulmonary defence against oxidative stress caused by various pollutants including DEP. PCNA may have contributed to the repair of DNA damage and to cell proliferation caused by, exposure to these pollutants.
Kreyling et al., (2002).	rats	Pulmonary	Tested the hypothesis that UFP may translocate from deposition sites in the lungs to the systemic circulation. Ultrafine Ir-192 radio- labelled particles (15 and 80 nm) were inhaled by young adult, healthy, male WKY rats ventilated for 1 h via an endotracheal tube. After exposure, excreta were collected quantitatively. At time points ranging from 6 h to 7 d, rats were sacrificed, and a complete balance of Ir-192 activity retained in the body and cleared by excretion was determined gamma spectroscopically.	The study indicates that only a rather small fraction of ultrafine iridium particles has access from peripheral lungs to systemic circulation and extrapulmonary organs. Therefore, the hypothesis that systemic access of ultrafine insoluble particles may generally induce adverse reactions in the cardiovascular system and liver leading to the onset of cardiovascular diseases needs additional detailed and differentiated consideration.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Li et al., (2002b)	In vitro (epithelial-cells)	Inflammation	Studied the mechanism of proinflammatory effects in the respiratory tract due to exposure to diesel exhaust particles (DEP) This effect is related to the particle content of redox cycling chemicals and is involved in the adjuvant effects of DEP in atopic sensitisation.	Demonstrated that organic chemicals extracted from DEP induce oxidative stress in normal and transformed bronchial epithelial cells, leading to the expression of haem oxygenase 1, activation of the c-Jun N-terminal kinase cascade, IL-8 production, as well as induction of cytotoxicity. The results show that epithelial cells exhibit a hierarchical oxidative stress response that differs from that of macrophages by more rapid transition from cytoprotective to cytotoxic responses.
Linnainmaa et al., (1997).	In vitro (rat liver epithelial cells)	Cytotoxicity	The in vitro cytotoxicity and the induction of micronuclei of two ultrafine titanium dioxide (TiO_2) samples was assessed in a rat liver epithelial cell (RLE) assay. Pigmentary TiO_2 was used as a control particle, and mitomycin C, a potent inducer of chromosome damage, was used as a positive control agent in the micronucleus experiments.	Neither of the ultrafine TiO_2 samples was toxic to the cells at the concentration range of 5-200 $\mu g/cm^2$. All samples had a slight decreasing effect on the frequency of micronuclei at the lowest treatment concentration of 5 $\mu g/cm^2$. The results suggest that ultrafine particles, similar to pigmentary TiO_2, have no direct clastogenic potential.
Madden et al. (2000)	Rats	Inflammation	Examined whether ozone can directly react with and affect DEO bioactivity. Exposed DEP to ozone in a cell-free in vitro system and then examined the bioactivity of the resultant DEP in a rat model of lung injury.	The results suggest that ambient concentrations of O-3 can increase the biological potency of DEP. The ozonised DEP may play a role in the induction of lung responses by ambient PM.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Maejima et al. (2001)	Mice	Immune responses	Assessed the potential enhancement by DEP of immune responses in mice. Female BDF1 mice (60 mice in each group) were exposed to diesel exhaust (particles, 3.24 mg/m^3; nitrogen dioxide, 1.0 ppm: DE group), Kanto loam dust (particles, 3.29 mg/m^3; nitrogen dioxide, 0.01 ppm: KLD group), diesel exhaust without particles (particles, 0.01 mg/m^3; nitrogen dioxide, 1.1 ppm: DEG group), or clean air (pollen and control groups) for 16 h/day, 5 days/wk for 24 wk, as well as to Japanese cedar pollen (JCP) (around 550,000 grains of JCP/m^3) for 2 days/wk in the same period. The control group was exposed to clean air alone throughout the experiment.	The results suggest that these air pollutants (DE, KLD, and DEG) enhance the production of IgE antibodies in mice, with similar adjuvant activities in each case. The fine particles and gas components are considered to have exhibited different enhancing mechanisms in mice as follows: (1) The fine particles augmented production of IgE antibodies through activation of T lymphocytes, and (2) the gas components exhibited almost no action on T lymphocytes, but directly induced disorders of the cytokine network and augmented the production of IgE antibodies.
Marano et al., (2002).	In vitro	Inflammation	The mechanisms of proinflammatory response induced by DEPS were elucidated using a human epithelial cell line (16-HBE). The obtained results give biological plausibility to the epidemiological findings.	Found that DEPs can be phagocytosed by HBE cells, inducing the release of cytokines. MAP kinase pathways (i.e., ERK1/2 and P38) were triggered as well as the activation of the nuclear factor NF-kappaB. Reactive oxygen species (ROS) were strongly incriminated in this response because DEPs induce the increase of intracellular hydroperoxides and antioxidants inhibit the release of DEP-induced cytokines, the activation of MAP kinases and NF-kappaB. Organic compounds adsorbed on DEPs seemed to be involved in the response and the production of ROS. Moreover, results show that DEPs can activate CYP1A1 in HBE cells.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Matsuo et al. (2001)	In vitro (human leukemic promyelocytic cells)	cytotoxicity	Studied the cytotoxicity of diesel exhaust particles (DEPs) toward human leukemic promyelocytic cells HL-60.	DEPs were found toxic and cytotoxicity increased in a dose-dependent manner. The results suggest that the cytotoxicity results from generation of reactive oxygen species by DEPs, which have been incorporated into cells.
Miller et al., (2001).	Mice	Inflammation	NiO or $NiSO_4$ aerosols were administered to C57BL/6J mice by intratracheal instillation or whole-body inhalation to study the effect of submicrometre particles on pulmonary injury. Bronchoalveolar lavage fluid was collected 18 hr after instillation and analysed for total and differential cell counts, cell viability, and total protein. For inhalation experiments, an acute, whole-body exposure was conducted, exposing mice to 6 - 72 hr of continuous submicrometre NiO aerosol (d(pg) = 50 nm; 340 µg Ni/m^3 or 24 - 72 hr of $NiSO_4$ aerosol (d(pg) = 60 nm; 420 µg Ni/m^3; d(pg) = 250 nm; 480 µg Ni/m^3.	Exposure to NiO produced no significant lung injury when either instilled or inhaled, whereas inhaled $NiSO_4$ caused significant increases in protein content

