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Epidemiology of the Effects of Residential Exposure to Ultrafine Particles from Vehicular Traffic

Susan E. O' Connell

PhD 2005

Cardiff University, School of Medicine

Department of Epidemiology, Statistics and Public Health

Summary

Cardiovascular and respiratory illnesses represent a large burden to the National Health Service and exposure to air pollution, specifically particulate pollution, has been put forward as a risk factor for the development and exacerbation of such illnesses. Vehicular traffic is thought to be the largest contributor of ultrafine particles to the atmosphere in urban areas.

This thesis aimed primarily to investigate whether healthy persons living in areas with high volumes of traffic presented with higher levels of certain cardiovascular and respiratory indicators (biomarkers of effect) that might be involved in causal pathways for disease.

Participants were recruited from the general Cardiff population by sending letters out to each household. A visit was made to each participant and measures of ultrafine particles were made indoors and outdoors at each location. Participants donated a blood sample which was then analysed to determine the levels of the various biomarkers of interest. Each participant answered a questionnaire designed to determine the personal perceptions of participants regarding traffic levels in their area.

The mean numbers of particles were compared for each area using analysis of variance (ANOVA) and Mann-Whitney U tests. Similarly for the biomarkers of effect, analysis of variance and Mann-Whitney tests were conducted in an effort to elucidate whether or not a difference in the levels existed between the exposed and unexposed groups. The presence of a dose-response relationship between exposure and effect was also investigated. Areas with higher levels of traffic had significantly greater concentrations of ultrafine particles but no differences were observed in the levels of biomarkers.

A separate but related investigation of a database of respiratory and cardiovascular admissions revealed a significant variation by category of road but the observed associations were not what were expected.

Acknowledgements

My sincere and heartfelt thanks go to my supervisor Ian, without whose constant advice and encouragement I could not have completed this thesis. To Barry Nix for his enduring patience and for his advice and help with the analysis stage of the study, it was very much appreciated. To Chris, because without his help, I would never have managed to complete the study in time.

To Rhys for being a constant source of support and encouragement, particularly towards the end and to my mum and dad for always being on the other end of the phone, I could not have made it through this without you.

To my friends, for helping me to keep it all together at the crucial moments and providing the all important shoulder to cry on, your support has been invaluable.

To Claire, thank you for the coffee breaks and helping me to keep focused on the final goal – we'll celebrate together when this is all over.

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Abstract

Cardiovascular and respiratory illnesses represent a large burden to the National Health Service in the UK. In recent times interest has turned to air pollution as a possible cause of such illnesses and a large amount of research has been conducted in an effort to elucidate the true extent of these associations. Both in the USA and in Europe, extensive research has resulted in a large body of evidence intimating that the respirable particulate fraction of air pollution is responsible, particularly ultra fine particles, to some degree, for increases in a variety of health outcomes including mortality, hospital admissions and emergency room visits and effects on pulmonary function. Possible mechanisms of effect have been also been put forward on the basis of results from experimental work suggesting that ultrafine particulate pollution elicits its effect by the generation of reactive oxygen species and/or inflammation. In many urban areas, legislation effectively controls emissions from industrial sources and as a result the most likely contributor of ultra fine particulate air pollution in towns and cities is motor traffic. Part A describes an epidemiological study comparing the levels of various biomarkers of effect in the blood of supposed healthy individuals who are estimated to be exposed to differing levels of ultra fine particles, generated from traffic, by virtue of their own area of residence.

Part B of the thesis uses an epidemiological database of hospital admissions to determine whether proximity to certain road types might affect one's likelihood of being admitted to hospital with a cardiovascular or respiratory illness.

Methods: Part A

Based on the volumes of traffic estimated to be using the roads, various streets were assigned to either an exposed group or an unexposed group.

Recruitment of participants to each group involved sending letters to each address on the relevant streets, outlining the study and the exclusion criteria and inviting residents to take part in the study. A total of 70 persons from the exposed areas and 51 persons from the unexposed areas were recruited to the study.

A visit was made to each home during which measurements of mean number of ultra fine particles/cm^3 were measured both inside the home of the participants and on the street outside their home using a P-Trak ultra fine particle counter. A brief questionnaire detailing the participants' personal perceptions regarding the traffic in their area was also administered.

Participants were then invited to provide a blood sample for analysis at the hospital outpatients unit and these blood samples were then passed on to the laboratories for analysis.

Statistical analysis was performed on all the data in an effort to elucidate any relationships between the particle numbers measured and the levels of the various biomarkers in the blood of participants.

Methods: Part B

A database was constructed using the Patient Episode Database for Wales and NHS Administrative Register databases. These contained respectively information on all hospital admissions for a variety of respiratory and cardiovascular diseases in the year 2002 as well as for the base population of Cardiff which consisted of every person registered with a Welsh GP in July 2002. The final database contained a category of road, defined by road type and distance from the city centre, for each person along with information on their age, sex, socioeconomic status and hospital admission date for those individuals from the base population who were admitted.

Results: Part A

Mean outdoor levels of particles/cm^3 between the exposed and unexposed groups, indoor levels between the two groups and indoor and outdoor levels within each group were compared using Analysis of Variance (ANOVA) and Mann-Whitney U tests.

The levels of each individual biomarker of effect were examined by constructing scatter plots for each one to examine how the individual measurements in the groups differed from each other. Comparison of medians was also conducted to investigate whether there was a difference between the exposed and unexposed groups in relation to the combined levels of biomarkers measured in each group. The presence of a dose-response relationship was also investigated by grouping the data according to the mean numbers of particles/cm^3 measured outdoors in each street and comparing the levels of biomarkers between exposure groups.

Results: Part B

Three different independent variables of interest, 1) road category, which combined road type and distance from the city centre; 2) distance, which only accounted for distance from the city centre; and 3) road type, which considered only the type of road on which the person lived were analysed to check whether they were associated with increased hospital admissions for a variety of cardiovascular and respiratory illnesses. A variety of other independent variables known to affect the associations, such as age, were included in each run of the analysis. Logistic Regression analysis was conducted on three different dichotomous variables to examine whether living on a given road type increased the odds of a person being admitted to hospital with a primary diagnosis corresponding to an ICD code of interest.

Conclusions

Although a statistically significant difference was found between the mean numbers of particles/cm^3 measured outdoors in the exposed and unexposed areas, no such difference was observed between the levels of the various biomarkers of effect examined. These findings suggest that despite the difference in mean particle numbers, no effect on the levels of biomarkers is apparent. Lack of numbers of participants willing to provide blood samples resulted in reduced power and therefore the general conclusion is that further investigation is needed before it can be stated with any confidence that the increased levels of particles observed in the exposed areas do not have any adverse health implications for the population.

There was no evidence of a dose-response relationship with respect to the levels of the biomarkers measured, although a statistically significant difference was found between the three groups examined in terms of the mean number of particles measured outdoors.

Although there were significant associations with hospital admissions for the independent variables road category, distance and road type, and although the results may be altered by residual confounding, these associations were not what were expected. It was therefore concluded that using road type as a proxy for exposure to traffic generated particulate pollution may not be appropriate in this setting.

Aims and Objectives

The primary aim of this thesis was to investigate whether healthy individuals residentially exposed to traffic generated particulate pollution showed any difference in the levels of certain biomarkers of inflammation and of oxidative stress as compared with similar non-exposed individuals. Two objectives relating to this aim were;

1. To make measurements of ultra fine particulates, both indoors and outdoors in an attempt to gain a better idea of the variations in ultra fine concentrations in different areas of Cardiff City. Further, to see if the variation observed was sufficient to categorize individuals into groups of differing exposure.
2. To investigate the relationships between exposure and biomarker concentrations in order to show the possible presence of any dose-response relationships.

A related and subsidiary aim of the thesis was to attempt to elucidate whether residing in certain urban areas increases an individual's likelihood of being admitted to hospital with one of a number of cardiac or respiratory diseases.

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Chapter 1
Particulate Air Pollution and the Links to Health Outcomes

1.1) An Introduction to the History of Air Pollution Research

'It is horrid Smoake which obscures our Church and makes our palaces look old, which fouls our Cloth and corrupts the Waters, so as the very rain, and refreshing Dews which fall in the several Seasons, precipitate to impure vapour, which, with it black and tenacious quality, spots, contaminates whatever is exposed to it.'
John Evelyn; Fumifugium 1661.[1]

In recent times, interest in the environment has grown for a number of reasons. One of the main reasons for the increase in interest is due to more attention being paid to the potential adverse health effects of environmental pollution. One of the main areas of concern is that of Air Pollution and its' potential effects on the respiratory and cardiovascular health of the population. There are many different sources and types of air pollution. Although long since recognised as a problem, air pollution really came to the fore during the great London Smog episode of December 1952 when, due to a particularly cold spell, people kept coal fires burning more than usual. The resulting increase in smoke from homes, in addition to the sulphurous emissions from industries, together with ideal weather conditions (i.e. lack of wind and freezing air temperatures) created thick pungent smog that lay over London for days. The word smog was coined by Dr HA de Vouex and referred to the sooty smoke filled fogs experienced in Britain. Smog had been occurring in Britain long before the 1952 episode and was actually seen as a tourist attraction of sorts, with tins of London fog available for purchase. Life in Victorian cities was very much influenced by coal smoke, to the point where the effects were more far reaching than the medical and physical, and even something as simple as being confused became known as 'being fogged' and London became widely referred to as 'The Big Smoke'. Finally, as a direct result of the increase in the number of deaths both during and immediately following the 1952 smog episode, air pollution was recognised as a threat to the health of the population. The primary result of the episode was to deliver the clear message that air pollution was not good for health and that steps needed to be taken to improve air quality.

The chairman of the Air Pollution Committee 1953-1954, Sir Hugh Beaver, presented a report stating that domestic fires contributed more smoke to the environment than industry. "The domestic fire is the biggest single smoke producer. In ratio to the coal burnt, it produces twice as much smoke as industry and discharges it at a lower level". In light of his findings, Sir Hugh Beaver recommended that the government create smokeless zones that prohibit smoke emissions, encouraging use of smokeless fuels, as well as areas of smoke control where the use of bituminous coal would be restricted. In spite of this, the government were reluctant to act and it was not until Gerald Nabarro won a ballot for a private members bill that allowed him to introduce a Clean Air Bill, which was based on Sir Hugh Beaver's original document, that the government paid much attention. Rather than allowing Gerald Nabarro's bill, they brought forward their own resulting in the Clean Air Act of 1956. The 1956 Act introduced 'smoke control areas' and more rigidly enforced smoke control in towns and cities. In 1968 the Clean Air Act was amended and extended and in 1993 the 1956 and 1968 Clean Air Acts were consolidated to form a single Clean Air Act governing air pollution as well as encompassing clean air legislation that had previously been governed by other Acts; for example part IV of the new Clean Air Act now regulated the quality of such fuels as petrol and diesel and part V facilitates the conduction of investigation and research relating to air pollution by local authorities. It does not, however, apply to industrial processes, which are covered by the 1990 Environmental Protection Act. As the effect of these Clean Air and Environmental Protection Acts began to come to fruition and smoke from industry and domestic fuel burning declined, there also came a rise in motor vehicles and their exhaust fumes and emissions resulting in another source of air pollution to be controlled. Nowadays the word smog is used with reference to the fogs polluted by traffic fumes and emissions and although never as severe as the Great London Smog, it still represents one of the major health threats to the general population.[2-4]

Since the UK joined the European Union in the 1970's, European legislation has been used to control the amount of pollution both from industry and transport. In response to EU legislation, the UK government developed the National Air Quality Strategy (NAQS), the purpose of which was to set targets for air pollutants of concern such as particulate matter. Many of these targets will need to be met by 2005, however a number of the major cities in the UK are unlikely to be able to meet the standards set out by the NAQS due to the high number of pollutant sources. As a result local authorities in cities where targets are not likely to be met have been forced to declare Air Quality Management Areas (AQMA's) and prepare an Air Quality Action Plan (AQAP) for improving air quality in these locations.

As with many urban centres the main source of emissions in Cardiff will be road transport and in line with all other local authorities in England and Wales, Cardiff County Council are actively involved in devising strategies to help them meet the statutory requirements with respect to Air Quality. Continuous monitoring of pollutants has shown that there should be no problem in meeting the standards and objectives for most of them, including PM_{10} (listed below), in Cardiff.[4-5]

Standard:	Objective:
$50_{\mu gm^{-3}}$ measured as 24 hour mean	$50_{\mu gm^{-3}}$ by 31/12/2004 maximum of 35 exceedences/year
$40_{\mu gm^{-3}}$ measured as annual mean	$40_{\mu gm^{-3}}$ by 31/12/2004

Table 1.1: Air Quality Standards for the U.K.

There are many different categories and types of air pollution and each bring their own problems. The principal pollutants found in the air include:

Carbon Monoxide

Carbon Monoxide (CO) is an odourless, colourless and tasteless gas that is produced as a result of the incomplete combustion of fossil fuels. CO is slowly oxidised to CO_2 once emitted into the atmosphere. Idling and decelerating traffic are a major source of CO with the contribution from traffic having increased from 60% in 1970 to 90% in 1992. Inhalation of CO can lead to reduced oxygen availability as it forms carboxyhaemoglobin by binding free haemoglobin molecules in the blood stream. There is a wide range of health problems associated with exposure to CO including exhaustion in young healthy adults and ECG changes in patients with ischemic heart disease.

Sulphur Oxides

Sulphur Dioxide (SO_2) is the primary pollutant associated with acid deposition following oxidation to Sulphuric Acid (H_2SO_4). Concentrations of SO_2 have decreased in the last number of years, as a result of the move away from coal use as a domestic fuel. In fact in many major cities there is now legislation in place banning the use of coal as a fuel in urban areas. In the UK however, the burning of fossil fuels in industry means that SO_2 is still being produced. High levels of SO_2 together with high levels of particulate matter are believed to have been jointly responsible for the increased number of deaths and hospital admissions for respiratory complaints during the London Smog of 1952.

Nitrogen Oxides

Nitrogen Oxides, collectively known as NO_x comprise several different gases such as Nitric Oxide (NO), Nitrous Oxide (N_2O) and Nitrogen Dioxide (NO_2) with NO_2 being the one that causes the most concern for human health. Approximately 50% of the atmospheric NO_2 in the UK is produced by motor vehicles resulting in higher levels of NO_2 in urban areas. NO_2 is a health concern because it is an airway irritant and high levels of NO2 can affect people with respiratory problems such as asthma and may possibly result in hospital admissions.

Volatile Organic Compounds

Volatile Organic Compounds (VOC's) comprise a broad range of organic compounds. The major problem with VOC's is their ability to generate ozone, particularly the short-lived compounds that contribute significantly to atmospheric photochemical reactions. Some VOC's however are potential health threats in themselves, for example Benzene, which is known to have carcinogenic and toxic effects. Most of the Benzene in urban air (~80%) comes from the benzene content and partial combustion of petrol in spark ignition cars.

Ozone

Ozone (O_3) is a highly reactive oxidising agent that is present naturally in the atmosphere as a result of the action of sunlight on NO_2. As already mentioned, VOC's also contribute substantially to the levels of ozone as a result of their contribution to the atmospheric photochemical reactions. Ozone is a secondary pollutant and evidence suggests that ozone levels have increased dramatically in recent years. In terms of the risk to the population, ozone is a highly reactive oxidising agent, which attacks biological materials including cell membranes and proteins and causes inflammatory reactions in the respiratory tract.

Particulate Matter

Essentially particulate matter is dust found suspended in the atmosphere. It is a complex mixture of organic and inorganic materials resulting from a variety of activities and arising from a number of different sources such as industrial processes as well as natural sources. These tiny particles of solid material or liquid aerosol are always present in the air but if their concentration becomes high they become an air pollution concern and as such a potential health concern. There are many different types of particulate matter and due to its varying sizes and its ability to be inhaled deep into the lungs; it's presence in the atmosphere poses a potentially serious health threat to the general population as well as to certain individuals within the population.

Cardiff and other cities in Britain should be able to attain the standards and objectives laid out by the Government and this is beneficial in terms of improving the quality of the air and the health of the general population. Unfortunately this doesn't change the fact that those living close to the side of very busy urban roads are potentially being exposed to high levels of both fine and ultrafine particles from traffic on a regular basis, levels of air pollution vary across urban areas with certain area like busy roadsides always likely to have higher levels of particulate pollution. It is a well known fact that exposure to pollution has potentially serious side effects, particularly for those with already compromised health and for the very young and very old.[6-7]

Pollutants in the air are much more difficult to treat and control than other pollutants and for standards to be achieved full co-operation from governments is required so as to limit the effects pollution in one country has in another. For example, Ozone is considered a trans-boundary pollution resulting in high levels of ozone in the UK that have been generated in countries across Europe and carried in on the winds. This means that European governments must support each other in an effort to clean up the air. With the evidence suggesting that particulate air pollution is a principal reason for aggravated respiratory and/or cardiovascular problems as well as for increases in hospital admissions and deaths it looks as if most countries are willing to set and comply with air quality regulations and standards. Unfortunately as long as traffic levels in urban centres continues to rise the risk presented by particulate pollution remains all too real and the prospect of even healthy individuals being at risk from the effects of prolonged exposure to particulates from traffic is becoming a more legitimate possibility.

Particle size is the most important parameter for characterizing the behaviour of aerosols. Most aerosols cover a wide range of sizes with a hundredfold range between the smallest and largest particles of an aerosol quite normal. Particles are generally described in four modes; nucleation mode, accumulation mode, fine particle mode and coarse mode. The nucleation mode consists of particles formed by condensation of hot vapours or nucleation mode are generally <0.2μm in diameter. The accumulation mode consists of particles that have grown from the nucleation mode as a result of the coagulation or condensation of vapours, resulting in particles ranging in size from 0.2μm-2μm in diameter. The fine particle mode simply consists of the combined nucleation and accumulation modes. The coarse mode consists of particles derived mainly from natural sources, such as from soil or sea spray and consists of particles greater than 2μm diameter.

Although their size, shape and density determine the ability of particles to remain suspended in the air, these properties will also influence where in the respiratory tract they are deposited and this will also potentially reflect the ability of the particles to cause damage. The ISO defines four main fractions of airborne dust; the inhalable, thoracic, respirable and high-risk respirable fractions. Briefly, the inhalable fraction consists of the mass fraction of total airborne particles inhaled through the nose and mouth; the thoracic fraction consists of the mass fraction of particles capable of penetrating the respiratory system beyond the larynx; the respirable fraction consists of the mass fraction of particles penetrating further still to the uncilliated airways or the alveolar region of the lungs where gas exchange occurs and; the high risk respirable fraction is a definition of the respirable fraction which is applied to the sick, the infirm and to children, i.e. those who are possibly more susceptible to the effects of the respirable fraction than are generally healthy individuals.[6-8]

Various studies have been carried out using different particle sizes as indicators of particulate air pollution. The most common measures of particles are; total suspended particles (TSP) which refers to the mass of airborne particles and consists of particulate matter as measured by a sampler with a cut-off point of between 25 and 40μm aerodynamic diameter; Respirable suspended particles as measured by a sampler with a 3.5μm cut-off point; Thoracic particles (PM_{10}) as measured by a sampler with a cut-off point of 10μm and; Fine particles ($PM_{2.5}$) as measured by a sampler with a cut-off point of 2.5μm.

1.2) Main sources of particulate pollution

There are many types of microscopic particles floating in the air, from re-suspended soil to photo-chemically formed particles. These airborne particles are examples of aerosols – a collection of solid or liquid particles suspended in gas. Aerosols are two- phase systems, consisting of the particle and the gas in which it is suspended. They include a wide range of phenomena such as dust, fume, smoke, mist, fog, haze, cloud and smog. The word aerosol was coined around 1920 as an analog to the hydrosol, a stable liquid suspension of solid particles.[9]

Aerosol	A suspension of solid or liquid particles in a gas. They are usually stable for at least a few seconds but in some case's they can last a year or more.
Bioaerosol	An aerosol of biological origin, including viruses, viable organisms such as bacteria or fungi and products of organisms such as fungal spores and pollen
Dust	A solid particle aerosol formed by mechanical disintegration of a parent material, such as by crushing or grinding.
Fume	A solid particle aerosol produced by the condensation of vapors or gaseous combustion products. These sub-micrometer particles are often clusters or chains of primary particles.
Mist and Fog	Liquid particle aerosols formed by condensation or atomization
Smog	A general term for visible atmospheric pollution in certain areas

Table 1.2: Sources of different types of secondary particles

There are two types of particles – primary and secondary. Primary particles are emitted directly into the atmosphere from sources such as open cast mining, non-nuclear power stations, vehicular emissions, cement factories or any industry predominantly using combustion processes. Combustion is the major source of air particulates and is a complex process, which produces distinct particles of varying size, in a number of ways. One common method is vaporization followed by condensation. They can also occur naturally, for example soil or sand particles, sea spray, airborne spores, pollen grains and their fragments.

