

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
<i>Principals</i>		
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
<i>Associates</i>		
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
<i>Research Associates</i>		
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
<i>International Expert Advisers</i>		
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)
- Gezondheidsraad: 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 comments 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 categorical 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.

References

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- Hastie T and Tibshirani, R. (1990) *Generalized Additive Models*. Chapman and Hall, London.
- Liang KY, & Zeger SL. (1986). Longitudinal data analysis using general linear models. *Biometrika*, 73(1), 13-22.
- McCullagh, P., and Nelder, J. A. (1989). *Generalized Linear Models*, Second Edition. Chapman and Hall, London

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.

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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.

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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 µg/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 µg/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.

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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

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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 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 signaling of the GM-CSF response.

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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.
Bonvallet 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.

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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 and 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.

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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

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
				lung cancer.

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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.

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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.

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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 <i>Haemophilus</i> 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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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.

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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Castranova et al. (2001)	Rats and mice	inflammatory	Investigated the effects of exposure 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 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.
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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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 ₂ O 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 leading 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 phenanthrene (the most prevalent polycyclic aromatic hydrocarbon in DEP) and anthracene were both capable of enhancing

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				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 sized 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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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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.

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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 µg/cm ² . All samples had a slight decreasing effect on the frequency of micronuclei at the lowest treatment concentration of 5 µg/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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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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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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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-dimethyldibenzothiophene, possesses estrogenic activity.

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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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Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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.

Table C-1. Summary of toxicological studies on ultrafine particles

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 µg/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.

Table C-1. Summary of toxicological studies on ultrafine particles

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 $\mu\text{g}/\text{m}^3$ 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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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 µg/m ³), 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 be involved in the attenuation of CYP2B1, GST, and catalase proteins and enzyme activities.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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 murine 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 µg/m ³ high dose: 3 µg/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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
Sato et al. (1999)	Rats	Pulmonary mutagenicity	Investigated pulmonary carcinogenesis mutagenicity of DEP. F344 rats were exposed to DEP 6 µg/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 µg/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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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 found 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 <u>aggravation of lung injury</u> .
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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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 µl 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 µg/m ³ (3 x 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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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.

Table C-1. Summary of toxicological studies on ultrafine particles

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.
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 damage. 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.

Table C-1. Summary of toxicological studies on ultrafine particles

Ref	Groups studied	Effects studied	Study Description	Findings/ Conclusions
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 Th ₁ 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)] 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 <i>Listeria</i> from the lungs. The results showed that exposure to DEP decreased the ability of macrophages to produce antimicrobial oxidants in response to <i>Listeria</i> , 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 <i>Listeria</i> 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 <i>Listeria monocytogenes</i> 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 <i>Listeria</i> 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 Th ₁ 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 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 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 the liver within 5 minutes after inhalation. Activity progressively increase in the bladder reaching about 25% of deposited activity by 45 minutes Peripheral Blood 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
<p>Brown et al 2002</p> <p>To characterise the deposition and clearance of technetium-99m-labeled ultrafine aerosol in subjects with COPD and healthy age matched volunteers</p>	<p>COPD Group 6 female, 4 male 45-70 years age range classified into bronchitic and emphysema groups</p> <p>Healthy Group 6 female, 3 male (data for one subject discarded due to equipment fault) 40-67 years age range.</p>	<p>¹ Inhalation from a mouthpiece.</p> <p>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.</p>	<p>Scintigraphy</p> <ul style="list-style-type: none"> • 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. • <p>Deposition</p> <p>A Deposition Fraction was calculated from inhaled and exhaled activity measurements.</p>	<p>Deposition Fraction was significantly greater for bronchitic subjects than healthy or emphysema subjects.</p> <p>Estimated Dose Rate for COPD subjects was found to be 70% greater than for healthy subjects mainly because of their higher minute ventilation.</p> <p>No accumulation of activity in the liver was observed.</p> <p>Clearance did was not significantly different between Healthy and COPD groups.</p>