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Minami et al. (1999)	guinea-pigs.	Cardiac	Investigated the mechanisms of cardiac arrest due to exposure to DEP. Examined the systemic effects of DEP on electrocardiographic (ECG) changes using guinea-pigs.	Found that intravenously administered dimethyl sulfoxide (DMSO) extract of DEP solution induced arrhythmias and deaths via complete atrioventricular (AV) block in guinea pigs. Fractions of DEP extracted by hexane, ethanol or methanol, 4-hydroxyphthalic acid 2-methyl ester, a compound isolated from methanol extract of DEP did not induce significant ECG changes in guinea pigs. As compared with fresh DEP solution, the DMSO/DEP solution used in the present study induced similar cardiac toxicity after being stored in a freezer at 4 degrees C for 3 days. Conclusions: Stable and water-soluble fractions of DEP may be responsible for cardiotoxicity.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Moller et al., (2002).	In vitro (alveolar macrophages from beagle dogs (BD-AM); macrophages from the cell line J774A.1	Inflammation	Studied the influence of fine and ultrafine test particles (UFP), such as TiO_2, elemental carbon, commercial carbon black, diesel exhaust particulate matter, and urban dust (UrbD), on cytoskeleton-related functions of macrophages, such as phagocytosis, phagosome transport mechanisms, and mechanical cytoskeletal integrity. The diameter of the test particles ranged from 12 to 220 nm and the Brunauer-Emmet- Teller specific surface area ranged from 6 to 600 m^2/g. Macrophages were exposed in vitro with 10-320 mug UFP/ml/10^6 cells up to 24 h.	While fine TiO_2 did not show any effect, macrophages were sensitive to UFP exposure. Urban dust and DEP (standard reference material 1650) caused comparable cytoskeletal dysfunctions to elemental carbon with high specific surface area. Cytoskeletal dysfunctions induced by DEP or UrbD could be reduced after washing the particles. All cytotoxic parameters showed only weak correlations with the specific surface area or the total number of UFP, which can result from the different types of particles and different surface compositions. Conclusions: UFP cause cytoskeletal toxicity in vitro in macrophages, which can cause cellular dysfunctions, such as impaired proliferation, impaired phagocytic activity, and retarded intracellular transport processes as well as increased cell stiffness and can result in impaired defence ability in the lung.
Mori et al. (2002)		Estrogenic activity	Studied estrogenic activity of the hexane extract of diesel exhaust particles (DEP).	Found that the neutral fraction of the hexane extract of DEP contains dibenzothiophene derivatives, one of which, 4,6-dimethyidibenzothiophene, possesses estrogenic activity.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Murphy et al. (1999)	In vitro (human cells)	Inflammation	Investigated the mechanisms of particle toxicity to the lung. The bioreactivity of carbon black (CB; 50, 40, 30, and 20 nm) and diesel exhaust particles (DEP, 30 nm) were examined with primary cultures of Clara and type II epithelial cells. All particle samples had different surface chemical compositions.	Bioreactivity was found to be related to CB particle size and hence surface area: the smaller the particle and larger the surface area, the more toxic the particles. Also, CB particles with the most complicated surface chemistry were the most bioreactive. Freshly prepared DEPs were equally toxic to type II and Clara cells and they became progressively less toxic to the type II cells with time. With all CB and DEPs, the primary epithelial cells internalised the particles, although this was noted most in cells of low functional competence.
Murphy et al., (1998).	In vitro (lung epithelium cells)	Inflammation	The comparative toxicological effects of diesel exhaust and other well-characterised particles (carbon black, amorphous and crystalline silica) on rat respiratory epithelium were investigated in the present study. The effects of small masses of particles (1 mg) delivered by intratracheal instillation were monitored by changes in components of lavage fluid.	Respirable, crystalline quartz, produced significant increases in lung permeability, persistent surface inflammation, progressive increases in pulmonary surfactant and activities of epithelial marker enzymes up to 12 weeks after primary exposure. Ultrafine amorphous silica did not induce progressive effects but it promoted initial epithelial damage with permeability changes and these regressed with time after exposure. By contrast, ultrafine/fine carbon black had little effect on lung permeability, epithelial markers or inflammation, despite being given at a dose, which readily translocated the epithelium. Similarly, diesel exhaust particles produced only minimal changes in lavage components. It is concluded that diesel exhaust particles are less

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				damaging to respiratory epithelium than silicon dioxide and that the surface chemistry of a particle is more important than ultrafine size in explaining its biological reactivity.
Nemmar et al., (1999).	Guinea-pigs	Inflammation	The effects of ultrafine polystyrene carboxylate-modified (fluorospheres) on inflammatory processes are being investigated in rabbit lungs. One millilitre of sterile NaCl (0.9%) containing 4 mg of ultrafine particles (UFP) was intratracheally instilled into anaesthetised rabbits. The control animals were only instilled with sterile NaCl (0.9%).	The results indicate that chemically inert, electrically charged UFP induce a pulmonary inflammatory process during which the release of SP and histamine from C- fibres and mast cells was enhanced after various stimuli. These latter mediators can also modulate the inflammatory process.

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Nemmar et al., (2001).	Hamsters	Cardiovascular	Studied the passage of radioactively labelled ultrafine particles after their intratracheal instillation. Hamsters received a single intratracheal instillation of 100 mug albumin nanocolloid particles (nominal diameter less than or equal to 80 nm) labelled with 100 mu Ci technetium-99m and were killed after 5, 15, 30, and 60 min.	In blood, radioactivity expressed as percentage of total body radioactivity per gram blood, amounted to 2.88 +/- 0.80%, 1.30 +/- 0.17%, 1.52 +/- 0.46%, and 0.21 +/- 0.06% at 5, 15, 30, and 60 min, respectively. In the liver, radioactivity amounted to 0.10 +/- 0.07%, 0.23 +/- 0.06%, 1.24 +/- 0.27%, and 0.06 +/- 0.02% at 5, 15, 30, and 60 min, respectively. Lower values were observed in the heart, spleen, kidneys, and brain. Dose dependence was assessed at 30 min following instillation of 10 µg and 1 µg Tc-99m-albumin per animal (n = 3 at each dose), and values of the same relative magnitudes as after instillation of 100 mug were obtained. Conclusions- significant fraction of Tc-99m- albumin, taken as a model of ultrafine particles, rapidly diffuses from the lungs into the systemic circulation.
Nemmar et al., (2002b)	Hamster	Cardiovascular	Studied the effect of ultrafine (60 nm) polystyrene particles on thrombus formation in a hamster model after intravenous and intratracheal administration of unmodified, carboxylate-polystyrene, or amine-polystyrene particles.	The results suggest that the presence of UFP in the circulation may affect hemostasis. The observed in vivo prothrombotic tendency resulted from platelet activation by positively charged amine-polystyrene particles.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Oberdorster (2000)	Rats	Pulmonary inflammation	Exposure of rats to laboratory-generated ultrafine carbonaceous (elemental, and organic, carbon) particles was carried out at a concentration of ca. 100 $\mu g/m^3$ for 6 h. Modulating factors of responses were prior low-dose inhalation of endotoxin in order to mimic early respiratory tract infections, old age (22- month old rats versus 10-week old rats) and ozone co-exposure.	Found that (i) ultrafine carbon particles can induce slight inflammatory responses (ii) LPS priming and ozone co-exposure increase the responses to ultrafine carbon; (iii) the aged lung is at increased risk for ultrafine particle-induced oxidative stress. Studies with ultrafine and fine TiO_2 showed that the same mass dose of ultrafine particles has a significantly greater inflammatory potential than fine particles. Conclusions: The increased surface area of ultrafine particles is a most important determinant for their greater biological activity. The propensity of ultrafine particles to translocate may result in systemic distribution to extrapulmonary tissues.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Oberdorster et al., (2002a)	Rats	Inflammation	Determined whether ultrafine elemental carbon particles translocate to the liver and other extrapulmonary organs following inhalation as singlet particles by rats. Ultrafine C-13 particles were aerosolised (CMDs of 20-29 nm (GSD 1.7)). Nine Fischer 344 rats were exposed to these particles for 6 h. in whole-body inhalation chambers at concentrations of 180 and 80 $\mu g/m^3$ animals each were killed at 0.5, 18, and 24 h postexposure. Six unexposed rats served as controls. Lung lobes, liver, heart, brain, olfactory bulb, and kidney were excised, homogenised, and freeze-dried for analysis of the added C-13 by isotope ratio mass spectrometry.	The results demonstrate effective translocation of ultrafine elemental carbon particles to the liver by 1 d after inhalation exposure. Translocation pathways include direct input into the blood compartment from ultrafine carbon particles deposited throughout the respiratory tract. However, since predictive particle deposition models indicate that respiratory tract deposits alone may not fully account for the hepatic C-13 burden, input from ultrafine particles present in the GI tract needs to be considered as well. Such translocation to blood and extrapulmonary tissues may well be different between ultrafine carbon and other insoluble (metal) ultrafine particles.
Osier & Oberdorster (1997)	Rats	Inflammation	Compared the response of rats exposed by intratracheal inhalation to "fine" (similar to 250 nm) and "ultrafine" (similar to 21 nm) titanium dioxide particles with rats exposed to similar doses by intratracheal instillation.	Animals receiving particles through inhalation showed a decreased pulmonary response, measured by bronchoalveolar lavage parameters, in both severity and persistence, when compared with those receiving particles through instillation. These results demonstrate a difference in pulmonary response to an inhaled vs an instilled dose, which may be due to differences in dose rate, particle distribution, or altered clearance between the two methods.