At present not a lot is known about how much secondary particles contribute to PM_{10} levels, however quite a bit is known about the gases from which they are formed and their sources *(Table 1:2)*. Secondary particles generally form as a sulphates or nitrates from other pollutants such as SO_2 and NO_2. The sources that emit SO_x and NO_x are generally located in urban areas with the exception of some major power plants located rurally. Secondary sulphate and nitrate particles take a number of days to form and can remain in the air for days or weeks which can result in the long range transportation of particles from the site of origin.

Particulate Matter *(PM)* consists of a broad range of both chemical and non-chemical constituents. Based on their composition it is sometimes possible to pinpoint the source of particular types of PM in given areas. [6-7, 9-10]

Secondary Particulates	Precursor	Source of Precursor
Sulphates	Sulphur Dioxide	Industry, Diesel vehicles, Marine Vessels
Nitrates	Nitrogen Oxides	Motor Vehicles, Combustion Sources
Organic Carbon Compounds	Various Hydrocarbons	Motor Vehicles, Industry, Vegetation

Table 1.3: Various components of particulate matter can be used to identify the source of the particles

The major chemical constituents of PM_{10} are:

Re-entrained Dust: The dust consists mainly of metal oxides such as aluminium, calcium, iron or titanium oxides with the concentrations depending on a number of factors such as the presence of industry in the area. Re-entrained dust consists of vehicular and road materials arising from brake wear, tyre wear and pavement material. This material is found mainly in the coarse particle fraction of PM_{10}

Sulphates: Sulphates occur as a result of the conversion of SO_x gases to particles and the most commonly occurring forms of sulphate include ammonium sulphate, ammonium bisulphate and sulphuric acid. These compounds are water-soluble and are almost exclusively found in the fine $PM_{2.5}$ fraction of particles.

Nitrates: Ammonium nitrate is the most commonly occurring nitrate in particles owing to the nature of these particles, they can easily evaporate in the atmosphere or after they have been collected on a filter as a result of changes in temperature or relative humidity.

Sodium Chloride: NaCl or salt is generally found in particles originating from coastal regions and can be found in the coarse fraction of PM_{10} in it's raw form and in the fine fraction after evaporation from sea salt or when re-suspended from melting snow.

Organic and Elemental Carbon: Organic carbon in particulates is made up of hundreds of different compounds all of which contain carbon atoms. Elemental carbon (soot or carbon black) consists of pure, graphite carbon and non-volatile organic materials such as PAH's.[6-7, 9-10]

Two other particles sizes which have more recently become the focus of attention are ultrafine particles and nanoparticles. The ultrafine particle fraction consists of particles smaller than 100 nm (nanometres) diameter and the nanoparticle fraction consists of particles with a diameter less than 50 nm.[6-10]

1.3) Traffic and Particulate Pollution.

Road transport is a major source of air pollution in the UK and contributes approximately 25% of the total Particulate Matter (PM) in the atmosphere. As is to be expected large cities and towns are most affected by these particulates due to the higher volumes of traffic present, with city centre traffic contributing between 30 & 40 % to the annual average PM_{10} concentrations.[7]

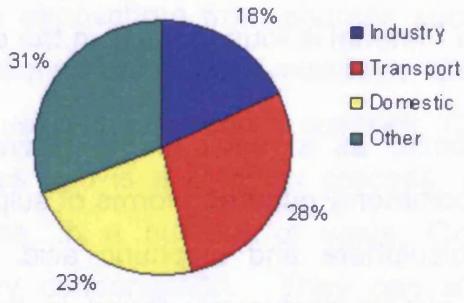


Fig 1.1: Numerous sources contribute to the levels of particulates in the atmosphere

There is also evidence to suggest that on days where pollution levels are high the contribution of road transport is often higher than usual. For example, analysis by Government experts shows that when particulate levels exceed health standards, the contribution by road traffic is in the range of 75-85%.[11]
Emissions from motor vehicles fall into two main categories;

1) Particles generated from vehicle exhaust
2) Vehicle related particles from tyre, clutch and brake wear

Emissions from vehicles depend on two main factors; the use of the engine and the type of fuel used. If an engine is running efficiently then the main emissions are carbon dioxide and water, slow or idling engines however are very inefficient and therefore emissions are mainly the products of incomplete combustion such as carbon monoxide and VOC's from petrol engines and particulates, smoke, VOC's and carbon monoxide from diesel engines. Products of incomplete combustion also result from cold engines with petrol engines taking up to 10km to warm up to complete efficiency and diesel engines taking up 5km to reach the same levels of efficiency. Diesel engines have a number of advantages over petrol engines; primary among which is the fact that diesel fuel contains more energy per litre than petrol and diesel engines take less time to reach an efficient working temperature. Diesel engines however are not without their own problems because although overall they emit fewer hydrocarbons, carbon monoxide and lead pollution than petrol cars they produce more noxious gases and significantly particulate matter. Diesel fuel is a mixture of many different hydrocarbon molecules. The combustion, both complete and incomplete, of diesel fuel forms a complex mixture of organic and inorganic compounds in the gas and particle phases. Those found in the gas-phase, under normal atmospheric conditions are the volatile organic compounds (VOC's) and despite the tendency to focus on the particle phase one must be aware that the gas phase also emits toxic compounds such as aldehydes, aromatics (e.g. benzene) and polyaromatic hydrocarbons (PAH's). Diesel particles also contain PAH's, nitro-PAH's, sulphates and metal. Diesel particles are complex and cover a range of sizes and morphologies with the ability to penetrate the lung, thus presenting a health threat. Like most engine combustion particles, diesel exhaust particles are quite fine with quite small aerodynamic diameters. The size distribution shows a significant fraction of the mass at about 0.3µm in the accumulation mode, which accounts for about 80-95% of the total mass. The formation of accumulation mode particles as aggregates of carbonaceous material and ash results in particles with a very large surface area and hence they are incredibly efficient at absorbing toxic elements such as PAH's to their surface.

With the ever-increasing numbers of vehicles on the roads in recent years and ever expanding knowledge regarding both the environmental and health effects of exposure, control of vehicular emissions has become a vitally important part of air quality management. Clearly, the best option is to prevent these emissions from being generated as far as is possible and this can be accomplished through several different means. For example since January 1993 all new cars sold in the EU have been fitted with a catalytic converter which resulted in the dramatic decrease in emissions of nitrogen oxides and other harmful pollutants. Diesel engines could be replaced with engines using alternative fuels such as propane or compressed natural gas. Diesel fuels themselves could be reformulated to reduce the amount of sulphur and other contaminant generating compounds they contain. Diesel fuel reformulation can be a relatively simple and straightforward process and the reformulation most commonly used today and required by the EPA for On Road diesel engines in 2007 is to reduce the sulphur content of diesel fuel from as high as 3000ppm to less than 15ppm. Another option is to use biodiesel, a clean burning alternative fuel, produced from domestic, renewable resources. Biodiesel contains no petroleum, but it can be blended at any level with petroleum diesel to create a biodiesel blend. It can be used in compression-ignition (diesel) engines with little or no modifications. Biodiesel is simple to use, biodegradable, non-toxic, and essentially free of sulphur and aromatics.

Although not strictly caused by traffic there are considerable quantities of dusts on road surfaces which arise from the ingress of soil on vehicle tyres and from the atmosphere, the erosion of the road surface itself and the degradation of parts of the vehicle – especially the tyres. Because these particles lie on a surface that readily dries and are subject to atmospheric turbulence caused by passing vehicles, this provides a ready source of particles that are resuspended into the atmosphere. [7, 12-14]

1.4) The Fate of Atmospheric Particles

Particles in the air are affected by a variety of forces and undergo turbulent coagulation, turbulent diffusive deposition and gravitational sedimentation. For example coagulation increases the size of dust particles, resulting in a decrease in concentration. Coagulation occurs when Brownian motion, differential sedimentation, or turbulent flows force two particles into contact and the particles stick to form an aggregate. The rate of coagulation depends on both the forces driving the relative motion of the particles and the hydrodynamic, van der Waals and electrostatic inter particle interactions.

There are numerous environmental impacts resulting from the presence of ultrafine particles in the atmosphere. Ultrafine particles are an important component of atmospheric aerosols and the beneficial effect of removing trace gases such as sulphur dioxide and volatile organic compounds as they can act as sinks for reactive species since their exposed surfaces can catalyse heterogeneous reactions.

As particle diameter becomes larger, particle concentration decreases. Ultrafine particles that originate via direct emissions from gasoline and diesel combustion coagulate quickly through diffusion. The rate of coagulation is dependent on the square of the particle number concentration. For example, a particle mass distribution of an urban aerosol has a bimodal distribution with a maximum in the accumulation range (0.1-1 or 2μm) and another in the coarse particle range (>1 or 2μm). Particles in the accumulation mode have a longer residence time than UFP's because removal by diffusion is negligible. These particles grow slowly by coagulation until they exceed 2μm where sedimentation and impactation become significant.[9, 15-17]

1.5) Particulate Pollution and Health

Although it has never been entirely clear what caused the excess deaths during the London Smog, it was noticed that most of the deaths were as a result of respiratory and cardiovascular diseases and of these deaths many were people who were already suffering from a pre-existing illness of some kind. It is widely accepted that the Smog episodes resulted in many people dying prematurely as a result of the increasing pollution in the air. Reanalysis of the data however has suggested that the number of deaths was underestimated with reassessment showing that the 1952 smog was accompanied by and followed by unusually high mortality rates for up to 2.5 months. Reanalysis of the data it has shown that a number of deaths that were attributed to influenza at the time could indeed has been due to the effects of air pollution.

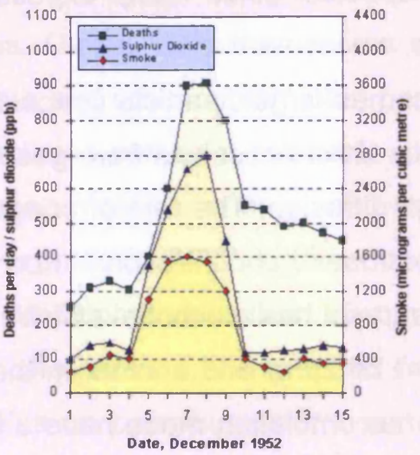


Fig 1.2: The relationships between smoke and sulphur dioxide pollution and deaths during the great London smog, December 1952, Source: Wilkins, 1954, taken from DEFRA[10]

Until recently, studies have concentrated mainly on the effects of PM_{10} (particles of <10µm) and epidemiological evidence appears to indicate a clear relationship between increased PM_{10} levels and morbidity and mortality from both cardiovascular and respiratory illness. Recently however inhalation research involving animals has demonstrated that the smaller particles such as $PM_{2.5}$ and below have the potential to be more damaging. It is now widely accepted that exposure to these two different particle fractions can pose significant health threats.

Immunological	Immune Cell Activation and Influx Inflammation Cytokine Activation Complement Activation
Biochemical	Oxidative Stress
Blood	Platelet Activation Increased Viscosity Increased Coagulation
Cellular	Apoptosis Macrophage Overload General Cell Function
General	Aggravation of Pre-existing Conditions such as Asthma and/or COPD

Table 1.4: Evidence suggests various possible effects of exposure to health pollution

While there is evidence to suggest that exposure to air particulate air pollution causes the worsening of symptoms and in some cases the premature death of persons with pre-existing cardiac illnesses, there remains little knowledge as to the mechanisms by which particles actually elicit their effects. Particulate pollution has been associated with cardiac arrhythmias and heart attacks and may possibly aggravate heart diseases such as congestive heart failure and coronary artery disease. Particles may also aggravate lung diseases such as bronchitis, causing increased medication use and doctor visits and increase susceptibility to respiratory infections. It is believed that they produce heart disease by damaging the endothelial barrier in the vascular system, activating leukocytes and platelets, initiating the formation of arteriosclerotic plaques, and stimulating an inflammatory response.

1.5.1) Particulate Pollution and the Respiratory System

Respiratory Disease: Respiratory Diseases are conditions that affect the nose, throat, larynx, bronchial tree and lungs. Among the most common respiratory diseases are asthma, bronchitis, emphysema, chronic obstructive pulmonary disease (COPD), influenza and pneumonia and they represent the most common reason for consulting a GP and also cause a large number of deaths. There are numerous factors known to both cause and aggravate respiratory diseases, chief among which is smoking, both active and passive. Air pollution may also be an influential factor. Depending on location, in the UK, every breath we take draws in between 0.5 million and 50 million particles into our lungs and these particles can be deposited in the lungs in an array of manners including sedimentation, inertial impactation, interception and diffusion.

Sedimentation	settlement by gravity, tends to occur in the larger airways
Inertial impactation	occurs when an airstream changes direction in large airways, particulary the nose
Interception	applies to irregular particles such as fibrous dusts like asbestos which are intercepted by collision with the bronchial walls.
Diffusion	this is the behaviour of very small aerosol particles which are randomly bombarded by the molecules of air.

Table 1.5: Methods of particle deposition in the lungs

The primary function of the lungs is rapid and efficient gas exchange, a function that is accomplished by a well co-ordinated interaction of the lungs with the central nervous system, diaphragm and chest wall musculature, and the circulatory system.

The lung's defence system is both mechanical and chemical. For the most part, inhaled particulate matter is either exhaled or trapped in the upper airways of the respiratory system and expelled. Some particles however make it past the initial defence mechanisms and enter the trachea and the lungs. These inhalable particles are sequestered in protective mucus secreted by the epithelial cells lining the airways and propelled, by the co-ordinated beating of cilia lining the surface, to the throat for expulsion. These so called pulmonary clearance mechanisms work together to protect the respiratory system from the effects of the majority of particles. These mechanisms however, can only deal with the inhalable and thoracic fractions ($PM_{15\text{-}10}$), i.e. those particles that are large enough to be trapped by the cilia or mucus in the larger vessels and airways. Respirable particles ($PM_{<3.5}$), which penetrate beyond these ciliated airways, cannot be cleared by the same mechanisms as the inhalable and thoracic fractions. These particles are deposited in the lungs and alveoli and can have potentially far-reaching consequences, particularly for the susceptible groups in the population. In the alveoli the main lung defence is provided by the macrophages, cells that are attracted to and ingest invading micro-organisms. Macrophages have many functions including; the secretion of defensive chemicals, the ability to attract other inflammatory cells to aid clearance and the ability to digest some organisms. Once they have ingested a particle, the macrophages transport it either up the airway or through the alveolar membrane into the lymphatic system that drains fluid from the peripheral lung to the lymph nodes and thence into the blood stream. This system has evolved to ensure that virulent organisms are either removed or carried to places where the body's immune defences are concentrated and where the organism can be most effectively neutralised. The effects of respirable particles in the lungs include; slowing down the exchange of carbon dioxide and oxygen in the blood, resulting in shortness of breath and straining the heart because it must work harder to compensate for oxygen loss. Other carcinogenic and non-carcinogenic effects have been seen in the post-mortem examination of the lungs of various animals exposed to insoluble, low toxicity particles.[7, 8, 18]

1.5.2 Particulate Pollution and the Cardiovascular System

Cardiovascular Disease: Cardiovascular Diseases (CVD's) are diseases that affect the proper functioning of the heart and blood vessels, chief among which are: myocardial infarction (heart attack), cerebrovascular diseases (stroke), trasient ischaemic attacks (TIA) and peripheral vascular diseases. According the the World Health Organisation, an estimated 17 million people die of CVD's, particularly heart attacks and strokes, each year. There is some evidence to suggest that smaller particles bypass the respiratory defence mechanism and cross into the bloodstream. The effects of this are currently unclear however some studies suggest particles in the cardiovascular system could be related to increases in heart rate and blood pressure, fibrinogen and blood coagulation factors; arterial vasoconstriction; inflammatory mediators and decreases in heart rate variability. A person's relative risk due to air pollution is small compared with the impact of established cardiovascular risk factors such as smoking, obesity or high blood pressure. In spite of this however, exposure to particulate air pollution presents a potentially serious public health problem because of the enormous number of people that are exposed over an entire lifetime.[19-21]

It is now accepted that exposure to particulate pollution, whatever the source, is a potential health risk for the susceptible population subgroups and there is a whole range of evidence to support this. In this setting susceptibility relates to the higher probability of any given individual developing certain diseases of a respiratory or cardiac nature under a given exposure or set of exposures, or indeed simply showing a worsening of symptoms of an already existing illness. Susceptibility describes the higher probability of an individual to develop certain diseases under a given exposure or set of exposures. This is, in most cases, associated to a higher sensitivity of these individuals to a specific exogenous or endogenous substance or a physical agent. With regard to particulate air pollution, susceptible population subgroups appear to include; children, those with industrial exposure, elderly and anyone with pre-existing cardiac and/or respiratory illnesses.

Chapter 2
Evidence of the Health Effects of Particulate Air Pollution: A Literature Review

As far back as the 1600's, there was some recognition of the potential problems posed by air pollution. Jon Evelyn attempted, in Fumifugium, to draw attention to the problem of air pollution in London and even suggested some solutions for dealing with the problem. Evelyn stressed the vital role which air plays in the well being of a body, highlighting how quickly the air we breathe reaches vital organs such as the lungs.

"Aer that is corrupt insinuates it self into the vital parts immediately; whereas the meats we take though never so ill condition'd, require time for the concoction, by which its effects are greatly mitigated; whereas the other, passing so speedily to the Lungs, and virtually to the Heart itself, is deriv'd and communicated over the whole masse".[1]

At the time the use of sea-coal, possibly so called because it was brought by sea to coastal centres in England during the 13th century, was widespread throughout London and according to Evelyn this created a 'hellish and dismall cloud' and the smoke and grime was making the air unbreathable. It was almost three centuries later however, after the Great London Smog of 1952 resulted in the premature death of more than 4,000 people, before anyone paid much attention and began to consider ways to combat air pollution.[1] Although it was becoming clear that air pollution was a problem, it was not clear how and why.

Over the centuries the nature of air pollution in Britain has changed drastically, thanks in part to legislation introduced after the 1952 smog. Back in the 1600's, John Evelyn wrote about the numerous effects of smoke, pointing out that it made churches and palaces look old, paintings, clothes and furnishings were discoloured, not to mention the health effects[1]. Nowadays the extreme air pollution episodes that left the cities buried under a thick blanket of smog for days appear to be a thing of the past. This does not mean, however, that air pollution is no longer a threat and just because pollution episodes are not as obvious as in the past, does not mean to say that they are a thing of the past.

Based on evidence, vast numbers of studies carried out in the last number of years, it would appear that there are still serious health implications of exposure to air pollution.

The first early attempts at keeping medical statistics would appear to be the Bills of Mortality, which would seem to have been brought into place as a result of the plagues that had ravaged London. Gathering together all the statistical information from the London Bills of Mortality, a draper, John Gruant attempted to comment on the effects of coal-burning on health. Much of the statistical material available to Gruant still exists today, allowing re-examination it his conclusions in light of modern knowledge, thus assessing the relevance of factors not then considered, such as meteorological factors[22].

Since then, numerous studies have been carried out, employing various different methodologies, in an attempt to determine the implications of exposure to air pollution. The primary study types of interest for this thesis are epidemiological in their methods, providing information as to the effects of exposure to air pollution in populations. However, the laboratory based studies such as chamber studies, *in vitro* and *in vivo* studies are not to be over-looked as they provide a large body of evidence as to the mechanisms of effect and the effects of exposure themselves at an individual and molecular level.

2.1) Epidemiological study designs

Generally, epidemiological studies of air pollution can be divided into acute exposure studies or chronic exposure studies. Acute exposure studies will be typically use short-term temporal changes in air pollution levels as the source of exposure variability whereas chronic exposure studies tend to employ spatial differences in pollution as the source of exposure variability. Acute exposure studies evaluate short term changes in health associated with short term changes in air pollution, with studies ranging from simple observations of changes in health over a single air pollution episode of one or more days, to sophisticated analysis of daily time series over several decades. Because these studies typically evaluate only short term temporal relationships (usually 1-5 days), the observed effects of pollution are usually interpreted as health effects of acute exposure. Chronic exposure studies, on the other hand, compare various health outcomes across communities or neighbourhoods with different levels of pollution. These studies are often interpreted as evaluating chronic and/or cumulative effects of exposure. In general time series studies can be classed as acute exposure studies whereas cohort studies are more likely to be chronic exposure studies.[23-24]

Time Series Studies

With regard to time series studies, the effect of interest, often a health outcome will be measured both before and after the suspected cause (e.g. an air pollution episode) has occurred. In its simplest form a time series study can be defined as a study consisting of a set of observations made sequentially in time or in other words the observation of the relationship between change in the average exposure level or intervention and the change in the disease rate for a single population. Due to the fact that time series methodology lends itself quite well to examining the effects of short-term fluctuations in air pollutant exposures on acute morbidity and mortality, they are quite frequently the design of choice for researchers wishing to use longitudinal study designs to investigate the relationship between ambient air pollutants and various health outcomes. Time series studies are stochastic in nature; what this means is that future outcomes cannot be exactly predicted from past values, i.e. one can only use evidence from time series studies to make an educated guess as to possible future outcomes.