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	<p>¹ Exposure chamber ² 1000 µg/m³ NaCl (Control) or H₂SO₄ 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 H₂SO₄ exposures were carried out 2 weeks or more apart.</p> <p>Symptoms Subjects were polled by questionnaire immediately after exposure and 18 hours after exposure regarding respiratory symptoms, nasal or eye irritation and odour.</p> <p>Plethysmography Thoracic gas volume, Airway Resistance report as Specific Airway conductance (SGaw) with pneumotachograph used to measure FEV₁ and FVC before (baseline) and immediately after exposure to the NaCl (control) or H₂SO₄ aerosol and 18 hours post exposure.</p> <p>Bronchoalveolar lavage (BAL)</p> <ul style="list-style-type: none"> • 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 H₂SO₄ aerosol exposures. • BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes expressing I, CD³⁺, CD⁴⁺, CD⁸⁺ antigens quantified. <p>Alveolar Macrophage Function Tests of:</p> <ul style="list-style-type: none"> • Antibody-dependent Cell Mediated Cytotoxicity • Superoxide ion release • Influenza virus inactivation 	<p>Symptoms Four subjects detected an odour or taste during H₂SO₄ exposure. No odour or taste was reported for NaCl exposure. Three subjects had cough and four subjects reported throat irritation during H₂SO₄ exposure. One subject had cough and three reported throat irritation during NaCl exposure. Subjects were asymptomatic 18 hours after exposure.</p> <p>Plethysmography No changes in FVC, FEV₁ or SGaw immediately after or 18 hours after exposure to NaCl or H₂SO₄ when compared to pre-exposure baseline measurements. There were no differences in lung function measurements between NaCl and H₂SO₄ exposures.</p> <p>Bronchoalveolar lavage (BAL)</p> <ul style="list-style-type: none"> • No significant differences in cell differential counts in BAL from right middle lobe and lingula. • Neither NaCl nor H₂SO₄ 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 H₂SO₄ 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. <p>Alveolar Macrophage Function No statistically significant difference was found in Alveolar Macrophage function between NaCl and H₂SO₄ exposures.</p>

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
			<p>for BL and BAL fractions.</p> <p>BL and BAL fractions were also analysed for concentrations of protein, IL-6, IL-8, PGE₂, α₁-antitrypsin and fibronectin. The concentration of Fibrogen additionally determined for BAL fraction.</p>	<p>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.</p> <p>Fibrinogen concentrations were lower in the CAPS exposed group.</p> <p>Concentrations of protein, IL-6, IL-8, PGE₂, α₁-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.</p>
Holgate et al 2002	<p>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.</p> <p>CONTROL GROUP 9 female, 16 male</p>	<p>¹ Exposure Chamber Diluted fresh diesel exhaust ² 100µg/m³ ³ 2 hours ⁴ 6 hours</p>	<p>Lung Function Lung Function measure before exposure, one hour after start of exposure and at end of exposure for Airway Resistance, FVC and FEV₁. Methods not reported.</p> <p>Peripheral blood Venous blood samples taken:</p> <ul style="list-style-type: none"> • before exposure • one hour after start of exposure • at the end of exposure • 6 hours after end of exposure <p>Samples analysed for leukocytes, neutrophils, lymphocytes and monocyte counts and hemoglobin levels.</p>	<p>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 FEV₁ for Asthmatic or Control Group measurements we found to result from diesel exhaust exposure.</p> <p>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.</p>

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
	<p>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.</p>		<p>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.</p> <p>Endobronchial biopsy 6 hours after exposure immunostained for inflammatory cell counts and inflammatory markers, RNA determination.</p>	<p>Bronchial Wash Control Group For diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> • 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. <p>Asthmatic Group For diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> • 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. <p>Bronchoalveolar Lavage Control Group For diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> • 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 <p>Asthmatic Group No significant differences in measured end points or diesel</p>

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
				<p>exhaust exposure compared to air.</p> <p>Endobronchial biopsy Control Group For diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> • 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 CD³⁺ cells in bronchial epithelium <p>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:</p> <ul style="list-style-type: none"> • submucosa eosinophil count which appears to be lower for diesel exhaust exposure; • Cytokine IL-10 levels significantly higher.
Kuschner et al (1995)	<p>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.</p> <p>Subjects were their own controls.</p>	<p>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).</p>	<p>Self Reported Symptoms Each subject was asked to record any symptoms and record his/her body temperature during the evening following the afternoon exposure.</p> <p>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 .</p>	<p>Self Reported Symptoms No subject reported any symptoms indicative of metal fume fever or body temperature elevation.</p> <p>Plethysmography No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure.</p>