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Pacheco et al., (2001).	In vitro (human airway epithelial-cells)	Inflammation	Examined the effect of DEP on IL-10 and TGF-beta, cytokines produced by macrophages and repressor (Tr-like) lymphocytes, which influence tolerance. Human PBMCs (n = 22) were incubated with 1-100 ng/ml of DEP, and suboptimally primed with LPS. IL-10 gene expression was assessed by the S1 nuclease protection assay, and production of IL-10, TGF-beta, TNF-alpha, IL-1beta and IL-4 stimulated CD23 was evaluated by ELISA after 24 and 48 h. The effect of the order of exposure to DEP and LPS was evaluated on IL-10 protein and mRNA in cells (1) preincubated with LPS followed by DEP, or (2) exposed first to DEP followed by LPS. IL-10 was further evaluated using benzo[a]pyrene and [alpha] naphthoflavone as a surrogate for the polyaromatic hydrocarbons (PAHs) adsorbed to DEP. Control cells were incubated with carbon black, without PAHs.	In PBMCs exposed to DEP with LPS, or preincubated with LPS before DEP, IL-10 production and mRNA fall significantly. TGF-beta is similarly suppressed, IL-1beta secretion is significantly stimulated, and IL-4 stimulated CD23 release rises in the atopic subjects. In contrast, when DEP is added prior to LPS, IL-10 production rises, and IL-1beta falls to zero. These effects on IL-10 are reproduced with benzo[a]pyrene and reversed by the coaddition of [alpha] naphthoflavone, its known antagonist. The carbon black fraction has no effect on IL-10 production. The effect of DEP on IL-10 can be inhibitory or stimulatory, depending on the order of exposure to DEP and LPS. Pro-inflammatory cytokines and factors rise when IL-10 is inhibited, and are suppressed when IL-10 is stimulated. These results are duplicated with benzo[a]pyrene, suggesting that the PAH portion of the DEP is the active agent.

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Reibman et al., (2002).	In-vitro (bronchial-epithelial-cells)	Inflammation	Assessed hypothesis that ambient PM of different size fractions collected from an urban environment (New York City air), would activate primary culture human bronchial epithelial cells (HBECs). Because of the importance of granulocyte-macrophage colony-stimulating factor (GM-CSF) on inflammatory and immunomodulatory processes, the study was focused on this cytokine.	Demonstrated that the smallest size fraction (ultrafine/fine; <0.18 μm) of ambient PM (11 $\mu g/m^2$), upregulated GM-CSF production (2-fold increase). The absence of effect of carbon particles of similar size, and the day-to-day variation in response, suggested that the chemical composition, but not the particle itself, was necessary for GM-CSF induction. Activation of the extracellular signal-regulated kinase and the p38 mitogen-activated protein kinase was associated with, and necessary for, GM-CSF release. These studies serve to corroborate and extend those on model particles. Moreover, they emphasize the role of the smallest size ambient particles in airway epithelial cell responses.
Rengasamy et al., (2003).	Rats	Inflammation	In this study, the effect of acute exposure of DEP on phase I and phase II enzymes of rat lung was investigated.	The study suggests that DEP may induce CYP1A1 and QR enzymes via a chemical effect, while the carbonaceous core may he involved in the attenuation of CYP2B1, GST, and catalase proteins and enzyme activities.

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Renwick et al., (2001a)	In vitro (macrophage cell line)	Inflammation	Investigated whether slowed clearance after exposure to ultrafine particles was due to a failure in alveolar macrophage phagocytosis. Particles utilised were fine titanium dioxide (TiO_2), ultrafine titanium dioxide ($UTiO_2$), carbon black (CB), or ultrafine carbon black (UCB), Cytotoxicity of particles was measured by means of MTT activity.	The results demonstrated that ultrafine particles impair macrophage phagocytosis to a greater extent than fine particles compared on a mass basis. Conclusions: Slowed clearance of particles, specifically the ultrafines, can in part be attributed to a particle-mediated impairment of macrophage phagocytosis.
Reynolds et al. (2000)	In vitro (human cells)	Inflammation	Studied bioreactivity of DEP using nonspecific (cell protein) and specific cell surface markers (gamma glutamyl transpeptidase and rT1(40) for type II and type I cells, respectively).	Both cell types proved resilient to all fractions of DEP analysed. Little difference in bioreactivity was observed between nonmodified and modified particles. However, more concentrated samples of soluble components removed from the DEP did contribute in part to the toxicity observed by DEP.
Reynolds & Richards (2001)	Rats		Study on acute up- or down-regulation of genes that are taking place in the rat lung in response to the small instilled mass of DEP.	DEP instillation caused a slight oedematous lung with no overt inflammation and ten out of a possible 207 (5%) rat stress acnes were repeatedly changed in response to DEP instillation. Conclusions: DEP elicits a low bioreactive response in a healthy rat lung.
Rudra-Ganguly et al., (2002).	In vitro (bronchial epithelial-cells)	Inflammation	Investigated the role of DEP extract and associated polycyclic aromatic hydrocarbons (PAHs) on prostaglandin synthesis in endotoxin-activated murine macrophages and in mitogen-stimulated fibroblasts.	Found that DEP and PAHs do not affect ligand-induced COX-2 gene expression, phospholipase activation, or arachidonic acid release in macrophages and fibroblasts but exert their inhibitory effect on prostaglandin production by preferentially blocking COX-2 enzyme activity.

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Sadakane et al (2002)	Mice	Allergic airway inflammation	Investigated airway inflammation induced by diesel exhaust particles and house dust mite allergen	The results suggest that the murine strain differences in the pathogenesis of allergic airway disease caused by mite allergen might be related to the local expression of the cytokines we screened. The aggravating effect of DEP may be mediated by an increase in the local expression of IL-5, RANTES, eotaxin, and the production of an antigen specific to IgG1.
Saito et al., (2002a)	Mice	Inflammation	Investigated the effect of diesel exhaust (DE) on cytokine expression in marine lung tissues. BALB/c mice were exposed to DE for 1 month at different dose levels of DE (low dose: diesel exhaust particles [DEP] 100 $\mu g/m^3$ high dose: 3 $\mu g/m^3$.	The results suggest that DE alters immunological responses in the lung and may increase susceptibility to pathogens, and that increased IL-4 expression by low-dose DE exposure may induce allergic reaction such as asthma.
Saito et al., (2002b)	In vitro (mouse alveolar macrophages)	Inflammation	Investigate the effects of diesel exhaust particles (DEP) and mycobacterial injection on macrophages by examining protein and mRNA expression levels of various cytokines, including tumour necrosis factor-alpha (TNF-alpha), interleukin (IL)- 1beta, IL-12, and IL-18 in BALB/c mouse alveolar macrophages (AM) and a macrophage cell line (RAW264.7)	The results show that DE exposure has complex and diverse effects on cytokine production by AM, and that longer exposure (> 8 hours) may suppress cytokine production by AM in vitro. Longer exposure of DE may therefore suppress the host defence in the lung and may increase susceptibility to lung infections such as mycobacterial infection.

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Sato et al. (1999)	Rats	Pulmonary mutagenicity	Investigated pulmonary carcinogenesis mutagenicity of DEP. F344 rats were exposed to DEP 6 $\mu g/m^3$ for 4 weeks. Oncogenes and related genes expressed in their lungs were surveyed using a cDNA microarray technique. Results were confirmed by northern blot analysis.	Expression of A-raf and proliferating cell nuclear antigen (PCNA) mRNAs was induced in rat lung by exposure to DE. These results suggest that A-raf and PCNA might contribute to pulmonary carcinogenesis in rats.
Stearns et al., (2001).	Rats	Inflammation	Used an in vitro model of type II lung epithelium to evaluate the cells' ability to take up ultrafine particles (titanium dioxide [TiO_2], 50 nm diameter). The human epithelial cell line A549 was grown on aclar substrates and exposed to 40 mug/ml TiO_2 particles for 3, 6, and 24 h before imaging with energy-filtering transmission electron microscopy.	After 3 h of TiO_2 exposure, cells internalised aggregates of the ultrafine particles which were observed in cytosolic, membrane- bound vacuoles. After 24 h of exposure there were considerably more intracellular aggregates of membrane-bound particles, and aggregated particles were also enmeshed in loosely and tightly packed lamellar bodies. Throughout 24 h of exposure a preponderance of particles remained associated with the free surface of the cells and were not internalised. The majority of membrane-bound vacuoles contained aggregates of particles and only occasionally did they contain as few as two or three particles, despite the use of several different approaches to assure the possibility for individual particles to be ingested and detected. There was morphologic evidence of microfilament disturbance, but no evidence of a decrease in internalised particles in cells pretreated with cyto D.