In terms of the data collection there are two different types of time series study; discrete time series are when observations are made only at specific times, usually equally spaced and continuous where observations are made continuously in time.

When analysing a time series study one would be well advised to employ some simple descriptive techniques before going ahead with the more sophisticated inferential techniques. By applying these simple descriptive statistics first, more often than not the main properties of any given series will be revealed, thus making it easier for a researcher to select the most appropriate methods by which to further examine the data. For example a time plot, which is a simple and easily produced graph plotting observations against time, will very often reveal important features of the series such as seasonality and outliers.[24-26]

Seasonal Effect	The time series may exhibit an annual or seasonal variation
Other Cyclic Changes	Some time series will exhibit variation at a fixed period due to other physical causes
Trend	Loosely defined as a long-term change in the mean level
Other Irregular Fluctuations	Once trends and cyclic variations have been removed from the time series it is left with a series of residuals which may or may not be random.

Table 2:1 Common features seen in time series data

Cohort Studies

The aim behind employing cohort methodology is to enable researchers to observe individuals over a period of time and determine the frequency of disease occurrence among them. Cohort studies have two defining characteristics; they are exposure based and are either prospective or retrospective. A retrospective cohort study is one where all the relevant exposures and health outcomes have occurred at the time of the study commencing whereas in a prospective study the relevant causes/exposures may or may not have occurred at the time of the study commencing. Certainly the cases of disease will not have occurred at the time the study begins and following the selection of the cohort, the researcher must then wait for the materialization of the disease within the cohort. Although fundamentally the methodologies are similar for both retrospective and prospective studies, there is one very distinct and important difference between them and that is the ability to control for confounders and effect modifiers. It is easier to control for confounders at the outset of a prospective study when planning the data collection, but in a retrospective study, because the relevant events have already happened, the researcher must make do with what information is available, making it much more difficult to identify and control factors other than those of interest that may have had an effect on outcome.

The word cohort is often used to describe a group of people who share a common experience or condition a group of people sharing the same year of birth might be described as a birth cohort. Within cohort studies there are two main types of cohort; closed/fixed cohorts and open/dynamic cohorts.

A fixed or closed cohort is a group of people who are followed from a certain point in time until a defined end-point. The starting point of observation may be the exposure defining event or a point in time related to it and a comparable point in the non-exposed group. The endpoint is the occurrence of the disease of interest, death from another cause, loss to follow-up or a defined common endpoint in time, whichever comes first.

An open or dynamic cohort consists of persons who may enter or leave the cohort at any time without compromising the integrity of the study design. Thus open cohorts consist of persons whose exposure status or other characteristics relevant to the disease may change over time.

A cohort study can, in theory, be used to estimate rates, relative risks or occurrence times. In most situations, however, relative risks and occurrence times cannot be measured directly because this would require that everyone in the cohort remain at risk and under observation for the entire duration of the follow-up period. Alternatively the average risk and occurrence time must be estimated using life-table (survival methods). Life table methodology is a procedure by which the death rate (disease rate) of a closed population is evaluated within successive small age or time intervals so that age or time dependence of mortality can be elucidated.[23-26]

Person Time	The sum of each individuals time spent in the population over the risk period
Relative Risk	Measures the extent to which someone with a risk factor is likely to get the disease in question compared to some-one with no such risk factor
Absolute Risk	Incidence rate of a group exposed to a risk factor
Attributable Risk	The difference in incidence rates between exposed and non-exposed groups.
Incidence Rate	Number of new cases over a period / Population at risk for the time period during which cases collected

Table 2:2 Common terms associated with cohort studies

Both time series studies and cohort studies have their strengths and weakness' in terms of their suitability for use and there are a number of factors which must be considered. The following is a table outlining the advantages and disadvantages of time series and cohort studies.

Advantages	Disadvantages
Time Series Studies	
Examines a number of variables/exposures simultaneously with an outcome	Uses basic statistical methods
Continuous data gives strengths to the results	Long term effects cannot be estimated
Different lags of exposure can be considered	May over estimate or under estimate the relationship
Confounders can be adjusted for	Only known confounders can be adjusted for
Cohort Studies	
Allow direct measurement of the incidence of disease in the exposed and non-exposed groups	Are inefficient for studying rare diseases, unless the attributable risk is high, huge numbers of people are needed in the study
Can account for the relationship between the exposure and disease, in particular they can confirm that the exposure occurred before the onset of the disease	If prospective they can be expensive and time consuming
When prospective, they can minimise bias in estimating the degree of exposure	If retrospective they require adequate records to be available
Can examine multiple effects of a single exposure	The validity of their results can be seriously affected by losses to follow up
Can enable calculation of attributable risk	Long term commitment by staff
Can enable calculation of the outcome for rare exposures	Measurement drift in labs, staff
Can indicate the natural history of disease	

Table 2:3 Advantages and disadvantages associated with different study methods

Legislation has resulted in a reduction in ambient particle concentrations primarily by reducing the level of the thoracic fraction (PM_{10}). With regard to the respirable fraction ($PM_{3.5}$ and below) however, it is quite conceivable that this decline has not been quite so substantial and there is a distinct possibility that the levels of respirable particles may in fact have increased in the past decade.

Certainly the focus of attention appears to have shifted away from the thoracic fraction and towards the seemingly more high risk respirable fraction.

Although this thesis is concerned primarily with the possible adverse health effects of respirable particles, very little evidence exists in the current literature and, as a result, what follows is an extensive review of the available literature examining the health effects of both short-term and long-term exposure to particulate air pollution using measurements of thoracic particles and, where available, respirable particles as indicators of the levels of particulate air pollution.

Commonly employed methods for measuring levels of particulate pollution include measurements of black smoke (BS), total suspended particles (TSP), coefficient of haze and direct measurements of particles.

The methodology used to measure black smoke concentrations was developed over 50 years ago and are fully described in ISO 9835. Briefly, particulate matter is collected on a filter over a 24 hour period and the darkness of the stain is measured using a reflectometer. The size fraction of the material measured is defined by three factors – the inlet size, sample tube and the sample flow rate. The main advantage with measuring black smoke concentrations is that the monitoring equipment is simple robust and relatively inexpensive.

Coefficient of Haze (COH) is a measure of visibility interference in the atmosphere and is calculated using optical analysers such as photometers or nephalometers. These optical particle monitors utilise the interaction between air borne particulate matter and visible, infra red or laser light.

Total suspended particulates are measured by drawing air through a glass fibre filter at a rate of between 40 and 60 ft^3/min. The suspended particulates are then calculated by dividing the net weight of the particulate by the total air volume samples and are reported in $\mu g/m^3$.

Direct measurements of particles can be carried out in a variety of ways, most common of which is the use of TEOM analysers. The tapered element oscillating microbalance (TEOM) is widely used throughout the world for measuring continuous concentrations of particulate matter. The TEOM instrument works on the principle that the frequency of oscillation of a glass tapered tube (the element) changes by an amount that is proportional to the mass of the tube and as a result any change in mass of the tube due to deposition of particles will result in a change in resonant frequency that is proportional to the additional mass.[10]

Many studies have recently associated airborne particle concentrations with increased mortality;[28-88] increased respiratory symptoms and illness;[89-108] increased hospitalisation or emergency department visits;[109-127] reduced pulmonary function[128-136] and school absence.[137-141]

2.2) Epidemiological Evidence: Acute Exposure Studies

Mortality studies

Focusing on specific air pollution episodes, early studies examined the effects of acute exposures, evaluating changes in health before during and after the air pollution episode. These air pollution episodes varied in duration, lasting anything from a few days to a few weeks. Examples of severe air pollution episodes that have been studied include episodes at the Meuse Valley, Belgium;[28] Donora, Pennsylvania;[29] and London, England.[30]

In October 1948, a four day air pollution episode occurred in Donora, Pennsylvania. At the time the Public Health Service surveyed a sample of the population of Donora, collecting health effects information which then enabled Ciocco et al to conduct a follow-up study in 1957. Essentially the findings of the follow-up study were a) that of persons who had reported an acute illness at the time of the pollution episode appeared to show higher subsequent mortality and morbidity than did persons who had not reported any such illnesses and b) that of those who reported an acute illness, those who reported more severe acute illness appeared to show higher mortality and morbidity than those reporting only mild complaints. As they stand, these results would appear to suggest that persons who fell ill at the time of the episode were more likely to develop subsequent illnesses or to die than persons who did not. However when persons who reported cardiovascular diseases predating the 1948 episode were removed from the comparisons the difference between the two groups, those reporting illness and those not, was narrowed substantially, suggesting that in fact those who had a pre-existing cardiovascular illness were more likely to have developed acute illness during the pollution episode and that it was the pre-existing condition that resulted in the subsequently higher mortality and morbidity. A lack of data regarding individual pollutants meant that there was no way to discern which pollutant or combination of pollutants was responsible for the effects seen.

Logan demonstrated a possible linkage between cardiopulmonary mortality and morbidity with episodes of extremely elevated concentrations of particulate and/or sulphur dioxide air pollution by presenting figures from the Registrar-General's weekly returns in conjunction with additional data which indicate that the incident had an almost catastrophic effect on health, where for a few days, the deaths rates reached levels only previously seen at the height of the cholera epidemic of 1854. Based on the available figures, Logan estimates that the result of one of the most infamous pollution episodes, the Great London Fog of 1952, was to bring about approximately 4,000 deaths in the greater London area. As for cause of death Logan calculated that deaths from bronchitis increased by over eight times while pneumonia deaths increased by almost three times in the days during and immediately following the episode.

Later studies, examining the effects of less severe episodes, suggested the presence of smaller mortality effects associated with less severe and lower levels of pollution.

Wichmann et al[31] carried out a retrospective study in which the effects of a smog episode that occurred in parts of the North-Rhine Westfalia region from January 17th to January 23rd were investigated. The study examined data collected for a six week period from January 3rd to Feb 13th and in an effort to allow comparisons, data was collected from the state of North Rhine Westfalia, including the areas that were not affected by the air pollution episode. Mortality data; morbidity data including hospital admissions; ambulance transports; outpatients and consultations in doctors offices' were collected, along with ambient air pollution data and meteorological data. The main features of the smog episode were elevated levels of SO_2 and suspended particles (S.P.'s). In the polluted area a daily average SO_2 level of 0.83 mg/m^3 and a maximum daily measurement of 2.17 mg/m^3 were reached while for SP the corresponding concentrations were 0.6 mg/m^3 for daily average and 0.85 mg/m^3 for a three hour value. On analysis an increase of 8% in the number of deaths per day for the polluted area is noted with an increase of only 2% in the control area, with a clear increase in deaths from cardiovascular disease for both areas. As for morbidity, hospital admissions increased by 15% in polluted areas, five times as much as the 3% increase in the control areas. The strongest increase, an increase of 28% was recorded in the number of cardiovascular or respiratory patients who were taken to hospital in an ambulance. Splitting the group according to illness showed that there was a 25% increase in the number of cardiovascular patients while the increase of 36% for respiratory patients was larger. As far as the outpatients were concerned there was increase of 12% in the number of outpatient visits for the polluted area compared with a 5% increase for the control area. No major changes were seen in the number of consultations in doctors' offices although this could be due to a number of factors. One reason for this lack of change may be due to the fact that people are more inclined to remain indoors during periods of bad weather, a theory supported by the fact that visits to the doctor were strongly correlated with temperature but not with air pollution. Another reason for the lack of change in numbers could be down to the fact that those who would ordinarily visit their G.P. first may have been possibly have been more affected by the pollution episode and therefore by-pass their doctors' surgery and go straight to the Accident and Emergency Department.

In 1995, Anderson et al[32] reported on the effects of a 1991 air pollution episode in London, England. In December of 1991, an anticyclone (an area of moving air of high pressure in which the winds rotate outwards) lay over most of Britain and Western Europe. The resulting cold and stagnant air conditions, along with the corresponding temperature inversion which meant that emissions from vehicles and power sources were trapped close to ground level and prevented from circulating and dispersing as normal, provided the ideal conditions for the development of one of the thick and lasting fogs long since associated with London. Data for ten weeks around the 1991 episode week and for the same period for four control years were examined. Although for all five years there appeared to be an increase in deaths around December the dominant feature was a large increase in mortality and morbidity in 1989-90, one of the control years. This increase was later found to be associated with an influenza epidemic. Although the largest increase in mortality over the five years examined did not occur during 1991-92, a small peak, late in the episode week was observed and showed the highest number of daily deaths in the ten week period. Apart from ischemic heart disease in the age group 0-64 years, log linear analysis showed that the relative risks were increased for both all age and diagnostic categories. Overall the results of log linear analysis appeared to indicate that during the air pollution episode week, London experienced more deaths and hospital admissions than would have been predicted using data from control weeks years and areas.

Fairley[33] explored the relationship between daily mortality and suspended particulates in Santa Clara county from 1980-1986 and found that on days with increased particle numbers, the mortality rates appeared to increase. Using coefficient of haze as a measure of particulates, he found that same day coefficient of haze (COH) measures appeared as a significant predictor of mortality in all regression models examined bar two. However Fairley took care to highlight the fact that the associations observed do not necessarily imply causation and that further investigation was warranted.

Kinney and Ozkaynak[34] reported results of a multiple regression analysis in which they examined the associations between aggregate daily mortality counts and environmental variables in Los Angeles County, California. Five pollutant variables were included in the analysis including KM, a measure of particulate optical reflectance. Significant associations were found with KM and daily mortality, however KM was also highly correlated with NO_2 and CO, which were both significantly associated with mortality, making it difficult to estimate the individual effect of KM on mortality.

Subsequent time series studies increasingly used more formal time series modelling, including Poisson regression and began to hint at the emergence of a consistent pattern. These included studies in Steubenville, Ohio;[36] Philadelphia, Pennsylvania;[35] St Louis, Missouri and Kingston, Tennessee;[37] Detroit, Michigan[38] and Cincinnati, Ohio.[39]

In Steubenville, Ohio Schwartz found that a 100μg/m^3 increase in particulate levels was associated with a 4% increase in mortality on the succeeding day and that no statistically significant association with sulphur dioxide was observed after adjusting for particles.

The study carried out in Philadelphia, PA, compared daily total mortality and cause specific mortality with TSP and SO_2 concentrations. An average of 48 people died in the city each day and approximately 65% of the deaths occurred in people over 65 years of age. Initial analysis of weather factors showed that hot days, previous days mean temperature; mean dew point temperature and winter temperature were all significant predictors of daily mortality. Including TSP in the model indicated that particulates on both current and previous days were highly significant predictors of daily mortality using two day mean as the index for particles (previous and current day), resulting in an stronger association as compared with either day on its own. Adding both TSP and SO_2 to the model caused the effect of SO_2 to be reduced in magnitude considerably and become insignificant. TSP on the other hand remained significantly associated with mortality. In an effort to test the hypothesis that TSP might be serving as a vector to deliver SO_2 into the respiratory tract, the sample was divided into days above and below the median SO_2 concentrations (18 ppb). The coefficient of TSP was 0.000707+/-0.000238 during low SO_2 days and 0.000645+/-0.000187 during the high SO_2 days, hence there is no evidence that TSP act as a vector for SO_2.

In Kingston, TN and St Louis, MO positive associations were found with PM_{10} and daily mortality, although the Tennessee results did not reach significance. In St Louis neither SO_2, NO_2 nor ozone were significantly associated with total mortality. What was interesting about this study was that for both St Louis and Kingston the maximum daily PM_{10} concentrations during the year were considerably lower than the National Ambient Air Quality Standard of a 24 hour average of 150µg/m^3. This may indicate that the PM_{10} was acting as a proxy for some other factor, but on examination the associations found did not appear to be confounded by either meteorological factors or other pollutants. It is therefore also a possible indicator of the ineffectiveness of the NAQS in protecting the health of the population and indicate the need to reassess the standards.

The results of the Detroit study were quite similar to those found in Philadelphia. Data from over 500 days in Detroit, Michigan were collected and a site-specific predictive model for TSP was developed. From this, concentrations of predicted TSP were assessed as predictors of daily mortality. Again, in Poisson regressions controlling for year and time trend; various temperature variables including previous day's temperature, hot days and humid days were predictive of variations in daily mortality. Adding air pollution variables to the model showed that previous days predicted TSP was a highly significant predictor of mortality, as was previous days SO_2. Considering both previous days SO_2 and predicted TSP in the model, only predicted TSP remained significantly associated with daily mortality while SO_2 became highly insignificant. Unlike the Philadelphia study, the two day mean (previous and same day) was a less significant predictor of daily mortality, as were both current day measures on their own.

In Cincinnati, Ohio, the relationship between airborne particles and daily mortality was examined in an effort to determine if the findings of higher relative risks for pneumonia, cardiovascular disease and the elderly observed in Philadelphia could be replicated. TSP was found to be a significant risk factor for mortality (RR=1.03; 95% CI=1.03-1.10), an association almost identical to that observed with TSP in Philadelphia. Similarly for cause and age specific mortality, the relative risks were higher for the elderly and for deaths from pneumonia and cardiovascular disease (RR=1.09; 95% CI=1.04-1.14, RR=1.16; 95% CI=0.95-1.42 and RR=1.08; 95% CI=1.03-1.14 respectively).

Although previous groups of studies have shown consistency in their results across different communities, critics have suggested that the effects seen may be due to modelling techniques or the result of confounding by long-term time trends, season, weather or pollutants other than particles. There has been some replication and reanalysis of time-series studies that have found that the results of the original studies could be reproduced, as well as new studies carried out in different communities in which similar observations have been made. There have also been some studies in which the results did not agree with previous findings.

In two separate studies Moolgavkar et al reanalysed the Steubenville and Philadelphia data and in contrast to the results reported by Schwartz et al, the reanalysis of the data by Moolgavkar did not produce the same results despite the fact that every effort was made to use exactly the same data as had been used by Schwartz.[40-41] For example, similar to the original study in Steubenville, Moolgavkar et al found that TSP was significantly associated with mortality in the subsequent day; they also found however, unlike Schwartz et al, that the inclusion of SO_2 into the model caused the effect of TSP to be substantially decreased and become statistically insignificant. Similarly in Philadelphia, when TSP was the only pollutant considered in the model it was associated with mortality, as was SO_2. On the other hand and again, contrary to the results found by Schwartz et al, when pairs of pollutants were examined, the effect of TSP was greatly diminished in all seasons except summer. When ozone, a pollutant not examined in the original study, was added, the effect of TSP was again attenuated in the summer, suggesting that it is not possible to distinguish which of the pollutants was responsible for the effect observed.

There are a number of credible reasons why the results found in Moolgavkar's studies did not agree with the findings of Schwartz et al. The primary and probably most likely explanation for the discrepancies in findings is that although Moolgavkar et al went to the same primary data source as Schwartz, they were unable to exactly replicate either the mortality data or the pollution data sets. If this was in fact the reason for the difference in the findings then it points to the theory that neither study is robust to minor alterations in the data which in turn makes it incredibly difficult to draw any firm conclusions from either group of studies.

Other reasons for the differences in findings include the fact that Moolgavkar analysed the data by season in an effort to control for possible confounding by weather, although Moolgavkar did reanalyse the data as a single series (not separated by season) and found that it made little difference to the findings. The inclusion of ozone in his analysis by Moolgavkar may also bear some explanation as to the differences observed. With regard to the Philadelphia data, when ozone is introduced into the model, the effect of TSP is greatly affected in the summer and in addition, the seasonal effect of ozone was concealed when the data was analysed as a single season which would give credence to Moolgavkar's decision to both to include ozone in his analysis and to analysis the data series by season and raises the question of what difference the inclusion of ozone would have made to the findings of Schwartz et al. Differences in methodology may also affect the results and explain some of the differences in findings. Moolgavkar et al criticise the decision by Schwartz to use generalised estimating equations (GEE) in their analysis, claiming that the sample size was too small and the lack of correlations among daily counts of mortality rendered the use of GEE methods inappropriate and as such Moolgavkar et al chose instead to carry out straight Poisson Regression.

Although Moolgavkar's aim was to reproduce the findings of Schwartz et al, the inability to do so shows up the weaknesses in the work, the primary weakness being the lack of robustness of the results thus making it incredibly difficult to accept the findings presented by either group and highlighting the fact that associations presented in various studies can often times be tenuous at best.

Despite large differences in socio-economic factors, climate, environmental and other conditions, time series studies conducted in more than twenty cities across the world have found positive associations with particulate pollution and various health outcomes.