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
		<p>³ Range of particle concentrations 2.76 - 37.0 mg/m³, mean 16.4 mg/m³.</p> <p>⁴ 15 to 120 minutes of exposure assumed resting.</p>	<p>Spirometry FEV₁ before exposure to zinc oxide fume (or air for control) and 18 hours post exposure.</p> <p>Total Lung Capacity Single breath helium dilution method</p> <p>Carbon Monoxide Diffusing Capacity Single breath method</p> <p>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</p> <p>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 and MIP1-α.</p>	<p>Spirometry FEV₁ was minimally lower from baseline post exposure but the reduction was consistent with diurnal fluctuations.</p> <p>Total Lung Capacity No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure.</p> <p>Carbon Monoxide Diffusing Capacity No statistically significant difference on lung function parameters between before exposure (baseline) and post exposure.</p> <p>Peripheral Blood Polymorphonuclear leukocyte (Neutrophil) concentration was not significantly different between the before and after exposure blood samples.</p> <p>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) concentration increase. Compared with air exposure the lymphocyte count was significantly higher for the post zinc oxide exposure samples</p>

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
				<p>but there was no significant difference in the ratios of the various lymphocyte phenotypes.</p> <p>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/m³ cumulative zinc exposure.</p>
Kuscher et al (1997)	2 female, 4 male. Non-smokers, 3 former smokers. 21-43 years age range	<p>¹ Mouth breathing face mask.</p> <p>² Purified magnesium oxide fume, 98.6% of particles by weight below 1.8μm in diameter.</p> <p>³ Range of particle concentrations 5.8-230 mg/m³, median 133.0 mg/m³</p>	<p>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.</p> <p>Spirometry FEV₁ before exposure to magnesium oxide fume (or air for control) and 18 hours post exposure.</p> <p>Total Lung Capacity Single breath helium dilution method</p> <p>Carbon Monoxide Diffusing Capacity Single breath method</p>	<p>Self Reported Symptoms None of the subjects reported symptoms post exposure with either air or magnesium oxide fume.</p> <p>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</p>

Table D- 2. Controlled Exposure Studies of Ultra Fine Particles

	Subject Details		End Points Measured	Results
			<p>Peripheral Blood Complete blood counts and differentials were obtained pre-exposure and 18 hour post exposure to determine neutrophil concentrations.</p> <p>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.</p> <p>BAL counts of macrophage, Polymorphonuclear leukocyte (Neutrophil) and Lymphocytes.</p> <p>BAL samples were analysed for concentrations of TNF-α, IL-1, IL-6, IL-8.</p>	
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 $\mu\text{g}/\text{m}^3$ ³ 1 hour of a cycle of 15 min moderate exercise followed by 15 min of rest. Random sequence 3 weeks or more apart.	<p>Spirometry (PEFR, FVC, FEV₁, FEF₂₅₋₇₅) immediately before and after each exposure.</p> <p>Peripheral blood collected 6 hours after each exposure. Ttotal cells, differential counts and platelet count determined.</p> <p>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:</p>	<p>Spirometry No difference in spirometry measurements made before and after exposures.</p> <p>Peripheral Blood Differences in diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> • Neutrophil and platelet count higher • HLA-DR+ lymphocyte count lower <p>Bronchial Wash Differences in diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> • 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
			<ul style="list-style-type: none"> cell type/counts albumin, total protein, LDH, IL-8, ICAM-1, methylhistamine and fibronectin. <p>Endobronchial biopsy 6 hours after exposure with biopsies taken from the anterior portion of the main carina and the subcarinae 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:</p> <ul style="list-style-type: none"> 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. 	<p>significant.</p> <p>Bronchoalveolar Lavage Differences in diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> higher B Cell proportion amongst total cells but no significant difference in proportions of HLA-DR⁺, CD³⁺, CD⁸⁺, 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. <p>Bronchial Biopsies Differences in diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> 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 in 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.
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
			<p>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.</p> <p>Endobronchial biopsy 6 hours after exposure</p> <ul style="list-style-type: none"> 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. 	<p>diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> 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. <p>Endobronchial Biopsy</p> <p>Differences in cytokine mRNA in bronchial tissue for diesel exhaust exposure compared to air:</p> <ul style="list-style-type: none"> 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 _x , 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	