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Stone et al., (2000).	Rats	Inflammation	Investigated whether UFP could invoke alterations in calcium influx in both macrophage cell lines and primary macrophages.	Effects on calcium fluxes induced by thapsigargin were seen with two very different ultrafine particles ultrafine latex beads and ultrafine CB- and were seen in both the human MM6 cell line and rat BAL cells. The induction of an oxidative stress by the ultrafine particles was supported by the ability of ultrafine latex beads to induce ROS production. Ultrafine carbo, black was round to induce enhanced calcium influx, partly through oxidative stress.
Takano et al., (2002a)	Mice	Inflammation	Determined whether acute inhalation exposure to DEP induced the expression of Cyp 1A1 in murine lung.	The results suggest that the lung expression of Cyp 1A1 can be a biomarker of acute inhalation exposure to DEP and may be implicated in an accelerated production of ROS and the subsequent aggravation of lung injury.
Takano et al., (2002b)	Mice	Inflammation	To provide experimental evidence for the epidemiological data, determined the effects of diesel exhaust particles (DEP) on lung injury related to bacterial endotoxin in mice.	These results provide the first experimental evidence that DEPs enhance neutrophilic lung inflammation related to bacterial endotoxin. The enhancement is mediated by the induction of proinflammatory molecules, likely through the expression of Toll-like receptors and the activation of p65-containing dimer(s) of NF-kappaB, such as p65/p50.

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Takenaka et al., (2000).	Rats, In vitro (macrophage cells)	Inflammation	Investigated the fate of agglomerated ultrafine particles in macrophages in vitro and in vivo. Metallic silver (Ag) was used as a test particle. For the in vitro study, J774 macrophage cell suspensions (200,000 cells in 400 µl medium) were plated in small chambers. Six hours later, 100 mu l of the silver-PBS suspension was added to each chamber. For the in vivo study using F344 rats, 50 µg Ag particles were instilled intratracheally. On days 1, 4, and 7 following instillation, rats were sacrificed and the lungs were examined morphologically.	Both, in vitro and in vivo studies suggested that agglomerated Ag particles remained in targets for a given period of time-at least up to 7 days.
Takenaka et al., (2001b)	Rats	Cardiovascular	Pulmonary and systemic distribution of inhaled ultrafine elemental silver (EAg) particles was investigated on the basis of morphology and inductively coupled plasma mass spectrometry (ICP-MS) analysis. Rats were exposed to EAg for 6 hr at a concentration of 133 $\mu g/m^3$ (3×10^6 cm^3, 15 nm modal diameter) and were sacrificed on days 0, 1, 4, and 7	Found that although instilled agglomerates of ultrafine EAg particles were retained in the lung, Ag was rapidly cleared from the lung after inhalation of ultrafine EAg particles, as well as after instillation of $AgNO_3$, and entered systemic pathways.

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Takizawa et al. (1999a)	In vitro (human bronchial epithelial cells)	inflammatory responses in the lung	Studied IL-8 gene expression, one of the important cytokines in inflammatory responses, by Northern blot analysis and run-on transcription assay.	DEPs have a potential to directly activate airway epithelial cells to produce and release inflammatory cytokines and mediators, and thus facilitate inflammatory responses in the lung. The results suggest that DEP activate NF-kappa B, which might be an important mechanism of its potential to increase the expression of inflammatory cytokines in vitro.
Takizawa et al. (2000)	In vitro (human bronchial epithelial cell)	Inflammation	Studied the effect of DEP on ICAM-1 (ICAM-1 plays an important role in the local accumulation of inflammatory cells) gene expression and surface expression in human bronchial epithelial cell line BEAS-2B.	DEP (5-50 µg/ml) showed a stimulatory effect on ICAM-1 mRNA levels. The results suggest that DEP induce up-regulation of ICAM-1 gene and this process might be largely dependent on oxidant- mediated NF-kappa B activation and p38-MAPK pathways.
Taneda et al. (2000)	In vitro (human cells)	Estrogenic and anti-estrogenic activities	Estrogenic and anti-estrogenic activities of diesel exhaust particles (DEP) were evaluated using yeast cells expressing the human oestrogen receptor and the responsive element regulating the expression of the receptor gene for beta -galactosidase.	Found that a suspension of whole DEP suspension is not estrogenic but that this preparation possesses the ability to reduce the oestrogen-dependent reporter activity. Conclusions- DEP contains heterologous compounds having anti-estrogenic activity. It is thought that those compounds in DEP can modulate the activity of oestrogen, leading to the disruption of balance between oestrogen and androgen.
Taneda et al., (2002).	NA	Estrogenic activity	Estrogenic and anti-estrogenic activities of two types of DEP, type-1 (old type) and type-2 (new type) were compared.	Found that both type-1 and type-2 DEP possess estrogenic and anti-estrogenic activities.

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Timblin et al., (2002).	In vitro (alveolar epithelial cells)	Inflammation	Demonstrated the development of dose-related proliferation and apoptosis after exposure of an alveolar epithelial cell line (C10) to PM or to ultrafine carbon black (UFCB), a component of PM	Found that the ultrafine particle component of PM is critical to its biological activity.
Tokiwa et al. (1999)	Lung tissues obtained from lung cancer patients	Carcinogenic	Investigated the fate of organic chemicals and carbon particles in the lungs in order to determine the mechanisms responsible for lung tumours.	The results suggest that carbonaceous particles, but not mutagens and carcinogens, promote the formation of 8-OHdG, and that as a mechanism, alveolar macrophages may be involved in oxidative damage. The oxidative damage may be due to the fact that the mutation is involved with the generation of a hydroxyl radical during phagocytosis, and the hydroxyl radical leads to hydroxylation at the C-8 position of the deoxyguanosine residue in the DNA.
Tokiwa & Sera (2000)	Lung specimens of patients with carcinomas	Carcinogenic	Investigated contribution of PAH in DEP to human lung cancer induction. Lung specimens of 112 patients with carcinomas were divided into two groups of higher and lower chemical concentrations and the findings were statistically analysed by adjusting for age, gender, stage, and smoking status and cell type.	The results suggest that tumours can be induced by continuous deposition of small amounts of environmental carcinogens in human lungs. Formation of 8-hydroxyguanosine (8-OHdG) is normally used as a biomarker of oxidative Conclusions: Carbonaceous particles, but not mutagens or carcinogens, promote the formation of 8-OHdG, and that as a mechanism, alveolar macrophages may be associated with oxidative damage, involving the generation of a hydroxyl radical during phagocytosis in the lungs.