Among these studies is the APHEA study (Air Pollution and Health: European Approach), which evaluated data from 12 European cities in 10 different countries and the APHEA 2 study, which expanded on the original APHEA study to cover 29 cities and were conducted in an effort to provide quantitative estimates of the short-term health effects of air pollution.[42-43] For both studies substantial heterogeneity was found among the cities, with large differences observed in means levels of various pollutants across different cities, for example the mean 24hr level of black smoke ranged from 15-292mg/m^3. In the first APHEA study some of the heterogeneity for the effects of black smoke and sulphur dioxide was explained by using a list of predefined explanatory variables, which included the levels of pollutant elevated as well as levels of other pollutants, geographical differences and meteorological factors such as annual mean or seasonal temperature and humidity. On top of which, it was noticed that separation between western and central eastern European cities generated more homogenous subgroups, although significant heterogeneity remained for the effect of SO_2 in western cities. Overall an increase of 50μg/m^3 in the one day pollutant levels was associated with an increase in daily mortality of 3% for black smoke and 2% for PM_{10} in western European cities. The corresponding figures for central eastern cities were 1% for sulphur dioxide and black smoke. Only one city, Bratislava, had PM_{10} data and an increase in 4% was found there. The size of the effects of particles found was compatible with those reported in other studies and the effect of sulphur dioxide on mortality was consistent with, and of a similar size to, the effect of particles. Evidence from three different approaches was presented suggesting that the effects of particles and sulphur dioxide are independent. Firstly, if the effect of one pollutant was a surrogate of the other then ranking the effects estimated for one pollutant by the mean level of the other, for example plotting the SO_2 coefficient for all cities ranked by their black smoke level should show a monotonic trend in the size of the effect. This was not found for either SO_2 or any particulate measurements. Secondly, models for the two pollutants during days with low and high levels of other pollutants were fitted and the pooled results indicated independent effects. Thirdly, the results of the two pollutant models showed a similar moderate decrease in the effects of both pollutants which remained significant.

The expanded APHEA 2 study showed effects of particles, which were comparable to those in the original study. The effects were more intense for older individuals with a percentage increase in mortality for a 10$\mu g/m^3$ increase in PM_{10} of 0.8% (95% CI = 0.7-0.9%) under the fixed effects model and 0.7% (95% CI = 0.5-1.0%) under the random effects models when considering deaths among the elderly (>65 years of age). The primary difference between the two studies was that in the second, a range of pollutants were considered, whereas the first examined only particulates and SO_2. In the second study NO_2 was found to reduce the mortality associations for a 10$\mu g/m^3$ increase in black smoke concentrations with a reduction in total mortality of 55% and a reduction of 48% in PM_{10} associations. On examining the heterogeneity observed in the effect estimates of both PM_{10} and black smoke by taking into account the potential effects modifier, the authors found that NO_2 was the most important effect modifier and that in a city with low long-term average NO_2 concentrations the estimated increase in daily mortality associated with a 10$\mu g/m^3$ increase in PM_{10} was 0.19% as compared with 0.8% for a city with high NO_2 concentrations. The ratio of PM_{10} to NO_2 was also found to be of some importance, with a lower ratio associated with a larger PM_{10} effect thus intimating that perhaps the effects of PM_{10} and NO_2 could not be separated. Several other variables appeared to be effect modifiers but only those explaining more than 10% of the heterogeneity were presented. Apart from NO_2, the most important effect modifiers appeared to be temperature, humidity and standardized mortality (A Standardised Mortality Rate is calculated as the number of deaths observed within an area divided by the expected number of deaths within that area, multiplied by 100. To calculate the expected number of deaths, for each age group, the standard age-specific death rate is multiplied by the local population in that age group. The numbers of expected deaths in each age group are then summed across all ages resulting in the expected number of deaths for the population). The heterogeneity of single-city effect estimates supports the hypothesis that the composition of particles differs among locations in a way relevant to their health effects. An indirect way to investigate the toxicity of particles according to specific aspects of their particular characteristics is to evaluate the potential effect modification of factors influencing their composition across a number of environments. The relation found between NO_2 (an indicator of pollution originating from traffic) and particles suggest that particles originating from vehicle exhausts are more toxic than those from other sources. A conclusion supported by the work of others such as Laden et al[44] who examined the elemental compositions of all the $PM_{2.5}$ filters from the Harvard Six Cities study. Using the elemental composition of size fractioned particles, Laden et al identified several distinct source related particle fractions of particles. They then examined the association of the fractions with daily mortality in each of the six cities and combined the city specific results in a meta-analysis to derive overall relative risks for each fraction. Using lead as a marker of mobile source particles (the study was conducted when lead was still in use) they found the strongest increase in daily mortality associated with the mobile source (lead) factor. In the combined analysis across the six cities, daily mortality increased by 3.4% (CI=1.7-5.2%) with each 10μg/m^3 increase in the two day mean of the mobile source factor. Similarly, although not significant, (CI=-1.1-5.2%), there was evidence of a 2% increase in daily mortality from ischemic heart disease.

Other European studies of note include; the three cities study in Switzerland where Wietliebach et al analysed daily mortality and air pollution data for three large Swiss urban areas – Zurich, Geneva and Basle which, in 1980, had a combined population of 1406000;[45] the four cities study in Poland;[46] and a time-series study conducted by Anderson et al, which aimed to investigate whether associations existed between air pollution and daily mortality.[47]

There were several specific aims of the Swiss study, including:

1) To investigate whether daily mortality counts were associated with ambient concentrations of particulate air pollution as appeared to be evidenced in previous studies

2) To investigate whether any of the estimated mortality effects were robust to the application of various regression techniques
3) The potential effect of confounding by other factors including time trends was to be assessed
4) To evaluate the effect of other pollutants on the outcome in order to determine whether particulate pollution was the sole pollutant responsible for the observed effect
5) To explore the linearity of the dose-response relationship between pollution and mortality

Series of daily counts were calculated for several categories including; total mortality, mortality for persons >=65 year, respiratory mortality and cardiovascular disease mortality. Three separate regression modelling techniques were used in this study, the first of which used standard Poisson Regression techniques and included several indicator variables for year, month and ranges of minimum temperature and humidity, in an effort to evaluate the potential impact of such confounders as time-trends, seasonal factors and weather. A second series of semi and fully non-parametric models and a third series of Gaussian regression models were also estimated. In an effort to account for the probable delayed and/or cumulative effects of particulate pollution, 3-day lagged moving averages were found to best fit the model. Even after controlling for seasonality and daily temperature and relative humidity variations, particulate pollution was found to be statistically significantly associated with counts of mortality in both Basle and Zurich and after controlling for time trends and seasonality, the estimated pollution effects did not appear to be sensitive to the type of model applied, or to the addition of other covariates to the models, indicating that particulate pollution may indeed have been responsible for the observed mortality effects. There did remain the possibility however, that other pollutants may have contributed to the observed effects and so regression models for a range of other pollutants were also estimated and mortality was found to be associated with TSP, SO_2 and NO_2 and when all pollutants were included in the model together, the regression coefficients became unstable and statistically insignificant. One wonders however, whether estimating two pollutant models for various pairs of pollutants would have been a more appropriate next step, especially given that SO_2 and TSP appear to be highly correlated and past evidence suggests that the same is true for TSP and NO_2. As it was, the results of the multi-pollutant model meant that no particular pollutant or combination of pollutant could be assigned responsibility.

Anderson et al[47] constructed a time series from 1987 to 1992 of daily counts of all age mortality, for all causes of death excluding accidents, in London with daily number of deaths used as the outcome variable. They also included mortality for respiratory and cardiovascular diseases (ICD 9 codes 460-519 and 390-459 respectively). The aim of the study was to determine whether or not outdoor air pollution had a significant effect on the level of daily mortality. The findings of the study showed associations between daily mortality and various indicators of air pollution. Black Smoke and Ozone showed the strongest associations which were independent of other pollutants, with a 2.5% increase in all cause mortality in the warm season for black smoke, an effect that remained virtually unchanged on inclusion of ozone in the model. For respiratory diseases, the effect of black smoke in the warm season almost doubled on inclusion of ozone, from a relative risk of 1.0064 to 1.0143., although the correlation matrix shows little or no correlation between ozone and black smoke. Although some associations were found with nitrogen dioxide and sulphur dioxide, these could partly be explained by their correlations with ozone or black smoke. The authors point out that pollution levels in London are much lower now than they were twenty years ago and as such there may not be a comparable effect nowadays. They do however acknowledge the potential for effects in the already vulnerable population and advise that consideration be taken when determining policies for reducing emissions of pollutants.

Outside of Europe and North America, time-series studies of mortality include studies from Brazil,[48-50] Mexico,[51] South Korea,[83-84] China, Hong Kong,[82, 85] Australia and New Zealand.[86-88]

In Brazil two time-series studies were carried out in Sao Paulo during the early 1990's looking at various aspects of air pollution and its effects. One concentrated on the levels of nitrogen oxides and the levels of mortality in children under five while the other examined the effects of PM_{10} on mortality in the elderly.[48-49] A third study, conducted in the late 1990's sought to combine and expand on the effects of the previous two studies and examined the daily mortality for a range of age-groups but with specific emphasis on the elderly

(>=65 years of age).[50] Using single pollutant models, Gouveia found that cardiovascular and respiratory deaths were associated with a range of pollutants including PM_{10}, with adjusted relative risks of 1.060 (95% CI = 1.005 – 1.158) and 1.038 (95% CI = 1.001 – 1.076) for respiratory diseases and cardiovascular disease respectively, for an increase in PM_{10} from the 10^{th} to the 90^{th} centile. In children under five years PM_{10} was not significantly associated with either mortality outcome in children. These results do not correspond with the results found by Saldiva et al who found that NO_x was positively and significantly associated with respiratory mortality in children under five. Reasons for the differences in results may have been the result of differences may been the result of differences in methodology, for example Saldiva et al used only one year of data as compared with the three years used by Gouveia. Using only one year of data makes it incredibly difficult to identify and control for such potential confounders as time-trends or seasonality. Both studies reported high co linearity between pollutants, and Gouveia et al were unable to report on the results of a multi-pollutant model, claiming that the results were too difficult to interpret. Saldiva et al on the other hand found that the regression coefficient for NO_x was not hugely altered across different models suggesting that the association between respiratory mortality and NO_x was robust relative to controls considered. So does this mean that the results of one study can be considered more reliable than the other? In fact what it means is that more investigation is needed and that while Saldiva et al may be correct in their assumption that NO_x is responsible for respiratory mortality in children, there still remain questions as to the accuracy of the results based on the lack of data. It may be that NO_x is masking the effects of other pollutants or synergistic interactions may be occurring between pollutants and despite the apparent robustness of the association of NO_x and mortality, the results cannot be taken as read. In terms of Gouveia's study, although considerably more data was analysed, three years of data may still be insufficient to reveal any major cyclic patterns in the data, although it will provide more information than could be seen when using a single year of data, thus making it more possible to control for such trends and perhaps resulting in more accurate estimates, for this reason perhaps more weight should be given to the results presented by Gouveia. On the other hand, Gouveia hypothesises that the lack of an association between mortality and pollution in children points to the possibility that Brazilian children may some-how be immune to the effects. It is more plausible however, that children, while not affected seriously enough to result in death are likely to show effects such as increased asthma, cough, wheeze and reduced pulmonary function (discussed in detail later), all of which may result in children being more susceptible to the effects of air pollution later in life.

A study in Mexico City found that a 10μg/m^3 increase in fine particles was associated with a 1.3% increase in total mortality on the same day (CI = 0.2 – 2.5) and a 1.4% increase after a four day lag (CI = 0.2 – 2.5).[51] Two pollutants models, combining fine particles first with O_3 and then with NO_2 found essentially the same results as the single pollutant models and for the three pollutant model a 10μg/m^3 increase in PM_{10} was associated with a 1.7% increase in all-cause mortality, a 2.3% increase in mortality in persons aged >65 years and a 3.4% increase in cardiovascular mortality. In spite of the fact that there were positive associations between fine particles and mortality, none reached significance and for a city with over three million motor vehicles, this lack of any significant association, in conjunction with the lack of an association with NO_2 is perhaps somewhat surprising.

In Hong Kong, Wong et al analysed four years of data from 1995 to 1998 to investigate the association between air pollution and mortality from respiratory and cardiovascular conditions.[82] During the four years of the study, 46% of all deaths could be attributed to either respiratory or cardiovascular causes. On investigation a significant increase in mortality was observed for all four pollutants examined (SO_2, O_3, NO_2 and PM_{10}). The increase ranged from 0.8% for a 10μg/m^3 increase in PM_{10} to 1.5% for a 10μg/m^3 in SO_2. For mortality from COPD, a 10μg/m^3 increase in all pollutants except SO_2 were significantly associated with and a significant increase in mortality from ischemic heart disease was associated with a 10μg/m^3 increase in concentrations of all four pollutants and ranged from an increase of 0.9% for a 10μg/m^3 increase in O_3 to a 2.8% increase in mortality for a 10μg/m^3 increase in SO_2, with a 1.3% increase in mortality from IHD associated with a 10μg/m^3 increase in PM_{10}. Further analysis was conducted in the form of a multi-pollutant model and for respiratory mortality only SO_2 and O_3 remained significant (R.R. = 1.015 and R.R = 1.010 respectively) after elimination of any non-significant pollutants (p>0.05), although only O_3 remained significant, with a stable RR for all three models and O_3 was also the only pollutant to remain significantly associated with COPD mortality. For IHD mortality, only NO_2 remained significant in the two, three and four pollutant models but its relative risks were smaller than in the single pollutant model. No significant effect for PM_{10} was observed for any outcome in the multi-pollutant model, not even for respiratory diseases or IHD, the two outcomes for which positive associations were seen in the single pollutant models. Although this lack of effect is surprising, there are some possible explanations; PM_{10} concentrations were highly correlated with NO_2 ($r = 0.78$) and moderately correlated with O_3 ($r = 0.538$) and so the effects of PM_{10} may have been masked by the effects of those two pollutants. Hong-Kong has high ambient levels of NO_2 and as mentioned previously, NO_2 is an indicator of pollution originating from, traffic, also known to be one of the primary sources of particulate pollution in urban areas and so perhaps there is the possibility of PM_{10} acting as a vector to deliver NO_2 to the lungs, rather than eliciting an effect of its own. Another reason for the lack of observed associations could be down to the composition of the PM_{10} in Hong Kong. Although it is recognised that diesel exhaust is a major source of PM_{10}, it appears that crustal dust and particles of marine origins are also large contributors to the PM_{10} levels, thus it is maybe possible that the lack of association was due to the PM_{10} consisting primarily of these crustal and marine particles, which in the past have not shown significant associations with mortality and if this were the case then perhaps $PM_{2.5}$ would have been a more appropriate measure of particulates in order that any possible toxic effects of particles from diesel exhaust could be evaluated.

Hong et al conducted a time-series study in Inchon, South Korea in an effort to examine the associations between particulate air pollution and mortality.[83] The study period was almost two years and measured 24 hour averages of PM_{10}, SO_2, O_3 and CO and 8 hour averages of O_3. Using a five day moving average, PM_{10} was found to be positively associated with all cause mortality, cardiovascular mortality and respiratory mortality although the associations were only statistically significant for all-cause and cardiovascular mortality. A $10\mu g/m^3$ increase in the five day moving average of PM_{10} was associated with an increase in all-cause mortality of 0.8% and in cardiovascular mortality the increase was 1.1%. In the multi-pollutant models however, PM_{10} was positively and significantly associated with respiratory mortality and a 10μg/m^3 increase in the five day moving average was associated with an increase of 1.5% in respiratory mortality. It is the opinion of the authors that the low frequency of non-malignant respiratory deaths was the reason for the non-significant association in the single pollutant model. It is possible however that the significant association with PM_{10} observed in the multi-pollutant model was the result of interactions with other pollutants. In this study PM_{10} was highly correlated with SO_2 and there was a near linear increase in relative risk when using PM_{10} and SO_2 as the pollution index, which may suggest that a joint effects model of PM_{10} and SO_2 would have been a better predictor of mortality risk than single pollutant models.

Another study in Korea, this time conducted in Seoul and Ulsan found, that in contrast to the Inchon study, the effects of TSP were significant in the single pollutant model, as were all other pollutants measured, but in the multi-pollutant model the effect was no longer significant.[84] Again particulates and SO_2 were highly correlated ($r = 0.42$ in Seoul and $r = 0.72$ in Ulsan) which may again suggest that a joint PM and SO_2 model may be a better predictor of mortality or, bearing in mind the different effects associated with different particle compositions it may well be that in order to determine the effect of combustion source particles, joint PM and gaseous pollutant models are needed, or perhaps simply examining gaseous pollutants as indicators of combustion source particulate pollution.

One of the major limitations of both studies is again the lack of data analysed and although in both studies there was control for factors such as season, time and weather, with limited data the cyclic trends in the time-series may not be easily identified and adequately controlled for. Other issues with this study include the fact that Lee et al used GEE methods, an approach that has previously been criticised by Moolgavkar et al for being used on data sets that were too small.

Xu et al analysed a single year (1989) of data from two residential areas in Beijing, China.[85] The use of coal as the primary fuel for heating was, at the time, widespread in both areas and so domestic coal burning was considered to be the main source of particles (measured as TSP) and SO_2 supported by the fact that negative correlations were observed between TSP and SO_2 and temperature and humidity. Ln(SO_2) was a significant predictor of daily mortality but no significant association with TSP could be discerned. In the two pollutant model, SO_2 remained significant following inclusion of TSP in the model. Again TSP and SO_2 concentrations were highly correlated (r = ~0.6) and similarly to the Korean cities, a large quantity of the ambient TSP concentrations in Beijing are largely derived from coastal and other natural sources, all of which again brings into question the importance of particle composition and implies that more work is required to be concentrated in the area of particle compositions. The lack of association with particulate pollution in the study may also have been the result of the masking of the effects of particles by SO_2 and so possibly suggesting that other pollutants should be used as proxy measures or that in fact the effects of an individual pollutant cannot readily be ascertained and that any observations observed may simply be taken as an indication of the possible effects of a complex mixture of pollutants only.

Employing the same methodology as that used in the APHEA project, Simpson et al examined associations between daily mortality and air pollution levels in Brisbane, Australia.[86] Same day pollutant averages were found to be most significantly associated with daily mortality with maximum 1hour and 24 hour black smoke particles and 1 hour and 8 hour average O_3 levels being the two pollutants that were most significantly associated with total daily mortality, particularly in the summer season (October to March). When analysed separately, by cause of death, the associations were no longer significant for respiratory illnesses and only maximum 1 hour bsp data was significantly associated with cardiovascular mortality. Analysis by age for the three mortality categories resulted in positive and significant associations in the >=65 years age group but not in the <65 years.

Morgan et al constructed a time-series for the period 1989 through 1993 examining the effects of outdoor air pollution on daily mortality in Sydney, Australia.[87] After adjusting for seasonal and cyclic factors an increase in daily mean particulate concentrations from the 10^{th} to the 90^{th} percentile was associated with an increase of 2.63% (95% CI = 0.87 – 4.41) in all cause mortality and an increase in cardiovascular mortality of 2.68% (95% CI = 0.25 – 5.16) although neither association reached significance.

Hales et al reported on an investigation of the relationships between mortality and ambient particle concentrations in Christchurch, New Zealand.[88] The relationship between mortality and weather variables was also investigated. An increase in previous days PM_{10} by 10µg/m^3 was associated with an increase of 1% in all-cause mortality and 4% in respiratory mortality and when the analysis was restricted to mortality in people >65 years a slightly stronger association between PM_{10} and respiratory mortality was observed. No significant associations were observed between PM_{10} and cardiovascular mortality.

Hospitalisation Studies and visits to general practitioners (GP)

In the same way that daily counts of mortality can and have been examined for associations with air pollution, hospital admissions can also be evaluated. Studies of the same severe air pollution episodes as previously mentioned have not only found associations with mortality but have also pointed towards increased morbidity and an increase in hospitalisations and related health care endpoints.

Numerous studies have analyzed emergency department visits and found them to be associated with particulate air pollution. In 1981 Samet et al reported a very small but statistically significant increase in emergency room visits for respiratory complaints in Steubenville, OH that were associated with elevated levels of particulate pollution and sulphur dioxide.[109] Samet wanted to ascertain whether emergency room visits could reasonably be used as a sensitive index of the short-term effects of air pollution. In the first instance, the magnitude of deviations from expected values within specific pollution and temperature strata were assessed. Secondly, a linear threshold model was implemented. For both techniques no consistent correlation of lagged and unlagged pollutant levels was found either with total emergency room visits or with visits for diagnosis other than trauma. For respiratory diseases, the regression model identified a significant effect of both unlagged TSP and SO_2 but not NO_2. Introduction of a 24 hour lag eliminated the effect of TSP and SO_2. According to the authors, this effect of TSP should be interpreted with caution because although the regression coefficients reached statistical significance at $p<0.05$, the contribution of the pollutant variables to the multiple R^2 is only 0.01 – that is 1% of the variance of respiratory disease is explained by TSP and SO_2.

Dependent Variable	Independent Variable[a]	B	ΔR^2
Respiratory Disease	Maximum Temperature	-0.08[b]	0.006
	Unlagged TSP	0.007[c]	0.011
Respiratory Disease	Maximum Temperature	-0.06[c]	0.006
	Unlagged SO_2	0.013[c]	0.012
All Diseases	Maximum Temperature	0.26[b]	0.120
	Unlagged TSP	0.011[c]	0.010

Results of Linear Multiple Regression Analysis

[a] Regression coefficients for each independent variable are shown

[b] p<0.01; [c] p<0.05

ΔR^2 the contribution to the multiple R^2 made by each independent variable

Table 2.4 Results of the linear multiple regression analysis (taken from Samet et al, 1981)

Other US studies that have found associations between emergency room visits or hospital admissions and particulate pollution include a series of studies carried out by Schwartz and colleagues, who have examined the associations between hospital admissions and air pollution in Seattle, Washington;[110] Tucson, Arizona;[111] and Detroit, Michigan.[112]

In Seattle significant associations were found between PM_{10} and emergency room visits for asthma. Previous days PM_{10} was a significant predictor of asthma emergency room visits while PM_{10} values two, three and four days before the visit were also significant predictors. Using the mean of the previous four days had the best explanatory power. Although one of the biggest peaks for asthma admissions occurred in March, a time when the pollen levels in Seattle were quite high and it would be reasonable to expect this to have an effect, excluding March from the analysis had little impact on the PM_{10} regression.

In Tucson, Arizona Schwartz found that PM_{10} and CO were associated with increased risk of cardiovascular hospital admissions. Admissions increased by 2.75% (CI=0.52-5.04%) for a 23μg/m^3 increase in PM_{10} and by 2.79% (CI=0.51%-5.41%) for a 1.66ppm increase in CO. In contrast, little association was seen with SO_2, NO_2 or ozone. In a two pollutant model containing both PM_{10} and CO the effect estimates were not hugely different from the single pollutant models with an effect estimate of 2.37% (95% CI=0.08%-4.72%) and 2.33% (95% CI=0%-4.72%) for PM_{10} and CO respectively. Little change to the effect estimates when examining the data by season was observed either.

In Detroit, although a previous study had found associations between air pollution and mortality, no information was given regarding morbidity and air pollution. Schwartz & Morris presented the results of their analysis of air pollution data (airborne particles, ozone, SO_2 and CO) and hospitalisation for cardiovascular complaints including ischemic heart disease, congestive heart failure and dysrhythmias. The rationale behind the study was simply that if air pollution did indeed have an effect on mortality, as was apparently evidenced by the results of the previous mortality study, then in all likelihood there would have to be an effect on hospitalisations for the same illnesses. The results showed that PM_{10} was a significant predictor of admissions for ischemic heart disease as were SO_2 and CO. Ozone did not appear to be a significant risk factor for cardiovascular hospitalisations. When considered in the two pollutant models, both SO_2 and CO became insignificant after controlling for PM_{10} while PM_{10} on the contrary remained significant after controlling for either SO_2 or CO. Pertaining to congestive heart failure, PM_{10} and CO were both associated with admissions while SO_2 and ozone were not. In the two pollutant models both CO and PM_{10} remained significant predictors of heart failure admissions. For dysrhythmias, no pollutant was a significant risk factor for admissions with PM_{10} on the previous day being the closest to a significant risk factor (RR=1.09, 95% CI=0.996-1.044, p=0.11).

PM_{10}	B	RR*	95% CI
Previous Day	0.0033 +/- 0.00126	NK	NK
Mean of previous four days	0.00367 +/- 0.00126	1.12	1.04 – 1.20

*Relative Risk for a 30µg/m^3 increase in PM_{10}

Table 2.5 Relative risks of hospitalisation for a 30µg/m^3 in PM_{10} in Seattle Washington

Model	Relative Risk	95% CI
Base	1.037	1.064-1.012
Same Day Temp	1.037	1.064-1.012
Cold Dry Days	1.037	1.064-1.012
Mean Daily Temp	1.033	1.059-1.009
2 Day Average Temp	1.037	1.063-1.012
Relative Humidity	1.037	1.064-1.012

Table 2.6 Relative risks of hospitalisation associated with different weather variables in Seattle, Washington

Two studies, carried out in Birmingham, Alabama and Minneapolis examining hospitalisation data were reanalysed by Moolgavkar et al who questioned the adequacy of the methods used in the original studies.[113-115]

Schwartz carried out a study examining the association between inhalable particles and ozone and hospital admissions for respiratory illness in the elderly (>=65 years).[113] Data on all hospital admissions in the Minneapolis – St Paul's area (population 2.46 million in 1990) were collected and for pneumonia and COPD admissions, daily counts were constructed for each day from January 1st, 1986 to December 31st, 1989, of admissions for pneumonia and COPD. Air pollution data included 24-hour average Ozone measurements for each monitor which were then averaged to give a single 24 hour measurement for all monitors. An alternative measurement, the average of the highest 1-hour readings for each monitor was also calculated. It is less clear as to the measurements of PM_{10} used for the study. Daily mean temperature and dew point temperature were also collected. For pneumonia, significant associations were found for both PM_{10} and Ozone independently with hospital admissions and when both pollutants were considered simultaneously there was little change in the estimated size of their effects. For COPD, ozone was negatively but insignificantly associated with hospital admissions, while for PM_{10} significant positive associations were found for COPD admissions when using PM_{10} measures for both the previous day and the concurrent days, although in the model using both days, the effects appear to be greater with a lag of one day. Significant associations were found between PM_{10} and both COPD and pneumonia and due to the statistical methods employed these associations were not confounded by seasonal patterns. Ozone on the other hand made an independent contribution to pneumonia admissions only and to COPD.

Likewise in Birmingham, Schwartz examined the association between inhalable particles and ozone and hospital admissions for respiratory illnesses in the elderly (>= 65 years).[114] Ozone measurements for the Birmingham study were the same as those used in the Minneapolis study and again there is some ambiguity as to the measurements of PM_{10} but it appears that 24 hour average was the measurement of choice. Daily mean and dew point temperature were again the weather variables examined. A significant association was seen between PM_{10} on the concurrent day and hospital admissions for pneumonia. Same day ozone concentrations on the other hand were not associated with pneumonia although some association was seen with ozone two days before admission. For COPD, a significant association with admissions was found while for ozone, previous days concentrations were weakly associated with admissions for COPD.

Pneumonia		
	Relative Risk*	**95% CI**
PM_{10}: Minneapolis^{1}	1.17	1.33-1.03
Minneapolis^{2}	1.17	1.33-1.02
Birmingham^{1}	1.19	1.07-1.32
Ozone: Minneapolis^{1}	1.19	1.33-1.02
Minneapolis^{2}	1.15	1.36-0.97
Birmingham^{1}	1.14	0.94-1.38

Table 2.7

Chronic Obstructive Pulmonary Disease		
	Relative Risk*	**95% CI**
PM_{10}: Minneapolis^{1+}	1.22	1.52-0.99
Minneapolis^{1+}	1.37	1.68-1.12
Minneapolis$^{2\#}$	1.18	1.49-0.93
Minneapolis$^{2\#}$	1.34	1.67-1.08
Birmingham	1.27	1.08-1.50
Ozone: Birmingham	1.17	0.86-1.60

*** Relative Risk for a 100µg/m^3 increase in PM_{10} and a 50 ppb increase in 24 hour average ozone measurement.**

1= single pollutant model; 2= two pollutant model

+= same day PM_{10} measurement; #= previous day PM_{10} measurement

Table 2.8

Tables 2.7 & 2.8: The combined results of the Minneapolis and Birmingham studies by Schwartz, separated by disease

Moolgavker again reanalysed the studies by Schwartz and again his reanalysis did not entirely confirm the original findings.[115] Using two more years of data and investigating the effects of other pollutants not considered in the original analysis, Moolgavkar et al found that although in a single pollutant model PM_{10} in Minneapolis was strongly associated with respiratory mortality, its effect was greatly attenuated on simultaneous inclusion of ozone, NO_2 and SO_2 in the models. In Birmingham, no consistent association of any pollutant with respiratory admissions was found, although only ozone and particulates were investigated due to the large amount of missing NO_2 and SO_2 data.

Despite finding an association however, Moolgavkar et al found that the stronger association was with ozone and that the association with PM_{10} was not robust to the inclusion of other pollutants, NO_2 and SO_2, not included in the original analyses. Variations in methodology and the amount of data were given as possible reasons for the difference in findings.

Across Europe, studies conducted in various cities have also reported associations between air pollution, including particulate pollution and hospitalisations for a range or respiratory or related health-care endpoints. The main body of evidence in Europe is again provided by the APHEA project. A range of air pollutants, including particles were found to influence the rates of hospital admissions, particularly for COPD but also for various respiratory diseases and asthma. Outside of the APHEA project, other studies to have found associations between particles and hospitalisation rates for respiratory and/or cardiovascular events include studies conducted in Athens, Greece;[116] London, England;[117] and Barcelona and Valencia, Spain.[118-119]

Pantazopoulou et al reported the results of a large population based epidemiological study, which assessed the short-term impact of air pollution on hospital emergency outpatient visits and admissions in the population of the greater Athens area. A positive association was found between the number of visits and air pollution levels for all pollutants measured but only NO_2 showed a statistically significant association. For emergency cardiac admissions the effect of all air pollutants used was statistically very significant. During the summer months air pollution did not appear to affect the daily number of cardiac admissions to a significant degree. Similarly the effects of air pollution on respiratory admissions were significant during the winter and insignificant during the summer.

Ponce de Leon constructed a time-series to examine the effects of a variety of pollutants, including particles measured as black smoke concentrations, on hospital admissions for a variety of respiratory diseases. After controlling for possible confounders such as long-term trends, seasonal fluctuations and an influenza epidemic, the results showed the most consistent association with respiratory admissions was observed for 0_3. Effects were observed with the other pollutants, including black smoke concentrations for some age-groups but the only statistically significant association observed was for black smoke lagged 1 day for children over the whole year and this was a negative association (RR=0.9815; 95% CI-0.9641-0.9993; p<0.005) a result thought, by the authors, to be due to the negative correlation between black smoke and 0_3 (r=-0.287).

Sunyer et al assessed the association between daily number of emergency room admissions for COPD and levels of black smoke and SO_2 in Barcelona. Positive associations were found with both SO_2 and black smoke and COPD emergencies in both summer and winter, although the association with black smoke during the summer was less evident. For an increase of $25\mu g/m^3$ in SO_2 an adjusted change of approximately 6% in the number of COPD emergencies during the winter and 9% during the summer was seen. For particulate pollution (as measured by black smoke) a similar association was seen in winter but an association of lesser magnitude was seen in the summer.

Ballester et al carried out a time series study with the intention of examining the short-term association between air pollution levels and hospital admissions for cardiovascular diseases.. Using the hospital admissions data for the two main urban hospitals in Valencia, the authors identified people of interest according to the ICD-9 code on their admissions information, selecting those who were admitted for cardiovascular diseases, heart diseases, cerebrovascular diseases and digestive diseases (excluding vascular related) as controls. Pollution data was collected from the city's air pollution monitoring network and consisted of 24 hour average levels of black smoke as well as measurements of other pollutants including; NO_2, SO_2, CO and ozone.

Analysis of the data involved using Poisson autoregressive regression to estimate the magnitude of association. Analysis was in two stages; firstly, a core model was built for each outcome variable, controlling for the main confounders and secondly estimation of the association of air pollutants was made by examining the delayed relation up to the fifth lag of pollutants. Although they claim their results suggest that current levels of air pollution and emergency cardiovascular admissions are positively and significantly associated with four of the five pollutants examined showing a statistically significant associations, the data presented suggests that in fact only three of the pollutants, Black Smoke, SO_2 and CO show significant associations, and then only in the hot semester. Ozone showed no significant associations for any outcome, NO_2 showed a clearer association with cerebrovascular diseases while CO was related with both groups of cardiovascular diseases. With regards to the two pollutant models the association of NO_2 with admissions for cerebrovascular diseases was the only one to remain unchanged after controlling for the other pollutants.

Meta-Analysis

The purpose of a meta-analysis is to either quantitatively or qualitatively combine the results of several studies in order that an overall effect of a given variable or intervention may be assessed. The benefit of a meta-analysis is that it may result in a stronger conclusion than be gained from individual studies on their own. A typical meta-analysis of a measure of effect computes an average coefficient and an estimated standard error corresponding to the coefficient. Often, a weighted average is computed, these weights can take two forms. One set of weights gives more emphasis to studies that had lower stochastic errors in estimating their regression coefficient. Usually it is assumed that the true risk may vary between studies because of differences in populations, effect modifiers etc. Hence a random effects model is usually fit with two components of variations. The first is within study variance and the second is the random study effect, incorporating the estimated variance in the true coefficient between studies.

Schwartz conducted a review of several studies, comparing the results found in different studies to the levels of confounders in the individual studies to assess the likelihood that the results are driven by inadequate control for those factors. The author then combines the results in a formal meta-analysis.[73]

The central concerns in the evaluation of time-series studies of daily mortality and air pollution are the possibility of confounding by some other pollutant and the possibility of confounding by weather and/or season. One other concern is the quality of exposure assessment. All of the studies reviewed by Schwartz have used data from outdoor monitoring stations and there is evidence to suggest that these do not accurately represent exposure levels and, within the studies evaluated, a variety of methods by which to measure PM levels have been used, ranging from the more accurate direct measurements of respirable particles to optical measurements that potentially miss some particles. Schwartz assigned these factors different weights for other pollutants present, and to the different methods used to address weather/season as a possible confounder. The studies being reviewed were divided into groups based on the type of analysis that was performed. The largest category consisted of eight locations where regression models were fit, relating daily mortality to air pollution and which all collected the air pollution data from population based monitors. A ninth study (Fairly[33]), carried out in Santa Clara is quite similar in that Poisson regression analysis was performed, it differs however in that it used coefficient of haze as its exposure variable. Four other studies using optical measures of particle concentrations and choosing to treat their data as Gaussian rather than using Poisson regression represent another category. One final estimate from London showed that mortality in the London administrative county in the four days ending December 9th was 2.1 fold elevated from the four days ending December 5th.

	Relative Risk	95% Confidence Interval
1	1.06	1.05-1.07
2	1.06	1.04-1.06
3	1.06	1.05-1.06
4	1.06	1.05-1.07
5	1.06	1.05-1.08
6	1.06	1.05-1.07
7	1.06	1.04-1.09
8	1.07	1.05-1.08
9	1.06	1.02-1.09

1-Unweighted meta-analysis, 2-Variance Estimates with inverse variance weights, 3-Inverse variance weights and quality weights, 4-Quality weights for all studies, 5-Locations with winter peaking pollutants (unweighted), 6-locations with winter peaking pollutants (weighted), 7-Above average mean temperatures (unweighted), 8-Above average mean temperatures (weighted), 9-no other pollutants

Table 2.9 Results of the Meta-analysis for each of the models used to calculate a combined relative risk and 95% CI

All of the studies reviewed reported an association between air borne particles and daily mortality with most of these associations were highly significant. The relationships are therefore either causal or they are the result of confounding and in an effort to ascertain the reason behind the observed relationships, Schwartz examined a variety of the most likely sources of potential confounding and found that the likelihood of confounding by any of the factors examined was reasonably unlikely. A primary example of factors that may affect the observed relationships are of course other pollutants such as SO_2, NO_2 or ozone and to counter this, most papers reviewed by Schwartz employed statistical methods to examine whether the effects of particles observed could in fact be considered independent or not. Two papers in particular, the Utah Valley study and the Santa Clara studies were carried out in locations where particle concentrations were moderate to high but where SO_2 concentrations were negligible. The finding of practically identical associations between particulate air pollution and daily mortality in these locations as in other locations is the strongest argument for the independence of particle effects. Overall the evidence suggests that it is unlikely that the associations between particles and daily mortality resulted from either confounding with other pollutants or inadequate control for confounding. Based on the review and analysis Schwartz presented, it would seem that direct and indirect epidemiological evidence suggests that commonly occurring levels of particulate air pollution are indeed associated with a range of adverse outcomes, including early mortality.

There has long been a question mark over the representativeness of US data for Europe.

In Europe, a WHO task group conducted a meta-analysis of time series studies of particulate matter.[54] According to agreement, meta-analytical estimates for the effect of particles (PM_{10}, $PM_{2.5}$, black smoke and coarse fraction) and ozone were estimated for the following health outcomes:

1) Daily all cause mortality, daily respiratory mortality and daily cardiovascular mortality as identified by WHO International Classification of Diseases (ICD)
2) Numbers of daily hospital admissions for respiratory and cardiovascular diseases, examined by age-group
3) Cough in individuals with underlying respiratory disease (for children and adults separately).
4) Use of medication in people with pre-existing respiratory conditions (analysing children and adults separately)

Following an extensive and detailed search of the available literature, a database was constructed in which to compile the relevant info. Each study was assigned a unique identifier which allowed users to select any set of results defined by various factors such as outcomes (e.g. mortality); disease; age-group, pollutant etc. Various criteria and guidelines were set out with respect to study selection and these include:

1) The decision was taken not to compromise any of the criteria of study selection in order that the number of studies available for meta-analysis might be increased in the event of a lack of studies fitting the predefined criteria.
2) Studies from outside of Europe would only be considered in the event that there were insufficient European studies available to include in the meta-analysis

3) No more than one estimate from any city was to be included in the meta-analysis and with this in mind the decision was taken to select only the most recently published study or, in the case of multi-city studies, to use a multi-city result where possible.
4) The initial analysis was to focus on simple rather than multi-pollutant models
5) The selected lag from the database would be used rather than specific lags or combinations of lags.

The meta-analysis itself calculated fixed and random effects summary estimates for each pollutant-outcome pair for an effect of 10μg/m^3 increase in the pollutant. Estimates of PM_{10} effects on all cause mortality were taken from thirty three separate European cities or regions, with 21 of the estimates taken from the APHEA 2 multi-city study. For a 10μg/m^3 increase in PM_{10} the random effects summary relative risk was 1.006 (95% CI=1.004-1.008). The number of estimates available for cardiovascular and respiratory mortality separately was much less, 17 and 28 respectively and the corresponding summary estimates were 1.009 (95% CI=1.005-1.013) and 1.013 (95% CI=1.005-1.020).

Similar results were found for black smoke concentrations and all cause mortality with an overall summary relative risk of 1.006 (95% CI=1.004-1.008) and for cardiovascular mortality and respiratory mortality the overall summary estimates were 1.004 (1.002-1.007) and 1.006 (0.998-1.015) respectively. For particles and hospital admissions sufficient numbers of estimate of the effect of PM_{10} were only available for respiratory admissions in the 65+ age group and based on the eight available estimates the relative risk for a 10μg/m^3 increase in PM_{10} was 1.007 (1.002-1.013).

With respect to fine particles only five estimates were available for all cause mortality.

Mortality		
	Relative Risk	95% Confidence Interval
PM_{10}		
All cause	1.006	1.004-1.008
Respiratory	1.013	1.005-1.020
Cardiovascular	1.009	1.005-1.013
$PM_{2.5}$		
All cause	1.0034	0.9915-1.0154
Black Smoke		
All Cause	1.006	1.004-1.008
Cardiovascular	1.004	1.002-1.007
Respiratory	1.006	0.998-1.015

Table 2.10: Relative risks for mortality

Hospital Admissions		
	Relative Risk	95% Confidence Interval
PM_{10}		
Respiratory >=65 years	1.007	1.002-1.013
$PM_{2.5}$		
Respiratory		
0-14years	1.091	0.9994-1.0391
15-64 years	0.9881	0.963-1.0135
65+	0.9926	0.9732-1.0125
Black Smoke		
Respiratory	1.006	1.001-1.010

Table 2.11: Relative risks for hospital admissions

Tables 2.10 & 2.11: Combined results of the meta-analysis, separated by mortality and morbidity taken from the meta-analysis

2.3) Epidemiological Evidence: Chronic Exposure Studies

Acute exposure studies provide little information about the long term effects of particulate air pollution such as how much life is shortened, how pollution effects longer-term mortality rates or pollutions potential role in the process of inducing chronic diseases that may or may not be life threatening. Chronic exposure studies evaluate the effects of low or moderate exposure that persists over long periods of time, as well as the cumulative effects of repeated exposure to elevated levels of pollution.