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Tsukue et al., (2002).	Mice	Allergic airway inflammation	To clarify the toxic effects of diesel exhaust (DE) on delivery in mice and on growth of young, C57Bl-strain females were exposed to 0.3, 1.0, or 3.0 mg diesel exhaust particles (DEP)/m^3 or filtered clean air (control) for 4 mo (12 h/day, 7 days/wk), After exposure, some females from each group were examined by necropsy, and the remainders were mated with unexposed males.	The results show that toxic substances in DE might cause abnormal delivery in mice, and that exposed females affected the growth and sexual maturation of their young.
Tsurudome et al. (1999)	Rats	Carcinogenic	Investigated carcinogenic mechanism of DEP. Examined the levels of 8- hydroxyguanine (8-OH-Gua), its total repair and the repair enzyme OGG1 mRNA in female Fischer 344 rat lungs, as markers of the response to ROS, after DEP was intratracheally instilled.	The 8-OH- Gua level in rat lung DNA increases markedly at an early phase after DEP exposure, by the generation of ROS and the inhibition of 8-OH-Gua repair activity.
Ushio et al. (1999)	In vitro (human cells)	Inflammation	Investigated the effect of DEP and formaldehyde (FA), on the production of pro-inflammatory cytokines (interleukin (IL)-1 alpha, IL-1 beta, tumour necrosis factor (TNF)-alpha and IL-8) by normal human dermal keratinocytes (hKCs).	These in vitro findings suggest that DEP may act as modulating factors of cutaneous inflammation by affecting the ability of keratinocytes to release pro-inflammatory cytokines.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
van Zijverden et al. (2000)	Mice	Immune responses	The study examined different particles, diesel exhaust particles (DEP), carbon black particles (CBP), and silica particles (SIP) for their immunomodulating capacity in both primary and secondary immune responses in female BALB/C mice.	It appeared that all particles acted as adjuvant, but the different particles stimulated distinct types of immune responses to TNP-OVA. It is concluded that DEP are able to skew the immune response toward the T helper 2 (Th_2) side, whereas SIP stimulate a Th1 response and CBP have a mixed activity, stimulating both Th_1 and Th_2 responses in this model
Veronesi & Oortgiesen (2001)	In vitro (human tracheal-bronchial epithelial cells)	Neurogenic inflammation	In this study, selected physicochemical characteristics (i.e., size, particle number, acidity, and surface charge) were measured on various field PM, derived from urban ambient (St. Louis, Ottawa, Canada), residential (Woodstove), volcanic dust from Mt. St. Helen (MSH), and industrial [oil fly ash (OFA) coal fly ash (CFA)l sources. The biological effects (i.e., increases in intracellular calcium ($[Ca_2+](i)$), cytokine release) of their exposure were measured in human, immortalised, tracheal- bronchial epithelial cells (BEAS-2B).	Exposure of BEAS-2B cells to each fraction produced an immediate, but differential increase in $[Ca_2+](i)$ and the subsequent release of the inflammatory cytokine IL-6, 4 and 16 h later. The results indicate that the surface charge (i.e., zeta potential) carried on PM's visible field particles predicts their differential release of the inflammatory cytokine IL-6 in cultures of human respiratory epithelial cells.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Whitekus et al., (2002).	Mice	Allergic inflammation	Tested the hypothesis that reactive oxygen species are involved in the adjuvant effects of diesel exhaust particles (DEP) in a murine OVA sensitisation model. Tested six different antioxidants, N-acetylcysteine (NAC), bucillamine (BUC), silibinin, luteolin, trolox (vitamin E), and ascorbic acid, for their ability to interfere in DEP-mediated oxidative stress in vitro.	The results indicate that NAC and BUC are capable of preventing the adjuvant effects of inhaled DEP and suggest that oxidative stress is a key mechanistic component in the adjuvant effect of DEP.
Wilson et al., (2002).	In vitro	Inflammation	Investigated interactions between transition metal salts and a surrogate environmental particle-ultrafine carbon black (UFCB).	In all experimental systems employed, the ufCB was found to be more reactive than its fine counterpart (CB). The findings suggest that (1) ultrafine particles and metals interact by chemical potentiation in a cell-free environment to generate ROS, (2) potentiation between ultrafine particles and metal salts is not observed in the presence of macrophages as iron is sequestered or chelated by the cells, (3) in the lung, ultrafine particles and iron salts interact in a potentiative manner to generate inflammation.
Yamazaki et al. (2000)	In vitro (human P4501B1)	chemical carcinogenesis	Investigated bioactivation of diesel exhaust particle extracts and their major nitrated polycyclic aromatic hydrocarbon components, 1- nitropyrene and dinitropyrenes, by human cytochromes P450 1A1, 1A2, and 1B1.	The results suggest that environmental chemicals existing in airborne DEP, in addition to 1-NP. 1,6-DNP. 1,8-DNP. 2-NF, and 3-NF, can be activated by human P450 1B1. Biological actions of air pollutants such as nitroarenes to human extrahepatic tissues may be of concern in tissues in which P450 1B1 is expressed.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Yang et al. (1999)	Rats	Lung inhalation	Investigated the effects of diesel exhaust particle (DEP) exposure on alveolar macrophage (AM) response to ex vivo and in vivo lipopolysaccharide (LPS) challenge. The roles of the insoluble particle and the organic compounds of DEP in altering pulmonary responses were evaluated by comparing the DEP-induced pulmonary responses to those of carbon black (CB), a carbonaceous particle with few adsorbed organic compounds, or to silica, a known pneumotoxic dust.	The results indicate that while DEP, CB, and silica all induce pulmonary inflammatory responses due to particle stimulation, only DEP suppress AM cytokine release in response to LPS stimulation. The contrasting cellular response with respect to DEP and CB exposures may be due to the presence of adsorbed organic compounds on DEP, which may contribute to the increased susceptibility of hosts to pulmonary infections after DEP exposure.
Yang et al. (2001)	Rats	susceptibility to pulmonary infection.	Tested the hypothesis that exposure to diesel exhaust particles (DEP) may increase susceptibility of the host to pulmonary infection. Male Sprague-Dawley rats received a single dose of DEP (5 mg/kg), carbon black (CB, 5 mg/kg), or saline intratracheally.	Exposure of rats to DEP, but not to CB, decreased the clearance of Listeria from the lungs. The results showed that exposure to DEP decreased the ability of macrophages to produce antimicrobial oxidants in response to Listeria, which may play a role in the increased susceptibility of rats to pulmonary infection. This DEP-induced suppression is caused partially by chemicals adsorbed onto the carbon core of DEP, because impaired macrophage function and decreased Listeria clearance were not observed following exposure to CB.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Yin et al., (2002b)	In vitro (rat alveolar cells)	Pulmonary immunity	Investigated the effects of DEP exposure on the functions of alveolar macrophages (AMs) and lymphocytes from lung-draining lymph nodes using a rat Listeria monocytogenes infection model. Focused on the effects of DEP exposure on AM functions, including phagocytic activity and secretion of proinflammatory cytokines.	The results suggest that DEPs retard bacterial clearance by inhibiting AM phagocytosis and weaken the innate immunity by inhibiting AM secretion of IL-1beta and TNF-alpha. DEPs may also suppress cell-mediated immunity by inhibiting AM secretion of IL-12, a key cytokine for the initiation of T helper type 1 cell development in Listeria infection.
Yoshida et al. (1999)	Mice	Reproductive system	Investigated the effect of the exposure to diesel exhaust on the male reproductive system of mice.	Ultrastructural changes were observed in Leydig cells of mice exposed to diesel exhaust (0.3 mg diesel exhaust particles (DEP)/m^3 through the airway, 12 h daily, up to 6 months) and reduction in LH receptor mRNA expression in Leydig cells was observed at a concentration of 1 mg DEP/m^3. Daily sperm production per gram of testis dose-dependently decreased with exposure to DE for 6 months; 29%, 36%, and 53% reductions were observed at 0.3, 1.0, and 3.0 mg DEP/m^3, respectively. A no- observed-adverse-effect level (NOAEL) was observed with approximately 30 μg DEP/m^3, which is lower than the WHO-recommended limit.
Yoshida et al., (2002).	Mice	Reproductive system	Investigated the effect of exposure of pregnant mice to diesel exhaust on male gonad development at the level of mRNA expression.	The data indicate that exposure of pregnant mice to diesel exhaust affects the expression of genes essential in the early stages of embryonic development.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Yoshino et al. (2002)	Mice	Immune responses	Investigated the effects of extracts of diesel exhaust particles (DEP) on Th_1 and Th_2 immune responses in mice.	The results showed that treatment with DEP and DIC-DEP increased both Th_1 and Th_2 responses to HEL. UNE-DEP facilitated Th_1 but not Th_2 responses, while MET- and AMM-DEP administration was followed by enhancement of Th_2 but not Th_1 responses. Neither HEX- nor BEN-DEP modulated Th_1 as well as Th_2 responses. These results suggest that DEP contain various compounds different in hydrophobicity, which may affect Th_1 and Th_2, Th_1 but not Th_2, and Th_2 but not Th1 immune responses.
Yoshino & Sagai (1999)	In-vitro (rats, lymph-node cells)	Rheumatoid-arthritis	Investigated the effect of diesel exhaust particles (DEP) on collagen-induced arthritis (CIA), which is an experimental model of autoimmune disease, in mice.	The results showed that administration of DEP enhanced both the incidence and the severity of CIA. The enhancement of the disease was associated with pronounced production of anti-CII IgG and IgG2a antibodies. Treatment with DEP also augmented proliferative responses of spleen cells to CII. These results suggest that exposure to DEP may influence autoimmune disease.
Yoshino & Sagai (1999a)	Mice	Immune responses	Examine whether oral administration of soluble antigen together with diesel exhaust particles (DEP) induced the systemic immune response in mice.	The results suggest that DEP may act as a mucosal adjuvant in the gut enhancing systemic Th_1 and Th_2 immune responses and might play a role in oral immunization and food allergy.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Yoshino et al., (2002).	Mice	Autoimmune arthritis	Investigated the effect of diesel exhaust particles (DEP) extracts on collagen-induced arthritis (CIA) in mice.	Found that DEP and DIC-DEP contain compounds, which enhance both Th_1 and Th_2 responses, while UNE- DEP and AMM-DEP contain chemicals, which augment Th_1 and Th_2 alone, respectively. Th_1- but not Th_2-modulating compounds from DEP, DIC-DEP and UNE-DEP Seem to influence CIA.

APPENDIX D. SUMMARY OF CLINICAL STUDIES ON ULTRAFINE PARTICLES

Table D-1. Dosimetry

Authors Purpose of Study	Subject Details		End Points Measured	Key Results
Nemmar et al 2002 Investigate whether the smallest particle fraction in an inhaled aerosol translocate from the lung to the circulation.	5 male non-smokers. 24 - 47 years age range	[1] Inhalation from a mouthpiece. [2] Technetium-99m-labeled ultrafine carbon aerosol with individual particles of the order of 5 - 10 nm. Subject inhaled approximately 100 MBq of aerosol in 5 breaths.	**Scintigraphy** Static acquisition (1 to 3 minutes) of lungs and thyroid followed by dynamic acquisition (5 to 25 minutes) of the abdomen and successive images of the whole body (50 to 60 minutes) **Peripheral Bood** Samples taken at 1, 5, 10, 20, 30, 45 and 60 minutes after exposure and for each time point sample gamma activity was measured and Thin Layer Chromatography conducted. **Urine** Thin layer chromatography conducted on a urine sample taken 60 minutes after exposure.	**Scintigraphy** 8% of deposited activity accumulated in he liver within 5 minutes after inhalation. Activity progressively increase in the bladder reaching about 25% of deposited activity by 45 minutes **Peripheral Bood** Radioactivity measured in all samples progressively rising with time to a plateau between the 10 minute and 20 minute samples. Thin Layer Chromatography results indicated that ^{99m}Tc bound to carbon particles were present in all blood samples together with a soluble ^{99m}Tc species. **Urine** Thin layer chromatography showed the presence of a soluble ^{99m}Tc species and the absence of any ^{99m}Tc bound carbon particles.

Table D-1. Dosimetry (Continued)

Authors Purpose of Study	Subject Details		End Points Measured	Key Results
Brown et al 2002 To characterise the deposition and clearance of technetium-99m-labeled ultrafine aerosol in subjects with COPD and healthy age matched volunteers	**COPD Group** 6 female, 4 male 45-70 years age range classified into bronchitic and emphysemia groups **Healthy Group** 6 female, 3 male (data for one subject discarded due to equipment fault) 40-67 years age range.	[1] Inhalation from a mouthpiece. [2] Technetium-99m-labeled ultrafine carbon aerosol Activity Mean Diameter of 61 ± 4 nm and Count Mean Diameter was 33 ± 2 nm. Inhalation continued until 25 µCi deposited in the lung.	**Scintigraphy** • Scans carried out following exposure and after 24 hours. Activity in the liver was quantified 2 hours after inhalation. • Scan divided into three regions of interest (ROI) as follows: • Central ROI being an area with dimensions equal to half the lung's width and one third of its height with one boundary roughly corresponding to the mediastinal surface and centered by height; • Peripheral ROI, area enclosing the whole lung less the central ROI; • Liver ROI being an area below the right lung. • **Deposition** A Deposition Fraction was calculated from inhaled and exhaled activity measurements.	Deposition Fraction was significantly greater for bronchitic subjects than healthy or emphysema subjects. Estimated Dose Rate for COPD subjects was found to be 70% greater than for healthy subjects mainly because of their higher minute ventilation. No accumulation of activity in the liver was observed. Clearance did was not significantly different between Healthy and COPD groups.

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
Frampton et al (1992)	2 female, 10 male, all healthy people lifetime non-smokers 20-39 age range	[1] Exposure chamber [2] 1000 µg/m^3 NaCl (Control) or H_2SO_4 aerosol. The aerosol generated had an average mass median aerodynamic diameter of 0.9 µm [3] 2 hours of a cycle of 10 min of moderate exercise performed on a bicycle ergometer in each half hour period. Alternate NaCl or H_2SO_4 exposures were carried out 2 weeks or more apart.	**Symptoms** Subjects were polled by questionnaire immediately after exposure and 18 hours after exposure regarding respiratory symptoms, nasal or eye irritation and odour. **Plethysmography** Thoracic gas volume, Airway Resistance report as Specific Airway conductance (SGaw) with pneumotachograph used to measure FEV_1 and FVC before (baseline) and immediately after exposure to the NaCl (control) or H_2SO_4 aerosol and 18 hours post exposure. **Bronchoalveolar lavage (BAL)** • Lavage fluid instilled/collected from a segmental bronchus of the right middle lobe and from a segmental bronchus of the lingula 18 hours after the NaCl (control) or H_2SO_4 aerosol exposures. • BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes expressingl, CD^{3+}, CD^{4+}, CD^{8+} antigens quantified. **Alveolar Macrophage Function** Tests of: • Antibody-dependent Cell Mediated Cytotoxicity • Superoxide ion release • Influenza virus inactivation	**Symptoms** Four subjects detected an odour or taste during H_2SO_4 exposure. No odour or taste was reported for NaCl exposure. Three subjects had cough and four subjects reported throat irritation during H_2SO_4 exposure. One subject had cough and three reported throat irritation during Nail exposure. Subjects were asymptomatic 18 hours after exposure. **Plethysmography** No changes in FVC, FEV_1 or SGaw immediately after or 18 hours after exposure to NaCl or H_2SO_4 when compared to pre-exposure baseline measurements. There were no differences in lung function measurements between NaCl and H_2SO_4 exposures. **Bronchoalveolar lavage (BAL)** • No significant differences in cell differential counts in BAL from right middle lobe and lingula. • Neither NaCl nor H_2SO_4 exposure indicated inflammation for those markers measured in BAL with no evidence of Neutrophil infiltration to the airway • A lower (but not statistically significant lower) percentage of T lymphocytes found in BAL following H_2SO_4 exposure when compared with NaCl exposure. This was accounted for by lower counts of $CD4^+$ phenotype but not significantly so. There were no significant differences in other cell type counts. **Alveolar Macrophage Function** No statistically significant difference was found in Alveolar Macrophage function between NaCl and H_2SO_4 exposures.

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
Ghio et al (2000) Hypotheses CAPS can cause a neutrophilic inflammation in the lungs of healthy humans.	38 healthy people, nonsmokers for at least 5 years. Subjects classified into one of four quartiles depending on air/CAPS exposure as follows: Quartile 1 - Exposed to filtered air - 8 subjects Quartiles 2, 3, 10 - each quartile comprised 10 subjects classified according to individual exposure level	[1] Exposure Chamber [2] Filtered airs or concentrated ambient air particles (CAPS) size range 0.1 - 2.5 µm. Quartile 1 - Filtered air. Quartile 2 - 47.2 µg/m^3 Quartile 3 - 107.4 µg/m^3 Quartile 4 - 206.7 µg/m^3 [3] 2 hour of a cycle of 15 min moderate exercise followed by 15 min of rest.	**Symptoms** **Plethysmography** Airway Resistance (Raw) immediately before and after exposure. **Spirometry** FEV_1, FVC, PEF immediately before and after exposure **Peripheral Blood** Samples taken immediately before and 18 hours after exposure. Erythrocyte, neutrophil, lymphocyte, monocyte, platelet, counts; haemoglobin, haematocrit, ferritin, fibrinogen levels; blood viscosity **Bronchoscopy with Lavage** Lavage sample instilled to a segmental bronchus of the lingula and collected in two fractions reported as Bronchial Lavage (BL) and BAL. BL and BAL were considered to reflect the environments of the bronchial and distal airways respectively. Lavage samples were taken 18 hours after the air or CAPS exposure. Total cell count and percentages of macrophage, neutrophil, lymphocyte, monocyte and epithelial cells were determined	**Spirometry, Plethysmograph, Symptoms** Subjects did not report symptoms after either air or CAPS exposure. No significant differences in FEV_1, FVC, PEF or Raw across the Quartiles. All spirometry measurements were normal. **Peripheral Blood** No changes between pre-exposure and post exposure or differences between the air and CAPs exposed groups were recorded for any marker except for fibrinogen concentration. A significant difference was found in fibrinogen levels between air exposed (Quartile 1) and CAPS exposed (combined Quartiles 2 - 4). A similar magnitude change was recorded for each of the CAPS exposed Quartiles indicating no dependence on dose. **Bronchoscopy with Lavage BL fraction** No significant difference between CAPS exposed groups and air-exposed group for total cell count, or proportions of macrophage, lymphocyte or epithelial cells. CAPS exposed groups had significantly higher numbers and proportions of neutrophils in the BL sample. Monocytes were also significantly higher in the CAPS exposed group. The concentration of protein was significantly lower in the CAP exposed group. **Bronchoscopy with Lavage BAL fraction** Total cell count in BAL was significantly higher for CAPS exposed groups compared with air exposed group. Expect for the proportion and count of macrophages and neutrophils