Mortality Studies

There are three primary cohort studies available for review and these are; The Harvard Six Cities Study;[55] a study conducted by the American Cancer Society[56] and a study of some 6,500 non-smoking Adventists.[57] All three are prospective cohort studies and measured the survival rates if individuals. These person based studies use subject specific information regarding known and/or suspected risk factors to control for potential confounding of the air pollution associations. The Harvard Six Cities study reported the results of the prospective follow-up study, designed to determine whether an association existed between air pollution and mortality. The study was carried out across six US cities in the early 1990's and controlled for various confounders such as individual smoking status, sex, age and other risk factors. The study population, selected at random from six communities, was restricted to 8111 white adults 25 through 74 years of age at enrolment, undergone spirometric testing, and had completed a standardized questionnaire. Enrolment occurred from 1974 (Watertown Massachusetts) to 1977 (Topeka, Kansas) and the total duration of follow-up was 14-16 years (111,076 person-years).

Air pollution data was collected from centrally located monitoring stations in each community, concentrations of TSP, SO_2, ozone and suspended sulphates were among the pollutants for which data was gathered.

Analysis showed that for all measures of air pollution, except ozone levels and aerosol acidity, ambient concentrations were highest in Steubenville and lowest in Portage and Topeka. Correspondingly, crude mortality rates and survival curves showed that mortality was highest in Steubenville and St Louis and lowest in Potage and Topeka, suggesting a possible association between air pollution and mortality.

Cox proportional hazard models showed that mortality was most strongly associated with cigarette smoking, which is perhaps not surprising, however increased mortality was also associated with having less than a high-school education and with increased body-mass index (increased BMI being especially true for women). Smoking, increased BMI and lack of education are all indicators of lower socio-economic status, which might explain some of the observed associations for the individual variables with air pollution.

Including the mean concentrations of each pollutant in the proportional hazards model showed a significant association between mortality and inhalable, fine or sulphate particles.

A second collaborative study, to test the hypothesis that mortality is associated with combustion source particulate air pollution was carried out linking individual risk factor data from the American Cancer Society (ACS) Cancer Prevention Study II (CPS-II) with national ambient air pollution data. The cohort consisted of a large group of adults drawn from 151 US metropolitan areas, who were followed prospectively over seven years from 1982-1989. Exposure to combustion source particles was estimated using measurements of sulphates and fine particles as indicator variables. Pollutant measurements used were mean concentration of sulphate air pollution for 1980 and median fine particulate concentration from 1979-1983. Three mortality categories were evaluated in this study: lung cancer, cardiopulmonary disease and all other causes. Cox proportional hazards models were estimated for each category and the models were then re-estimated after separating the data according to participants' smoking status and gender. The purpose of the re-estimation was to evaluate the robustness of the estimated effects found in the original model. The possible effect of confounding by climatic differences across the different metropolitan areas was evaluated by adding weather variables to the model, which accounted for hot or cold conditions. Adjusted mortality risk ratios were calculated for cigarette smoking as well as for differences in pollution levels between the most polluted and least polluted areas.

Cause of Death	Current Smoker	Sulphates (19.9µg/m^3)	Fine Particles (24.5µg/m^3)
All	2.07 (1.75-2.43)	1.15 (1.09-1.22)	1.17 (1.09-1.26)
Lung Cancer	9.73 (5.96-15.9)	1.36 (1.11-1.66)	1.03 (0.80-1.33)
Cardiopulmonary	2.28 (1.79-2.91)	1.26 (1.16-1.37)	1.31 (1.17-1.46)
All other	1.54 (1.19-1.99)	1.01 (0.92-1.11)	1.07 (0.92-1.24)

Table 2.12: Adjusted mortality relative risk (95% confidence intervals) by cause of death for cigarette smoking and for a difference in pollution. Table taken from Pope et al, 1995.[56]

For current smokers the risk ratios were estimated using sulphate data and, correspond to the risk of death for a current smoker with 25 years of smoking 20/day as compared with a never-smoker. For sulphates and fine particles the difference in pollution was equal to the difference between the relevant pollutant levels in most polluted area when compared with the least polluted area.

An association between mortality and air pollution was observed and the association persisted even after adjustment for age, sex, race, and smoking, exposure to passive smoke, occupational exposure, education, BMI and alcohol use. When compared with the relative risks for smoking the relative risks for air pollution and mortality appear quite small, however it is no surprise to find that the associations with cigarette smoking were present and larger than those observed for air pollution, particularly for lung cancer.

Statistically significant associations ($p<0.001$) with sulphates were observed for all-cause, cardiopulmonary and lung cancer mortality while significant associations were also found for all cause and cardiopulmonary mortality but not lung cancer when using fine particles as the pollution index. To evaluate whether the lack of association between fine particles and lung cancer was as a result of using different study areas or different pollution measures, the estimates were recalculated after restricting the models to use data only from the areas where both sulphate and fine particle measures were available and both fine particles and sulphate measurements were included in the model. The adjusted risk ratio using the two pollutant models for lung cancer was 1.44(1.11-1.86) and for cardiopulmonary disease was 1.20(1.08-1.34). The results obtained from the two pollutant model were not hugely different from the initial results obtained thus suggesting that sulphates are more strongly associated with lung cancer mortality than are fine particles.

Separation of the data by gender and smoking resulted in little change to the relative risks obtained using combined data. For example for all deaths using unseparated data the relative risks associated with differences in fine particles was 1.17(95% CI=1.09-1.26) and when the data were separated by gender, the relevant results were for women RR=1.16(95% CI=1.02-1.32) and for men RR=1.18(95% CI=1.07-1.30) thus suggesting little or no difference in the relative risk of mortality associated with fine particles for either men or women. The same appeared to be true when the data was separated by smoking status, with similar relative risks for never-smokers and ever-smokers. The results of this study indicate that air pollution, both sulphates and fine particles, were associated with and approximate difference of 15 to 17% between mortality risks in the most polluted and the least polluted areas.

A third prospective cohort study, the Adventists Health study of Smog (AHSMOG), also examined the relationship between long-term ambient concentrations of particulate air pollution and mortality. The study followed 6,338 non-smoking California seventh day Adventists (SDA) from 1977. To take part in the study the SDA's were required to have lived at least ten years within five miles of their residence at the time of enrolment and to be residing in one of the three California air basins of San franciscon, South Coast (Los Angeles and eastward) or San Diego. Using mortality data from 1977-1987, no associations of ambient particulates and increased risk of mortality were found. The data were then updated to include mortality through 1992 and re-examined.

	PM_{10} (above 100µg/m^3)	PM_{10} (mean)
All Causes		
Male	1.12 (1.01-1.24)	1.11 (0.98-1.26)
Female	0.94 (0.86-1.03)	0.94 (0.84-1.04)
Cardiopulmonary		
Male	1.09 (0.95-1.24)	1.10 (0.94-1.30)
Female	0.90 (0.80-1.01)	0.92 (0.80-1.05)
Non-malignant Respiratory		
Male	1.28 (1.03-1.57)	1.23 (0.94-1.61)
Female	1.10 (0.91-1.33)	1.10 (0.86-1.40)
Lung Cancer		
Male	2.38 (1.42-3.97)	3.36 (1.57-7.19)
Female	1.08 (0.55-2.13)	1.33 (0.60-2.96)

Table 2.13: Adjusted mortality relative risks and (95% CI) by cause of death for an interquartile range difference in PM_{10}(100) and PM_{10} (mean)

The relative risks were calculated for a difference in the inter-quartile range of the pollutant across the study population. PM_{10} showed a significant association with all natural causes of mortality in males. In simple pollutant models none of the other pollutants examined were significantly associated with all cause mortality and addition of the other pollutants to create two pollutant models did not have any great effect on the PM_{10} coefficient.
The robustness of the observed associations was investigated by conducting sub-group analysis for CRC for PM_{10}(100) and because the separate sex models resulted in similar coefficients the combined sex model was used. The associations of CRC mortality with PM_{10} was similar in past smokers and never smokers (RR=1.23; 95% CI=0.9-1.66) and (RR=1.17; 95% CI=1.00-1.37) respectively for an inter-quartile range increase of PM_{10}(100).

There are a number of strengths to the Harvard Six Cities study, the primary one being the fact that it controls for smoking. By its nature, the Adventists cohort study also controls for the effects of smoking in the sense that none of the participants were smokers. Many studies either do not or cannot adjust for smoking status and this is a major point of criticism especially given that this study showed the strongest association between mortality and smoking. All three studies appear to control adequately for smoking however. The American Cancer Society study even controlled for various different aspects of smoking status including passive smoking. In all three studies controlling for smoking did not appear to affect the air pollution coefficients.

Confounding of potential air pollution associations by factors other than smoking can affect the outcomes. In all three cohort studies the effects of a wide variety of potential confounders are examined including sex, occupational exposure, BMI, alcohol consumption and education. None of these factors appeared to substantially affect the associations observed between air pollution and mortality in any of the three studies.

In the six cities study, analysis conducted for sub-groups defined according to sex, smoking status and occupational exposure showed positive associations and the differences between the groups was not statistically significant. In the American Cancer Society study a lack of differences between the sexes was noted with respect to occupational exposure suggesting a lack of confounding by occupational variables as historically men are more likely to be employed in jobs with high industrial exposure to dusts and fumes. However, without specific employment information it is impossible to say whether that was the case for this particular cohort.

Abbey also found that accounting for such potential confounders as education, BMI and prior heart attack/stroke or diabetes did not substantially change the PM_{10} coefficient. Overall it would seem that all three studies have adequately controlled for a wide variety of potential confounders and in doing so have found that the associations with particulate pollution persist.

One of the limitations of the American Cancer Society study was that it relied on death certificates to identify cause of death. It has previously been noted that cross-coding between pulmonary and cardiovascular deaths occurs quite often (i.e. deaths due to respiratory disease are recorded as cardiovascular or circulatory deaths)[142-143] and to avoid the potentially biased and instable results by analysing cardiovascular and respiratory deaths separately, the authors combined them into on all encompassing group of cardiopulmonary deaths. This may however lead to the masking of an effect for one or other specific group or over estimation of the actual effect of the individual groups.

A reanalysis of the Six Cities Study and the ACS study was conducted with the objective of conducting a rigorous and independent assessment of the findings of the two studies by replicating and validating the original data and results and testing the robustness of the original results to alternate analytical approaches. Overall the analysis found that the quality of the original data could be assured and the original results replicated. In terms of the robustness of the results to alternative approaches the reanalysis did not find that the original results were substantially changed thus supporting the findings of an association between indicators of particulate air pollution and mortality in the original studies.[144]

Symptom and Disease Studies

While the number of studies examining long-term effects of air pollution on mortality may be few and far between the same cannot be said for studies evaluating associations between particulate air pollution and chronic respiratory symptoms and disease. In North America researchers examining the respiratory health of school children have found associations with both fine and ultrafine particles.

Dockery et al conducted a study, designed to examine the relationship between long-term, intermittent exposure to particle strong acidity and the respiratory health of children.[89] Twenty-four, predominantly suburban or rural communities, with homogenous, relatively stable populations and no major local sources of pollution were selected. Particle strong acidity was found to be associated with significantly higher reporting of bronchitis in the past year (Odds Ratio = 1.66, 95% Confidence Interval = 1.11 – 2.48 for the range of 52 $nmol/m^3$), as was annual mean sulphate (OR = 1.65, 95% CI = 1.12-2.42 for the range of 7$\mu g/m^3$). None of the pollutants were significantly associated with higher reporting of asthma, attacks of wheeze, persistent wheeze, chronic cough or chronic phlegm and there was a significant inverse association between chronic cough and PM_{10}. Gaseous acids were associated with a significantly higher risk of asthma (OR = 2.00, 95% CI = 1.14 – 3.53) and showed a positive association with higher reporting of attacks of wheeze, persistent wheeze and any asthmatic symptoms. Ozone was not significantly associated with a higher risk of any of the respiratory symptoms of interest although there was a suggestion of a higher risk of chronic cough associated with 24hr average ozone (OR = 1.29, 95% CI = 0.87 – 1.91).

To study possible chronic effects of air pollutants, Peters et al initiated a ten year prospective cohort study of Southern Californian children, with a study design focused on four pollutants: ozone, particulate matter, acid and NO_2.[91] According to their results, asthma prevalence increased by grade in females but not in males. The risk of physician diagnosed asthma was higher for males, blacks, children of parents with asthma, those with insurance and those living in houses with smokers or water damage. Risks were lower for Asians and those living in homes with plants. Patterns for current asthma were similar, but pests were associated with risks. Those spending more time outdoors did not have significantly higher risks for respiratory conditions. Bronchitis risk was higher in males, in those with parents with asthma, and those with hay fever, household mildew and health insurance. Risks were lower for blacks, Asians and those with carpet in their bedroom. There was a similar pattern for cough, with having asthmatic parents, hay-fever and house-hold mildew. The overall adjusted prevalence of specific chest conditions reported was analysed by community. Prevalence rates varied considerably between communities, with no obvious relationship to community air quality. In particular a community with comparatively good air quality, Atascadero, had the highest rates of asthma and bronchitis while the lowest rates occurred in Mira Loma, a community with comparatively poor air quality.

Based on the 1986-1990 exposure data, prevalence of wheeze was associated with exposure to NO_2 (OR = 1.48, 95% CI = 1.08 - 2.02) and acid (OR = 1.55, 95% CI = 1.09 – 2.21) in males only. Similarly, based on the 1994 exposure measurements a positive association of NO_2 (OR = 1.54, 95% CI = 1.08 – 2.19) and acid (OR = 1.45, 95% CI = 1.14 -1.83) was seen with wheeze in boys only. The overall results concerning outdoor air pollution show no consistent or large excesses of morbidity in subjects who lived in the most polluted communities and/or had the highest estimated exposures. According to the authors this might indicate: 1) little effect of even the most severe outdoor pollution, which seems unlikely given the volume of evidence to the contrary; 2) an increase of uncontrolled risk factors in cleaner communities offsetting any reduced risk from pollution which seems improbable; 3) an inability to detect important effects because of exposure misclassification, inadequate sensitivity of health measures, or bias in diagnostic practice between communities which would appear to be the most plausible explanation; or 4) effects of self-selection of place of residence inherent in cross-sectional comparisons.

In Europe, a study in Switzerland found that respiratory symptoms and bronchitis in children were associated with all air pollution parameters, including particles. Braun-Fahrlander et al conducted a cross-sectional survey of 4,470 children across ten Swiss communities in order that they might evaluate the potential associations of air pollution with respiratory symptoms and bronchitis in much the same way as had previously been done by Dockery et al.[92-93] Eight different respiratory and allergic illness and symptoms were evaluated including; chronic cough, dry nocturnal cough without infection, asthma and current wheeze. The air pollution measures included annual means of PM_{10} and NO_2 as well as measures of SO_2 and ozone. Each symptom was analysed separately to calculate the estimated relative increases of odds over the range of exposures observed. For PM_{10} the levels ranged from $10 \mu g/m^3$ in the least polluted community to $33 \mu g/m^3$ in the most polluted community. The odds of chronic cough were estimated to increase by a factor of 3.1(95% CI=1.62-5.81); for nocturnal dry cough without infection the odds were estimates to increase by a factor of 2.9(95% CI=1.69-4.89) and for bronchitis and conjunctivitis symptoms, the odds were estimated to increase by a factor of 2.2(95% CI=1.21-3.87) and 2.1(1.29-3.44) respectively. For asthma, current wheeze, sneezing attacks during the pollen season and a reference symptom, diarrhoea, no significantly positive associations were found.

In order to assess whether children with a family history of asthma and allergies resulted in stronger effects of air pollution, separate regression analysis was performed and for children with a reported family history of respiratory and conjunctivitis symptoms and bronchitis, stronger associations were indeed found, indicating perhaps that these may represent a susceptible population subgroup. For children with a family history of such symptoms the adjusted rate for chronic cough differed by 12.5% between the most polluted and least polluted communities as compared to a 3.8% difference for children with no family history of such symptoms.

Every reasonable effort was made to control for confounding in this study and for all examples, the associations found for air pollution with different symptoms appeared to remain stable. Although PM_{10} was found to be significantly associated with a range of symptoms, so to were NO_2 and SO_2 and due to the fact that all three pollutants were highly correlated according to the author (data not shown) it cannot be stated with any great certainty whether PM_{10} was solely responsible for the effects seen, especially as only data from single pollutant models was presented in this paper, and as acknowledged by the authors, each pollutant variable must be considered to represent the complex mixture of outdoor air pollution or multiple pollutant models must be constructed in order to the sensitivity of the associations found for individual pollutants to the inclusion of other pollutant variables.

In Germany a connection was found between diseases of the upper respiratory tract in Leipzig children and their exposure to suspended particles and other pollutants.[94] The aim of the study, conducted by von Mutuis et al was to investigate the importance of high and moderate levels of air pollution on the incidence of upper respiratory symptoms in 9-11 year old children living in Leipzig, East Germany. Study participants included all fourth grade students (n=1,854) from a random sample of 39 schools in Leipzig, Germany. Daily assessments of mean and maximum air pollution concentrations, temperature and humidity were available from air monitoring stations next to the respective schools. The air pollution measurement techniques were chemiluminescence for NO_x, beta-adsorption for PMs and ultra violet (uv) fluorescence for SO_2. During the winter months the concentrations of SO_2 and PMs increased considerably as a result of private coal burning for heating. SO_2 daily maximum levels ranged from 40µg/m^3 to 1,283µg/m^3 while daily maximum concentrations of PMs ranged from 53µg/m^3 to 1,040µg/m^3 although these levels dropped substantially in the summer months. During the high pollution period (October to March) the European standards for SO_2 were exceeded on 52% of test days, on 51% of test days for NO_x and on 28% of test days for PMs.

	Daily Mean		Daily Maximum	
Period	OR	95% CI	OR	95% CI
High: NO_x	1.53*	1.01-2.31	1.51	0.94-2.41
PM	1.28	0.83-1.99	1.62*	1.08-2.45
SO_2	1.72*	1.19-2.49	1.26	0.80-1.96
Com.	2.10*	1.30-3.37	1.91	1.01-3.63
Low: NO_x	1.82*	1.21-2.73	1.26	0.85-1.86
PM	1.00	0.66-1.50	1.13	0.75-1.70
SO_2	1.40	0.95-2.07	0.99	0.66-1.47
Com.	2.16*	1.23-3.81	1.72	0.77-3.86

*$p<0.05$

Table 2.14: Odds ratios from logistic regression analysis (taken from von Mutius et al[94])

All models controlled for paternal education, passive smoke exposure, number of siblings, temperature and humidity. Single pollutant models with NO_x, SO_2 and PMs respectively were all associated with a significantly increased risk of developing upper respiratory illnesses during the high concentrations period. The association for SO_2 and NO_x reached statistical significance only for daily mean; conversely only daily maximum levels of PMs were significantly associated with an increased risk during the high pollution period.

The results of the study suggest that high mean concentrations of SO_2 and moderate levels of particulate matter and NO_x are associated with an increased risk of developing upper respiratory illnesses in school children. After controlling for several potential confounders/effect modifiers including type of household heating, temperature and humidity, high mean concentrations of SO_2 and NO_x were significantly related to an increased risk of having upper respiratory symptoms at the time of examination. The association between upper respiratory illness and particulate matter however was only seen with maximum concentrations. These findings suggest that high continuous exposure rather than peak levels of SO_2 may increase the prevalence of upper respiratory illness whereas PMs may exert their adverse effects mainly through peak exposure.

A combination of high levels of different pollutants however resulted in the highest risk, suggesting that effects of individual pollutants are difficult to establish. The findings may also indicate that the high levels of bronchitis and respiratory symptoms that have previously been reported in East German school children may in part be the result of recurrent upper respiratory illnesses.

Schwartz used chronic respiratory symptom questionnaires and physician examinations from the first National Health and Nutrition Examination Survey (NHANES 1) to assess the relationship between long-term average TSP and chronic respiratory illness.[96] NHANES 1 could be used for this study because it covered a wide cross-section of the country and a large number of cities, making accidental confounding much less likely. In multiple logistic regression models, asthma was associated with pack-years of cigarettes smoked. Bronchitis was associated with pack-years also but dummy variables for current smoking and former smoking were also predictive. For dyspnoea, cigarettes per day, pack-years and former smoking were significant. For respiratory illnesses, current smoking, former smoking and pack-years were predictive. When TSP was added to the models, it was significantly associated with the risk of chronic bronchitis and of respiratory illness but was not associated with the prevalence of asthma or dyspnoea and when the model was restricted to never smokers, TSP remained significantly associated with the prevalence of bronchitis and was a marginally significant predictor of respiratory illnesses.