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
			for BL and BAL fractions. BL and BAL fractions were also analysed for concentrations of protein, IL-6, IL-8, PGE_2, α_1-antitrypsin and fibronectin. The concentration of Fibrogen additionally determined for BAL fraction.	which were significantly higher in the CAPS exposed group, the counts of other cells were not significantly different. Monocytes counts were also higher on average amongst the CAPS exposed individuals but the differences between the group means were not sufficient to support a statistically inferred difference. Fibrinogen concentrations were lower in the CAPS exposed group. Concentrations of protein, IL-6, IL-8, PGE_2, α_1-antitrypsin and fibronectin were not significantly different between air and CAPS exposed groups although amongst the individuals of the two higher CAPS exposed quartiles IL-8 concentrations were lower than for the air exposed group but not sufficiently so to infer a statistical difference.
Holgate et al 2002	**ASTHMATIC GROUP** 5 female, 10 male 23-52 years age range mild atopic asthma Positive skin tests to at least one common airborne allergen Non-smokers Not all asthmatic subjects completed the full experimental program. **CONTROL GROUP** 9 female, 16 male	[1] Exposure Chamber Diluted fresh diesel exhaust [2] $100\mu g/m^3$ [3] 2 hours [4] 6 hours	**Lung Function** Lung Function measure before exposure, one hour after start of exposure and at end of exposure for Airway Resistance, FVC and FEV_1. Methods not reported. **Peripheral blood** Venous blood samples taken: • before exposure • one hour after start of exposure • at the end of exposure • 6 hours after end of exposure Samples analysed for leukocytes, neutrophils, lymphocytes and monocyte counts and hemoglobin levels.	**Lung Function** A modest but statistically significant increase in Airway Resistance at the end of exposure to diesel exhaust amongst the Asthmatic Group. A modest but statistically significant increase in Airway Resistance was found after one hour and at the end exposure to diesel exhaust amongst the Control Group. No significant changes in FVC or FEV_1 for Asthmatic or Control Group measurements we found to result from diesel exhaust exposure. **Peripheral blood before and after** Only the results for before exposure and 6 hours following exposure to air and diesel exhaust are presented. Neither diesel exhaust nor air exposures produced any significant changes.

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
	19-42 years age range Normal lung function. Negative skin prick tests to common airborne allergens Not all control subjects completed the full experimental program.		**Bronchial Wash and Bronchoalveolar Lavage** performed 6 hours after exposure lung liquid assessed for cell type/counts and inflammatory marker concentrations, albumin and total protein, RNA determination. **Endobronchial biopsy** 6 hours after exposure immunostained for inflammatory cell counts and inflammatory markers, RNA determination.	**Bronchial Wash** **Control Group** For diesel exhaust exposure compared to air: • Significant higher neutrophil relative count; • No significant differences in other cell types except relative macrophage count which is reported as lower for diesel exhaust exposure; • No significant differences in total protein or albumin; • Significantly higher cytokine IL-6 and chemokine IL-8 levels, otherwise no significant differences in soluble inflammatory mediators. **Asthmatic Group** For diesel exhaust exposure compared to air: • No significant differences in counts of any cell type except relative eosinophil count which is reported as lower for diesel exhaust exposure; • No significant differences in total protein or albumin or soluble inflammatory mediators. **Bronchoalveolar Lavage** **Control Group** For diesel exhaust exposure compared to air: • Significant higher lymphocyte count; • No significant differences in relative cell counts of other cell types except relative macrophage count which is reported as lower for diesel exhaust exposure; • No significant differences in total protein or albumin **Asthmatic Group** No significant differences in measured end points or diesel

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
				exhaust exposure compared to air. **Endobronchial biopsy** **Control Group** For diesel exhaust exposure compared to air: • Significant higher VCAM-1 and P-Selectin expressed on endothelium; • Significant increase in IL-8 mRNA; • No significant difference differences in inflammatory cell count in bronchial submucosa; • Significantly lower CD^{3+} cells in bronchial epithelium **Asthmatic Group** Comparison of paired biopsy samples taken after diesel exhaust exposure and air exposure from the same subject found no significant difference in any end point measured except: • submucosa eosinophil count which appears to be lower for diesel exhaust exposure; • Cytokine IL-10 levels significantly higher.
Kuschner et al (1995)	6 female, 8 male healthy people; 3 never smoked; 2 former smokers (quit more than 5 years before study; 9 current smokers; mean age 35.6 years with standard deviation 7.9 years. Subjects were their own controls.	[1] Mouth breathing face mask. [2] Purified zinc oxide fume with median primary particle diameter ranging between 0.008 and 0.04 µm and a mass mean diameter of 0.17 µm OR medical grade air (control).	**Self Reported Symptoms** Each subject was asked to record any symptoms and record his/her body temperature during the evening following the afternoon exposure. **Plethysmography** Thoracic gas volume, Airway Resistance (Raw) and methacholine provocative dose before exposure to zinc oxide fume (or air for control) and 18 hours post exposure .	**Self Reported Symptoms** No subject reported any symptoms indicative of metal fume fever or body temperature elevation. **Plethysmography** No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure.

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
		[3] Range of particle concentrations 2.76 - 37.0 mg/m^3, mean 16.4 mg/m^3. [4] 15 to 120 minutes of exposure assumed resting.	**Spirometry** FEV_1 before exposure to zinc oxide fume (or air for control) and 18 hours post exposure. **Total Lung Capacity** Single breath helium dilution method **Carbon Monoxide Diffusing Capacity** Single breath method **Peripheral Blood** Polymorphonuclear leukocyte (Neutrophil) concentration determined prior to lung function testing, that is before exposure to zinc oxide fume (or air for control) and 18 hours post exposure **Bronchoalveoar lavage** Lavage fluid instilled to a segmental bronchus of the right middle lobe 20 hours after the air (control) or zinc oxide fume exposures. BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes and proportions of T Cell, $CD4^+$, $CD8^+$ and B Cell phenotypes. BAL samples were analysed for concentrations of TNF-α, IL-1β, IL-6, IL-8, IL-10 and MIP1-α.	**Spirometry** FEV_1 was minimally lower from baseline post exposure but the reduction was consistent with diurnal fluctuations. **Total Lung Capacity** No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure. **Carbon Monoxide Diffusing Capacity** No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure. **Peripheral Blood** Polymorphonuclear leukocyte (Neutrophil) concentration was not significantly different between the before and after exposure blood samples. **Bronchoalveoar lavage** Grouped data showed a significant higher count of Polymorphonuclear leukocyte (Neutrophil) cells following the zinc oxide fume exposures relative to the air (control) exposure. When the data were stratified according to cumulative zinc oxide exposure (a proxy for dose) and dose-response relationship was apparent Linear regression of the data found that cumulative zinc exposure was a statistically significant predictor of Polymorphonuclear leukocyte (Neutrophil) concentration increase. Compared with air exposure the lymphocyte count was significantly higher for the post zinc oxide exposure samples

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
				but there was no significant difference in the ratios of the various lymphocyte phenotypes. TNF-α, and IL-8 were significantly higher following zinc oxide exposure compared with air exposure. Linear regression found that cumulative zinc exposure was a statistically significant predictor of an increase in TNF-α, and IL-8 concentrations. The linear regression also indicated a threshold of about 500 mg.min/m^3 cumulative zinc exposure.
Kuscher et al (1997)	2 female, 4 male. Non-smokers, 3 former smokers. 21-43 years age range	[1] Mouth breathing face mask. [2] Purified magnesium oxide fume, 98.6% of particles by weight below 1.8μm in diameter. [3] Range of particle concentrations 5.8-230 mg/m^3, median 133.0 mg/m^3	**Self Reported Symptoms** Each subject was asked to record any flulike symptoms of myalgias, fatigue and rigors and record his/her body temperature during the evening on day of exposure. **Spirometry** FEV_1 before exposure to magnesium oxide fume (or air for control) and 18 hours post exposure. **Total Lung Capacity** Single breath helium dilution method **Carbon Monoxide Diffusing Capacity** Single breath method	**Self Reported Symptoms** None of the subjects reported symptoms post exposure with either air or magnesium oxide fume. **Spirometry, Total Lung Capacity, Carbon Monoxide Diffusing Capacity, Peripheral Blood Bronchoalveoar lavage** No significant differences in any of the end points measured between air and magnesium oxide exposure

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
			Peripheral Blood Complete blood counts and differentials were obtained pre-exposure and 18 hour post exposure to determine neutrophil concentrations. **Bronchoalveoar lavage** Lavage fluid instilled to a segmental bronchus of the right middle lobe 20 hours after the air (control) or magnesium oxide fume exposures. BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes. BAL samples were analysed for concentrations of TNF-α, IL-1, IL-6, IL-8.	
Salvi et al 1999	Healthy non-smoker. 4 female, 11 male. 21-28 year age range. Normal lung function. Negative skin prick tests to common airborne allergens	[1] Exposure chamber [2] Air (control) or diluted fresh diesel exhaust PM_{10} 300 $\mu g/m^3$ [3] 1 hour of a cycle of 15 min moderate exercise followed by 15 min of rest. Random sequence 3 weeks or more apart.	**Spirometry** (PEFR, FVC, FEV_1, FEF_{25-75}) immediately before and after each exposure. **Peripheral blood** collected 6 hours after each exposure. Ttotal cells, differential counts and platelet count determined. **Bronchial wash and bronchoalveolar lavage** Bronchial wash (BL) and bronchoalveolar lavage (BAL) performed for bronchus of middle lobe or lingua 6 hours after exposure. Samples analysed for:	**Spirometry** No difference in spirometry measurements made before and after exposures. **Peripheral Blood** Differences in diesel exhaust exposure compared to air: • Neutrophil and platelet count higher • HLA-DR+ lymphocyte count lower **Bronchial Wash** Differences in diesel exhaust exposure compared to air: • Significantly higher neutrophil count • Macrophage count and lactic dehydrogenase activity showed a tendency to be greater though not statistically

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
			• cell type/counts • albumin, total protein, LDH, IL-8, ICAM-1, methylhistamine and fibronectin. **Endobronchial biopsy** 6 hours after exposure with biopsies taken from the anterior portion of the main carina and the subcarninea of the third and fourth generation airways on the right side or from the posterior part of the main carina and coreesponding subcarinae on the left side. The biopies were immunostained for quantification of: • counts of neutrophils, lymphocytes (CD^{3+}, CD^{4+}, CD^{8+} cells), macrophages, eosinophils inflammatory cell counted seperately for epithelium and submucosa; • proportions of blood vessels stained for ICAM-1, VCAM-1, E-selectin, P-selectin, LFA-1 ligand and VLA-4 ligand.	significant. **Bronchoalveolar Lavage** Differences in diesel exhaust exposure compared to air: • higher B Cell proportion amongst total cells • but no significant difference in proportions of HLA-DR^{+}, CD^{3+}, CD^{8+},CD^{25+}cells • Methyl histamine and fibronectin concentrations significantly higher • No difference in concentrations of total protein, albumin, IL-8, C3a, C5a and soluble ICAM-1. **Bronchial Biopsies** Differences in diesel exhaust exposure compared to air: • Neutrophil count in epthelium and submucosa higher • Mast cell count in submucosa hugher; • Total T cell count higher in epithelium and submucosa comprising greater numbers of CD^{4+} cells in the epithelium and submucosa and CD^{8+} cells in the epithelium. • No differences in the number of activated T Cells (CD^{25+}), macrophages, eosinophils or B Cells. • Markedly higher proportion in proportion of blood vessels staining for ICAM-1 or VCAM-1 • No difference in data for E-selectin and P-selecti • Cells expressing the LFA-1 ligand were higher in the epithelium and submucosa • Cells expressing the VLA-4 ligand were higher in the submucosa though not significantly so.
Salvi et al 2000	See Salvi et al 1999	See Salvi et al 1999	**Bronchial wash** performed 6 hours after exposure and total RNA extracted from cells	**Bronchial Wash** Differences in cytokine mRNA in bronchial wash cells for

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
			and RT-PCR ELISA used to quantify relative changes in mRNA for of IL-1β, IL-4, IL-5, IL-8, TNF-α, IFN-γ and GM-CSF synthesis. **Endobronchial biopsy** 6 hours after exposure • immunostained for quantification of, GRO-α,, IL-4, IL-5, IL-6, IL-8, TNF-α,,GM-CSF and ENA-78; quantification was performed separately for the epithelium and submucosa. Total RNA extracted from tissue samples and RT-PCR ELISA used to quantify relative changes in mRNA for of IL-1β, IL-4, IL-5, IL-8, TNF-α,, IFN-γ and GM-CSF synthesis.	diesel exhaust exposure compared to air: • Significantly higher proportions of IL-8 mRNA • No difference on the mRNA levels of IL-1β, IL-4, IL-5, TNF-α, IFN-γ or GM-CSF. • **Endobronchial Biopsy** Differences in cytokine mRNA in bronchial tissue for diesel exhaust exposure compared to air: • Significantly higher proportions of IL-8 mRNA • IL-5 mRNA higher but just short of statistical significance; • No difference in the mRNA levels of IL-1β, IL-4, TNF-α, IFN-γ or GM-CSF. • Higher levels of the chemokines IL-8 and GRO-α in the bronchial epithelium.

APPENDIX E: SUMMARY OF POLLUTANT LEVELS MEASURED IN EPIDEMIOLOGICAL STUDIES

Ref	City	$NC_{0.01–0.1}$, #/cm^3	$NC_{0.01–2.5}$, #/cm^3	$MC_{0.01–2.5}$, µg/m^3	$PM_{2.5}$, µg/m^3	PM_{10}, µg/m^3	TSP, µg/m^3	Black smoke, µg/m^3	NO, µg/m^3	NO_2, µg/m^3	SO_2, µg/m^3	CO, mg/m^3	O_3, µg/m^3
Osunsanya, 2001	Aberdeen, UK	avr:10241, range: 740 - 60 636				avr: 13; range: 6 - 34							
Pekkanen et al, 1997	Kuopio, Finland	avr: 44300				avr: 18		avr: 13	avr: 9	avr: 28	avr: 6	avr: 0.6	
Pekkanen et al, 2002	Helsinki, Finland	max: 50310			max:39.8	max:76.8				max: 67.5		max: 1.0	
Penttinen, 2001	Helsinki, Finland	avr: 14500			avr: 8.4	avr: 13.5			avr: 16.7	avr: 25.3		avr: 0.4	
Tiittanen et al, 1999	Kuopio, Finland	range: 6980 – 40200			range: 3 - 55	range: 5 - 122	range: 5 – 234	range: 2.9 - 21.2		range: 5 – 46	range: 0 - 5.2	range: 0.1 - 1.0	range: 0 – 50
von Klot et al, 2002	Erfurt, Germany	avr:17300, range: 3272 – 46195	avr: 19326 range:3564 – 53023	avr: 30.3 range: 3.6 – 133.8	avr: 35.1 range:4.0 – 108.1	avr: 45.4 range:4.7 – 172.4				avr: 46 range:8 – 119	avr:24.0 range:0.1 – 114.7	avr: 0.9 range:0.3 – 3.0	
Witchmann et al, 2000	Erfurt, Germany	avr: 15773 ± 10321	avr: 17966 ± 11373	avr: 25.8 ± 21.4	avr: 26.3 ± 20.8	avr: 38.2 ± 26.4	avr: 8.9 ± 28.1			avr:36.4 ±15.3	avr:16.8 ± 18.7	avr:600 ± 500	