	OR	95% CI
Chronic Bronchitis	1.07	1.02-1.12
Respiratory Illness	1.06	1.02-1.10
Never Smokers		
Chronic Bronchitis	1.11	1.02-1.21
Respiratory Illness	1.07	0.996-1.15

Table 2.15: Odds Ratio for a 10µg/m^3 increase in annual TSP Concentrations

Abbey conducted a study that extended previous results of exposure to long term estimated ambient concentrations of PM_{10} and compared the findings with those previously reported for other pollutants.[97] The study was conducted on a pre-existing cohort of Seventh-Day Adventists. Briefly, the cohort consisted of 3,914 participants who completed a detailed lifestyle questionnaire as well was a standardized respiratory symptoms questionnaire. Development of new cases of airway obstructive disease (AOD) and chronic bronchitis were significantly associated with $PM_{10}(100)$ ($PM_{10}(100)$ refers to exceedance frequencies of ambient concentrations of PM_{10} above 100µg/m^3). The relative risk for developing new cases that were associated with an increase of 1,000 h/y in average annual exceedance frequencies above 100µg/m^3 for both AOD and chronic bronchitis was 1.17(CI=1.02-1.35 and CI=1.01-1.351) respectively. The relative risk for asthma was 1.30 (CI=0.97-1.73). Chronic bronchitis was also separated into chronic cough with sputum and cough only. The relative risk of a 1,000 h/y increase in $PM_{10}(100)$ for chronic cough with sputum 1.21(CI=1.02-1.44) and for cough only was 1.16 (CI=0.96-1.40).

The regression coefficients for mean concentration and for exceedance frequencies above 40, 50 and 60µg/m^3 were not statistically significant for AOD. The cut-off, 100µg/m^3 was statistically significant for both AOD and chronic bronchitis. The relative risks from elevated PM_{10} were higher for asthma than for AOD or chronic bronchitis but they were not quite statistically significant for any of the cut-offs, a finding that resulted from the smaller number of new cases however the relative risk approached statistical significance ($p=0.06$) for the cut-off of 100µg/m^3. Excess concentrations at all cut-offs (40, 50, 60, 80 and 100µg/m^3) were statistically significant for AOD and chronic bronchitis. No statistical significance for excess concentrations at any cut-off level was noted for asthma. The first set of analysis excluded individuals who had definite symptoms in 1977. For these individuals to be included and to ensure that reversal of symptoms as well as development of symptoms were accounted for, Abbey used a change in the symptoms severity score between 1977 and 1987 as an outcome variable for the three diseases.

PM_{10} was positively and significantly associated with an increasing severity score for AOD and for asthma. With respect to change in the bronchitis severity score, exceedance frequencies above 100µg/m^3 were not quite statistically significant but excess concentrations were. Sensitivity analyses were conducted by rerunning the multiple logistic regression models on those with and without occupational exposures to dust and fumes. These sensitivity analyses indicated a stronger association between PM_{10} and the development of definite respiratory symptoms in those with occupational exposure to dust and fumes; the coefficients were 16%, 23% and 18% lower for development of AOD, chronic bronchitis and asthma respectively than the coefficients for the entire group. The coefficients were 78%, 95% and 70% higher for those who had been exposed to dusts or fumes.

Previous analysis of TSP's on this cohort found statistically significant increased risks of AOD symptoms to be associated with exceedance frequencies of PM_{10} above 100μg/m^3. Increased risk of definite symptoms of asthma was not statistically significant for any cut-off or for mean PM_{10} concentrations.

Lung Function Studies

Lung Function, also referred to as pulmonary function, is a measure specific to respiratory health and therefore it is possible that measures of lung function may better reflect the more subtle effects of air pollution than can be captured by measuring symptoms. Common measurements of lung function include forced expiratory volume (FEV), forced vital capacity (FVC) and peak expiratory flow (PEF). Diminished FVC is commonly found in individuals with restrictive lung diseases as well as in individuals with obstructive lung diseases. FEV is also anticipated to be diminished in patients with restrictive or obstructive lung diseases. With respect to $FEV_{1.0}$/FVC ratio, patients with obstructive disease tend to show a reduced $FEV_{1.0}$/FVC ratio whereas patients with restrictive diseases will generally have a normal to slightly increased ratio. Studies that have evaluated associations between measures of lung function and particulate pollution levels in the USA include analysis of children's lung function data from both the first and second National Health and Nutrition Examination Surveys (NHANES) and analysis of data from children's lung function studies collected from twenty-four cities.[128-129]

By combining pulmonary function data with ambient particulate matter data from the national database maintained by the USEPA, Chestnut examined the relationship between ambient levels of particulate matter and pulmonary function in adults across forty nine US cities.[128] Exploration of this potential relationship involved two components; 1) exploration of the existence, if any, and shape of the relationship which entailed the use of graphical techniques and regression analysis and 2) testing the stability of the results by examining the effect of factors such as alternative independent variables, adjustments in the different sample cities, threshold models and the possibilities that different subgroups would be differently affected. A basic set of independent variables expected to be related to pulmonary function was selected for initial analysis including; physiological variables, potential occupational dust exposure and TSP.

A nonlinear relationship was seen when FVC, expressed as the percentage difference from expected was plotted against TSP and a similar relationship was seen for $FEV_{1.0}$. No apparent relationship was seen between FVC and TSP at low levels and a negative relationship existed at higher TSP levels starting between 60 and 80μg/m^3. The linear regression results apparently indicated that a statistically significant negative relationship existed between FVC and TSP and between $FEV_{1.0}$ and TSP. Extended regression analysis was performed with the aim of exploring whether the observed inverse relationship between pulmonary function and TSP was perhaps the result of correlations between TSP and factors that had been excluded in the initial analysis e.g. weather or socioeconomic factors. The only significant factor for both FVC and $FEV_{1.0}$ was residence in the South, which was associated with lower pulmonary function. The TSP coefficients in both the FVC and $FEV_{1.0}$ regressions were slightly larger than in the first regressions, which did not include additional variables. Eliminating locations with the lowest levels of TSP did not change the estimated coefficients for either pulmonary function measure.

Raizenne et al reported on the health effects of repeated, intermittent long-term exposures to directly measured particle strong acidity on pulmonary function in children.[129] City-specific mean pulmonary function values, adjusted for sex, age, height weight and interaction of sex with height were calculated and the adjusted FVC ranged from 2.381 to 2.521. The city specific adjusted means of the five pulmonary function parameters were highly correlated, with Pearson correlation coefficients ranging from 0.99 for the association of $FEV_{1.0}$ with forced expiratory volume at three quarters of a second ($FEV_{0.75}$) to 0.5 for the association of forced expiratory flow between 25% and 75% of FVC. Particulate air pollutants, including measurements of particle strong acidity were associated with decreased pulmonary function levels among children. Particle strong acidity was associated with a 3.5% decrement in FVC (95% CI = -4.9 to -2.0). Other measures of pulmonary function including $FEV_{25\text{-}50\%}$ and PEFR showed decrements similar to that for FVC.

All ozone parameters were associated with a decrease in pulmonary function; daytime mean ozone showed the strongest association. Adding particle strong acidity into the model resulted in a substantial attenuation of the daytime ozone effects suggesting that the association between daytime ozone and adjusted FVC is partially explained by the autocorrelation of daytime mean ozone with particle strong acidity (Particle strong acidity relates to the acidity of individual components of particles such as sulphuric acid). Air pollution was associated with children whose measured FVC was <85% of predicted. Particle strong acidity across the range of exposures was associated with a nearly threefold increase in the proportion of children with and FVC <85% of predicted (OR=2.5, 95% CI=1.8-3.6). The association of particle strong acidity and low FVC was not altered by an adjustment for ozone. Particle strong acidity had a weaker association with an $FEV_{1.0}$ to FVC ratio less than 85% of predicted (OR=1.1, 95% CI=0.6-2.0). The magnitude of effect on lung function observed in this study is comparable to the results obtained by other researchers.

Peters et al analyzed the relationship between air pollution levels and cross-sectional pulmonary function test results in 3,293 subjects across twelve Southern California communities.[130] Statistically significant relationships were found between air pollution level and pulmonary function tests in girls only. PM_{10}, O_3 and NO_2 were all associated with decreased pulmonary function, with PM_{10} associated with decreased FVC and $FEV_{1.0}$ and MMEF when the results of adjusted pulmonary function tests were regressed on 1986-1990 ambient air pollution data (adjusted for potential confounders such as passive smoking, presence of a gas cooker). When using the 1994 air pollution data significant effects were again seen only in females and the coefficients were generally larger. $PM_{2.5}$ exposure was associated with statistically significant decrease in all four measures of pulmonary function and the associations were stronger than for PM_{10}. Additional multivariate modelling of the female data was carried out in an effort to determine which of the 1994 pollutants (O_3, PM_{10}, $PM_{2.5}$, NO_2 and acid) was most correlated with each pulmonary function test. The relationships between pollution levels and pulmonary function outcome were quite similar for some of the pollutants, thus raising the question of whether the effects can in fact be separated. Regression of the adjusted pulmonary function measurements of 1994 ambient data, two pollutants at a time showed that no two pollutant models fit significantly better that the best single pollutant model for FVC, $FEV_{1.0}$, or PEFR. For MMEF however, O_3 in combination with either PM_{10} or NO_2 fit better than the best single pollutant model ($PM_{2.5}$). Of these two-pollutant models, the best fitting one included peak O_3 and PM_{10}. Investigation of the effect of spending time outdoors resulted in more negative coefficients for both males and females who spent more time outdoors.

Although the primary aim of the study was not to examine pulmonary function, a study by Dockery et al considered the association of air pollution with commonly used measures of pulmonary function – $FEV_{1.0}$ and FVC. However in this case, $FEV_{0.75}$ and MMEF, potentially more sensitive measures of pulmonary function, were also evaluated. In contrast to results in other studies however, no significant associations were found for any of the pulmonary function measures investigated. Among the pollutants considered in this study, three measures of particulate matter were considered – $PM_{2.5}$, PM_{15}, and TSP and for each one a monthly mean was calculated. For each child an air pollution exposure for the previous year was calculated. The first stage of the analysis involved adjusting for various covariates including sex, indicators of parental education, maternal smoking. In a similar outcome to a previous study on the same group by Ware et al,[100] maternal smoking was negatively associated with all lung function measures bar FVC but the two measures not examined in the previous study, $FEV_{0.75}$ and MMEF showed the strongest associations perhaps supporting the theory that they are more sensitive markers of the effects of air pollution on pulmonary function. Investigation of the associations with air pollution concentrations showed that only TSP concentration was associated with decreases in pulmonary function, with $FEV_{0.75}$ showing the strongest negative association. When considering possible sensitive subgroups, FVC was only 0.3% lower among children with asthma or wheeze, which if taken at face value, would suggest that these children's lung function was not sufficiently different from the norm for them to be classed as susceptible; however the $FEV_{1.0}$ was 4.5% lower. $FEV_{0.75}$ was 4.3% lower and MMEF was 10.6% lower, again supporting the decision of the authors to investigate these more sensitive markers. To assess the associations between air pollution and these children deemed to be susceptible, children with decreased pulmonary function were compared to those without but these separate regressions of the adjusted pulmonary function levels for children with and without wheeze did not show any associations.

Achermann-Liebrich et al examined the effect of long-term exposure to a variety of air pollutants including PM_{10}, on the pulmonary function of a sample of adults in Switzerland.[131] The population based sample, consisting of 8,651 adults, was drawn from eight different areas of Switzerland in an effort to represent a range of conditions such as urbanization and air pollution. Two measures of particulate pollution were investigated in this study, PM_{10} and TSP. Only 1993 data were available for PM_{10} and a 12 month average was used as an indicator of exposure while annual averages for TSP from 1991 were used. According to the results of a correlation test, TSP and PM_{10} were highly correlated (r=0.95) suggesting that TSP is a reasonable proxy measure for PM_{10}. It appears that the correlation coefficient has been calculated using TSP data from 1991 and PM_{10} data from 1993, suggesting that the 1993 data is correlated with the 1991 data so that it is possible to use 1993 PM_{10} data as an index of exposure to particulate pollution in 1991 as the authors have done in this study.

The study attempted to examine the effects of air pollution on FVC and $FEV_{1.0}$ and for the mean values of all pollutants except ozone, a significant association was found in healthy, never-smokers. Examining the sensitivity of the results for FVC led to little change in the associations, even after the addition of various potential effect modifiers such as education, occupational exposure or sibling asthma among others.

Given the location of this study, in terms of the varying geographical profiles the authors felt it was worth investigating the possibility that altitude may have been a confounder. Two areas of the study were at altitudes of over 1,000 metres and these areas also corresponded to the areas of least pollution. To examine the possible effects of altitude, the analysis was carried out minus these two areas and it was found that the regression coefficient remained within 20% of the originals with PM_{10} remaining virtually unchanged, but the regression coefficients decreased substantially in value to the point where they were no longer significant. It was expected that the exclusion of these two locations would weaken associations between air pollution and pulmonary function and although acknowledging the fact that living at altitude would have an effect on lung function, the authors felt that the altitude in this study was moderate and that they were unaware of any effects on lung function at these levels. Given that these two areas were also the areas of lowest pollution, however, it seems surprising that the regression coefficients were reduced to insignificance and it would seem to point towards an almost certain effect of altitude and as such it may have been worthwhile exploring this avenue and little further, especially in light of the fact that exclusions of the two areas with the lowest particle levels by Chestnut et al[128] in a previous study did not significantly alter the results.

Overall, the study found that air pollution was negatively associated with pulmonary function and that the results did not appear to be confounded by other factors apart from altitude. Although it was difficult to tell if any particular pollutant was responsible for the observed effects, based on the figures presented by the authors, of the mean percent difference from predicted FVC in asymptomatic never smokers versus annual average air pollution concentration in each study area, the case would appear to be strongest for PM_{10} as the association shows "the strongest evidence of a continuous gradient of effect across the range of exposure". For a 10µg/m^3 increase in PM_{10} annual average, a 3.4% decrease in FVC and a decrease of 1.6% in $FEV_{1.0}$ was predicted.

Mortality Displacement and Reduced Life Expectancy

"The setting of appropriate air quality guidelines requires knowledge of the population burden of illness and premature death avoidable by control of specified air pollutants. However, most epidemiological studies of the mortality impacts of specific air pollutants have been based on daily time-series data. These do not allow estimation of the usual population indices of premature mortality, based on changes in annual age-specific death rates." This is the opinion of McMichael et al who published a paper in 1998 highlighting the inappropriate use of daily mortality analyses in attempting to estimate the longer-term mortality effects of air pollution.[61]

Although evidence from time-series studies of mortality appear to leave little doubt that brief elevations in a wide variety of air pollutants result in extra deaths as well as perhaps a reduction in life-expectancy, these extra deaths appear to be occurring disproportionately in the old and infirm sectors of the population. This phenomenon is known as 'mortality displacement' or 'harvesting' and suggests that many of the excess deaths observed in time-series studies are in fact the result of time of death displacement. The principal behind mortality displacement is straight-forward; it theorises that the associations observed between air pollution and mortality are actually more of a reflection of the 'harvesting' of a pool of already frail individuals and as a result the days subsequent to high air pollution days would be expected to show a reduced effect of air pollution. From a public health perspective, what is needed is the ability to estimate the person-years of life lost in order that air pollution regulations can be more suitably defined to protect the population.

One of the first studies to try and model the harvesting effect was conducted by Spix et al who analysed total daily mortality and air pollution data collected in Erfurt, Germany during the 1980's.[122] To determine whether a harvesting effect was present, interaction terms of pollutants with mean numbers in the previous 2-21 days were examined. Spix et al found that a small harvesting effect appeared to modify the effect of air pollution resulting in a high mortality about two weeks prior to an air pollution episode depleting the 'at-risk' pool to the point where the air pollution episode resulted in fewer deaths than might otherwise have been expected.

Borja-Aburto et al found an association between $PM_{2.5}$ and mortality, with an acute increase in mortality on the high air pollution day, followed by a second peak in mortality four days after the pollution event.[51] They attribute this bimodal association to 'harvesting' stating that the initial mortality peak on the same day was the result of the deaths of susceptible persons while the second peak in mortality was as a result of the delayed effects of pulmonary defences, cardiovascular complications and other homeostatic changes among individuals less susceptible to the effects of air pollution.

Zeger et al similarly raised the question of whether the increased mortality was only among extremely frail persons whose life expectancy, even in the absence of pollution, was likely to be short.[60] Rather than attempting to assess whether the mortality associations observed in time series studies are the result of harvesting, Zeger et al come at it from another approach, instead they construct a model that ignores any information in the time-series data that may be influenced by short-term harvesting. The theory behind this approach is that by separating both the pollution and mortality data into components with variation occurring at different time scales they can then rely on the longer term components to estimate the effect of pollution in mortality. Applying their model to a Philadelphia time-series with the aim of calculating estimates of the pollution

mortality association in the time-series without considering the information that would be affected by harvesting, Zeger et al found that the associations between total suspended particles and mortality in Philadelphia reflects factors other than harvesting. The results of this study highlight the point made by McMichael et al that the results of time-series studies are often inappropriately used when defining the mortality risk associated with air pollution exposure.
Schwartz et al suggest that the difference in effects of air pollution reported by cohort studies compared with time-series studies may represent the effect of chronic exposure to air pollution and they attempted to investigate "how the association between particulate air pollution and mortality and morbidity varies as the time-scale of exposure varies".[145] Schwartz et al' supposition was that an increase in numbers of daily deaths due to air pollution would be followed by a decline if in fact air pollution was responsible for advancing death by no more than a few days and that accordingly averaging the numbers over a week would cancel out (or partially cancel) the two effects. As such the basis of their analysis is that if they could separate the correlation between air pollution and daily deaths into characteristic frequency ranges, then the presence of an association at lower frequency ranges would be indicative that not all the deaths that are associated with air pollution are being advanced by only a few days, i.e. harvesting is not the only factor affecting the mortality levels. By separating the time-series data of daily deaths, air pollution and weather into long wavelength, mid-scale and residual short-term components and using the mid-scale components by which to assess the associations between air pollution and mortality and examining four different mid-scale components and by comparing their results to the results found in a previous time series study in Boston, Mass. Schwartz et al's study provide some evidence both of short-term harvesting and of larger effects when short-term harvesting is excluded from the analysis. Two reasons for the latter effect are that they a) reflect the impact of longer term effect of exposure to longer-term average pollution concentrations or b) increased recruitment into the susceptible population caused by air pollution.
Later work by Schwartz attempted to design an analytical approach to testing this hypothesis that much of the mortality associated with particulate matter is only being advanced by a short period of time.[146] The supposition made by Schwartz was that the population could be divided into two pools, a low-risk

group and a high-risk group. He theorises that any short-term increase in the mortality rate in the high-risk pool, should it not be balanced by a corresponding increase in the net recruitment rate, would result in depletion of the high risk pool. Such a situation would arise as a result of a transient environmental factor such as a heat wave or an air pollution episode, events which may have an adverse effect on those in the high-risk pool but have little effect on the healthy population (i.e. those in the low risk pool). This scenario could only occur if the air pollution episode did not result in an increase in the net recruitment to the 'at risk' group, perhaps by increasing the rate of serious respiratory or cardiovascular illnesses, then the harvesting effect would be much less.

To test this 'harvesting hypothesis' Schwartz examined deaths occurring inside and outside hospitals separately, suggesting that if the deaths attributed to particulate air pollution are primarily in desperately ill people (i.e. that short-term harvesting is occurring) then these people would be more likely to die in hospital. What he found was that deaths outside the hospital had larger effect size estimates – 1.23% (95% C.I. = 0.8% - 1.65%) as compared with 0.77% (95% C.I. = 0.42% - 1.13%). Seeing as those persons that are hovering close to death (i.e. those likely to die within a few days) are disproportionately in hospital it would be expected that the greater impact would be seen in this group. The results presented by Schwartz are consistent with the possibility that particle exposures increase the risk of sudden death due to other triggers and that there was no evidence to support the theory of harvesting. The results could be seen to support the hypothesis that the associations with mortality reported in time series studies may partially reflect the effect of chronic exposure to particles and not simply the effect of short-term exposures.

Dominici et al[59] examined the possibility that increased mortality associated with air pollution is the result of 'mortality displacement' or 'harvesting'. Working under the assumption that air pollution events will affect only those already at risk one would expect the highest air pollution-mortality associations to be in the days immediately following the event. In fact Dominici et al's results appear to show the opposite – with the strongest associations occurring between ten days and two months after an air pollution episode. This study draws attention to the fact that the larger relative rates at longer timescales may partly reflect a greater biologic impact on chronic exposures than on acute exposures and the author points out that estimated relative risks from two studies examining chronic exposures are larger than estimates from time-series models suggesting that the most harmful effects of air pollution occur over much larger timescales than can reasonably be studied using time series methods.

Zanobetti et al took the analysis of the harvesting phenomenon a step further and attempted to assess the issue of mortality displacement in a multi-city hierarchic modelling approach, using data from 29 European cities collected as part of the APHEA 2 study rather than a single city analysis favoured by previous researchers.[67] The results of the study supported previous findings that the effect of air pollution on mortality could not entirely be attributed to short-term harvesting.

And what of those in the low-risk pool – is there any effect of air pollution on the health of these people?

Brunekreef attempted to determine the reduction in life expectancy for Dutch men by using data from two US cohort studies.[58] He calculated a joint relative risk of 1.10 per 10μg/m^3 difference in long term exposures to fine particles and used this to estimate the effect of fine particles on life expectancy for a hypothetical Dutch male population. A difference of 1.11 years in life expectancy between the 'exposed' and the 'clean air' groups was found suggesting that the effects of long-term exposure to relatively low concentrations of fine particles might possibly result in a reduction in life expectancy of more than a year. Brunekreef advises caution when interpreting the results however, drawing attention to the fact that the database used to calculate the relative risks was small and therefore the RR's may well be inaccurate and need to be revised as more data is added in the future. The results were also sensitive to extrapolation beyond the age-range actually studied. Expanding the analysis to include to further five year age-groups resulted in a difference in life-expectancy between the two groups of 1.51 years. This sensitivity to extrapolation led Brunekreef to question whether the results were sensitive to which life table was used for analysis and to investigate this possibility he similarly conducted the analysis using the life table of United States white men 1969-71 and came up with a difference in life expectancy of 1.31 years, slightly bigger than the 1.11 year estimated for the Dutch men. Brunekreef acknowledges that the reason for this may be that the populations studied in two cohorts may not be representative of the white American men in general and that another way to compare populations is to compare the percentage of subjects who were found to die in the cohort studies during follow-up, with the percentage estimated to die over a given period in the hypothetical populations making up the life tables. In Dockery's six cities cohort study, 830 deaths (approximately 22.6%) were reported over a 14-16 year follow-up of the 3668 men. Calculations based on the Dutch 1992 life table showed a death rate of 22.5% over a 15 year period while calculations based on the 1969-71 life table for US white men showed a death rate of 26.8% for the same period suggesting that the Dutch 1992 life table data are more comparable with data from Dockery et al's study than are the data from the 1969-71 life table for US white men.

Intervention studies

Pope reported in 1989 on the effects of a unique natural experiment that occurred in the Utah Valley.[106] The Geneva Steel Mill is the primary industrial source of fine particulate pollution in Utah County. When in operation the mill emits approximately 82% of all industrial sources of PM_{10} and contributes between 47 and 80% of total emissions. During the winter months (Dec-Feb) of 1985/86 while the Geneva mill was still in operation, the 24 hour PM_{10} levels exceeded the TSP standard of 150µg/m^3 on 13 occasions, with the highest single day concentration of 365µg/m^3. Conversely, when the steel mill closed as a result of industrial dispute, the levels of PM_{10} did not once exceed 150µg/m^3 during the winter of 1986/87. During the winter of 1987/88, after the mill reopened, the 24-hour PM_{10} levels this time exceeded 150µg/m^3 on ten occasions, with a single day high of 223µg/m^3. This intermittent operation of the steel mill provided the unique opportunity to look for links between PM_{10} levels and various health effects. Initial comparative analysis indicated that during the months when exceedances of the 24-hour PM_{10} standard of 150µg/m^3 occurred, the number of admissions for children aged 0-17 was almost triple the number for months with no exceedances, while adult admissions were up by approximately 44%. This comparative analysis does not, however, take into account other factors such as weather conditions. Regression analysis showed that for all hospital admissions there was a link between the PM_{10} levels and admission numbers. In fact 59% of the variance in monthly admissions for various respiratory illnesses was explained by current and lagged monthly mean PM_{10} levels. This was increased to 83% on inclusion of current and lagged mean low temperature in the model. Neither comparative nor regression analysis revealed any associations between the control variables and PM_{10} levels or the closing and reopening of the steel mill. The results indicated that hospital admissions for respiratory illnesses were strongly associated with PM_{10} levels. This association is much stronger for children than for adults and somewhat stronger for bronchitis and asthma than for pneumonia and pleurisy. These associations were particularly strong with monthly lagged variables suggesting that the health effects of particulate pollution are cumulative and that it takes time before they are manifested in inpatient hospital admissions data.

On September 1, 1990, the Irish Government banned the marketing, sale and distribution of bituminous coal within Dublin City, providing Clancy et al the ideal opportunity to examine the effects of this intervention on the levels of TSP, as well as effects on numbers of deaths within Dublin city.[62] The effect of the intervention was an immediate and permanent reduction in average monthly particulate concentrations. Although this was fifteen years ago and with the increase in population and traffic in Dublin and in Ireland as a whole, over the past decade, and it would therefore be interesting to conduct a follow-up study and investigate whether the lower levels of particulate pollution have persisted.
Clancy and colleagues compared air pollution, weather and deaths for 72 months before (Sept 1, 1984 – Aug 31, 1990) and after (Sept 1, 1990-Aug 31, 1996) the ban by season. Substantial changes were noticed in the age distribution of the Dublin population during the study period, this was adjusted for by calculating the directly age-standardised death rate, adjusted to the 1991 Irish census population. The effect of the ban on coal sales on population standardised death rates was assessed as an interrupted time series, adjusting for covariates. Overall, mean black smoke levels fell by about two thirds after the introduction of the ban on coal sales, while mean SO_2 concentrations fell by about a third. There was no difference noted on mean temperature before or after the ban but mean relative humidity was significantly lower before the ban than after the ban. An average 5042 non-trauma deaths per year (adjusted to the 1991 Irish population were recorded before the ban compared with 4639 deaths

after – a reduction of 403 deaths. Of these, there were 120 fewer respiratory deaths, 312 fewer cardiovascular deaths but an increase of 29 deaths from other causes. After adjustment for weather, epidemics and death rates in the rest of Ireland, the 5.7% decline in non-trauma death rates, predicted 287 fewer deaths overall. From the results shown in the table below, the ban on coal sales in Dublin County Borough resulted in a substantial decrease in average concentrations of particulate air pollution, as measured by black smoke, by 35.6μg/m^3. After adjustment for age distribution of the population using known predictors of death and death rates in the rest of Ireland as an index of unmeasured secular changes in deaths, it was estimated that there were about 243 fewer cardiovascular deaths and 116 fewer respiratory deaths per year in Dublin.

	Unadjusted % change (95% CI)	p	Adjusted % Change (95% CI)	p
		Total		
Non-trauma	-8 (-6.2 to -9.8)	<0.0001	-5.7 (-4.1 to -7.2)	<0.0001
		Cause Specific		
Cardiovascular	-13.4 (-10.8 to -15.9)	<0.0001	-10.3 (-8 to -12.6)	<0.0001
Respiratory	-16.1 (-11.6 to -20.4)	<0.0001	-15.5 (-11.6 to -19.1)	<0.0001
Other	1.4 (4.6 to -1.6)	0.36	1.7 (4.2 to -0.7)	0.17
		Age Specific		
	-8.1 (-3.7 to -12.3)	<0.001	-7.9 (-3.6 to -12)	<0.0001
	-8.6 (-9.6 to -12.3)	<0.001	-6.2 (-3.5 to -8.8)	<0.0001
	-7.6 (-7 to -8.1)	<0.001	-4.5 (-2.3 to -6.7)	<0.0001

Table 2.16: Change in the mortality rates for Dublin County Borough for 72 months before and after the ban of coal sales. (Results presented for age-standardised total, cause-specific and age specific mortality rates) Results taken from Clancy L. et al.[62]

2.4) Evidence to support the health effects as a result of exposure to traffic generated particulate pollution

Currently there are only a small number of studies that have attempted to link particles from vehicular sources with adverse health effects on the population.[44, 99, 149-154] As mentioned previously Laden et al[44] carried out some work on particles collected from filters during the Six Cities Study, analysing them and identifying certain characteristics of vehicular particles. It is thought that, since the introduction of tough legislation for industries, one of the primary sources of particulate pollution in cities is motor traffic and it is therefore somewhat surprising that so few attempts have been made to investigate the potential associations with health outcomes. There are a small number of studies, primarily conducted in Europe (mostly in the Netherlands) whereby researchers have attempted to examine the effects living in areas with high volumes of traffic on the respiratory and cardiovascular health of the population.

Roemer & Wijnen conducted a study in Amsterdam that attempted to determine whether or not people living close to busy streets were exposed to higher levels of air pollution that were those living on quiet streets.[99] Pollution data was obtained from background monitoring stations and from roadside monitoring stations. Correlation coefficients between the background stations and between the background station and the roadside station lay within the same range, suggesting that the background stations do not correlate any better with each other than they do with roadside measures. This is a little surprising as the roadside stations recorded higher concentrations of all air pollution components except O_3 than the background stations and in light of this one would expect the background stations to correlate less well with the roadside stations. Most air pollutants were more strongly related to mortality in the 'traffic' population than in the total population when using measurements from either the background station or the roadside station as the pollution index. The authors do not present the data for the traffic population using the measurements from the roadside station and so it is not possible to tell how the relative risk differs from that obtained for the traffic population when using the background site as the pollution index. The authors suggest that the lower variance in daily air pollution concentrations at the background stations when compared with the concentrations measured at the roadside stations might be a reason for the higher effects estimates found. If this lower variance observed is indeed a reason for the higher effects estimates, it would appear to support the theory that long term exposure to low levels of particles may be more harmful than short term (acute) exposure to high levels of particles. Surprisingly the authors did not show the relative risks and confidence intervals for the traffic population using the traffic sites as I would have thought that these two groups would have been of most interest especially given that the total population RR's and confidence intervals are perhaps not what were expected. Also the paper is extremely vague in terms of the data presented which raises questions as to the quality of the data and the methods used.

Hoek et al aimed to assess the relation between traffic related air pollution and mortality in participants of the Netherlands Cohort study on Diet and Cancer (NLCS) by focusing on traffic related air pollutants, which have been shown to be related to chronic morbidity.[149] Motorised traffic emissions result in small scale spatial variations and affect both urban and regional background air pollution concentrations. In other words, high concentrations of pollutants from motor vehicles are recorded at short distances from major urban roads. Using this knowledge Hoek et al quantified small scale spatial variations in air pollution concentrations by classifying proximity of residences to major roads and participants living within 100 metres of a free way or within 50 metres of a major urban road were deemed to be exposed. Using data from two previous Dutch studies it was estimated that participants living within 100 metres of a freeway were exposed to a black smoke concentration of 4.4μg/m^3 and a nitrogen dioxide concentration of 11μg/m^3 while participants living within 50 metres of a major urban road were estimated to be exposed to 13μg/m^3 black smoke and to 8μg/m^3 nitrogen dioxide. A consistent association between cardiopulmonary mortality and living near a major road and a less consistent association between mortality and the estimated ambient background concentration of the two indicator pollutants, black smoke and nitrogen dioxide, was found. The relative risk estimates for living near a major road were not sensitive to inclusion of different confounders. One possible reason for the less consistent associations between mortality and estimated background concentrations of indicator pollutants is that the wrong pollutants were chosen as indicators. Traffic emissions contain many pollutants including ultrafine particles, diesel soot and nitrogen oxides (other than NO_2) and it is possible that any one of these may be responsible for the mortality associations observed, with ultrafine particles the most likely candidate given the probable associations between ultrafine particles and cardiovascular events.

Von Vliet et al conducted a cross sectional study to investigate whether motor vehicle exhaust from traffic on freeways had any effect on the respiratory health of children attending schools less than 1km from major freeways in the South Holland Province.[152] They found that for children living within 100 metres of the freeways there was a tendency for symptoms such as chronic cough, asthma attack, wheeze and rhinitis to be more prevalent and that the prevalence increased with increasing truck density. These associations were found to be related to the concentrations of black smoke measured in schools suggesting that long-term exposure to traffic related air pollution, particularly diesel exhaust may increase chronic respiratory symptoms in children.

Using the same group of children, Brunekreef et al examined the lung function of the children and found that lung function was associated with truck traffic density and to a lesser extent with traffic density. Similarly to respiratory symptoms, lung function was observed to be more strongly associated with traffic density in children less than 300 metres from a main road.[153]

A British study, examined the relationship between residence close to major roads, traffic flow and the risk of hospital admissions for asthma in children under the age of five years in Birmingham, UK.[154] One of the unique factors for this study was that there were three groups of children examined; cases consisted of all children between the ages 0-4 years who were resident in Birmingham and admitted to any hospital in the West Midlands region between April 1998 and March 1989 with a diagnosis of asthma. A hospital control group consisted of a random sample of Birmingham resident children of the same age range as he cases and admitted to any West Midlands region hospital with a non-respiratory diagnosis. Finally the community controls consisted of a random sample of Birmingham children aged 0-4 years who were registered with a Birmingham GP. The study found that children admitted to hospital for asthma were significantly more likely to have high traffic flow along the nearest adjacent segment of main road than were either the hospital control group or the community control group. These children admitted for asthma were also significantly more likely to live less than 200 metres from the nearest main road. No significant difference was found between the two control groups for either traffic volume of proximity to main roads. Although no measures of pollution were included in this study, the combination of a significant relationship between admissions for asthma and both proximity to main roads and traffic volume, it would appear that the logical assumption to be made from this must be that traffic is in some way related to asthma and in light of the evidence supporting an association between respiratory illnesses and air pollution, it is not unreasonable to assume that the exhaust emissions could be the causal factor – a theory supported by the linear trend observed between hospital admissions and traffic flow.

Overall the evidence from epidemiological studies points overwhelmingly towards an association between particulate air pollution and cardiovascular and/or respiratory events, although there has been no real effort in most of the studies to differentiate between the various sources of particles and so the results may be somewhat misleading.

Scant though it is, there is some evidence to suggest that exposure to particles generated by motor vehicles, particularly those with diesel engines, is a risk factor for the development or aggravation of such cardiovascular and respiratory events. This limited evidence is not enough to provide evidence of a strong and definite association between particulate pollution from motor traffic, rather it is evidence of a possible link and an intimation that much more research is needed in the area of air pollution generated by traffic before any firm conclusions can be made.

2.5) Biological Effects of Particulate Air Pollution

Consistent association have been shown between particulate air pollution and exacerbations of illnesses of a respiratory nature as well as increased mortality from respiratory and cardiovascular causes and meta-analyses of the various epidemiological studies suggest that these associations are unlikely to be the result of confounders and therefore imply that the observed associations represent cause and effect.[155]

2.5.1) Particle Clearance Methods

An important defence mechanism in the airways against inhaled particles is the mucociliary escalator.

Beyond the ciliated airways the mucociliary escalator is less well formed and as such the macrophages are the most important factor in clearing inhaled particles. If particles cross the epithelium and enter the lung interstitium they are no longer likely to be cleared by the normal processes and will either remain in the subepithelial regions close to responsive cell populations, such as interstitial macrophages, fibroblasts and endothelial cells or be taken to the draining lymph nodes.

Particle Clearance - The Mucociliary Escalator

The mucociliary escalator serves as the primary defence mechanism for the lung, trapping inhaled particles and transporting them out of the lung to maintain a sterile environment. In a normal healthy lung the epithelium is lined with ciliated cells, from the bronchioles to the trachea. The escalator works by a complex interaction between cilia, small hair like projections on the surface of the respiratory epithelium cells and mucus. The mucus traps inhaled particles and is then propelled, by the cilia, in a cephalic direction to be either expectorated (coughed out) or swallowed. The mucus is secreted by goblet cells and is controlled by at least eight mucin genes. Different mucin genes are expressed by the same or distinct cells of the airway epithelium and glands. Some inflammatory mediators have been shown to upregulate mucin genes, one of which is IL-6, shown to increase the level of steady state mRNA of mucin 2 (MUC2) gene in the normal human airway epithelial cells. Lung infections are minimised by and efficient mucociliary escalator clearance mechanism, which is supported by antibacterial substances within the upper respiratory tract and the activity of alveolar macrophages in the more distal lung regions.[156-157]

Particle Clearance – Beyond the Mucociliary Escalator

For a number of reasons, inhaled particles are not always trapped in the larger airways and inhaled particles deposit in large numbers beyond the ciliated airways – in the terminal airways and proximal alveoli where the net air flow is zero and where, for very small particles, deposition efficiency increases because of the high efficiency of deposition by diffusion. In this region the airways divide and become smaller in diameter, the composition of the epithelium also changes becoming thinner with less ciliated cells and increasing numbers of Clara or non-ciliated bronchiolar cells. Here the mucociliary escalator is less well formed and as a result macrophages and other cells play an important role in the removal of particles. Macrophages work by phagocytosing particles and migrating to the start of the mucociliary escalator, bound for the gut and thus clearing particles from the lung. Clara cells are also present in high numbers from the terminal bronchiole to the alveolar duct. Clara cells have no cilia but their cytoplasm is filled with biosynthetic organelles. Clara cells are known to have a role in detoxification with the number of cells increasing in response to increased exposure to pollutants.[156]

2.5.2) Particles, Oxidative Stress and Airway Inflammation: A Complex Relationship

Currently more experimental than epidemiological studies exist which examine the potential effects of ultrafine particles on various biological systems including the inflammatory and immune response systems, and although this research is still very much in it's early stages some common themes and ideas have been found in the data, primarily focusing on the inflammatory and oxidant potential of particles,[157-186] and to a lesser degree, the blood coagulant potential.[187-194]

Inflammation

Inflammation is a complex reaction to localized injury or other trauma. The inflammatory response involves various immune system cells, as well as numerous mediators. A variety of inflammatory mediators are released by cells of the innate or acquired immunity during an inflammatory response. These mediators serve to trigger or enhance specific aspects of the inflammatory response. Problems with particles may arise for a number of reasons, for example, when particles damage macrophages, stimulate the release of inflammatory mediators from macrophages or epithelial cells, activate compliment or are present in sufficiently large surface area burden. Inflammatory mediators are released by tissue mast cells, blood platelets and a variety of leukocytes including; neutrophils, macrophages/monocytes, eosinophils, basophils and lymphocytes. Evidence suggests that the inflammatory response lies at the core of the pathogenesis of many diseases arising from particle exposure. Particles are the same as any other substance in that a high enough dose of them will be toxic. Low doses of particles can also have a toxic effect depending on their composition. All particles can be assumed to have a threshold level, below which the lungs can deal with them i.e. the number of macrophages damaged is low enough not to cause a perturbation and the released inflammatory mediators are low enough in concentrations so as not to stimulate appreciable inflammation. Studies by Ferin and Li found that ultrafine Carbon Black particles had higher inflammogenicity that fine carbon black. Ferin also showed that TiO was highly inflammogenic and rapidly crossed the epithelium causing interstitial inflammation.[156, 164-165]

Oxidative Stress

An imbalance between oxidants and antioxidants, in favour of the oxidants, potentially leading to damage, is termed oxidative stress. Oxidants are formed as a normal product of aerobic metabolism, but can be produced in elevated levels under pathophysiological conditions. The importance of this process lies in the reactivity of the molecules involved. Under normal circumstances electrons orbit around the atoms in opposite directions, when an atom has a single unpaired electron, its reactivity increases markedly and it is referred to as a free radical. Due to the increase in reactivity these free radicals can react indiscriminately with neighbouring molecules. Oxidation and often inactivation of target molecules are the result of this process of electron stealing and if these reactions are numerous, extensive cellular damage can occur. Oxidative damage to the blood/gas barrier of the lung would play havoc with the process of gaseous exchange. The human lung has been shown to have an extracellular antioxidant defence mechanism, antioxidants react preferentially with free radicals, giving low toxicity products; as such the extent of the damage is related to the availability of neutralizing antioxidant defences. Lung lining fluid has been shown to contain a range of similar low molecular weight antioxidants as blood plasma including; reduced glutathione, ascorbic acid (Vit. C), uric acid and α-topopherol (Vit. E). In addition to these low molecular weight antioxidants, lung lining fluid contains antioxidant enzymes such as superoxide dismutase and catalase, as well as metal binding proteins such as caeruplasmin and transferrin. Ambient air contains a range of pollutants, including particulates, which either contain free radicals themselves or have the ability to drive free radical reactions. As a result, air pollution appears to give rise to oxidative stress, particularly in the susceptible population, resulting in the initiation of various responses, one of which is the influx of inflammatory cells particularly neutrophils, which are known to release reactive oxygen species when activated, to the lung. Based on their own previous findings that increased numbers of neutrophils were sequestered in the pulmonary microvasculature in smokers and patients with acute exacerbations of COPD and that sequestered neutrophils are primed to release reactive oxygen species, Rahman et al[168] tested the hypothesis that an oxidant imbalance is present in the plasma of smokers and patients with asthma and COPD by measuring the trolox equivalent anti-oxidant capacity (TEAC) of plasma. They found that chronic smokers had lower levels of TEAC in plasma, as did patients with acute exacerbations of COPD as well as asthma patients hence intimating towards the presence of an oxidant imbalance and oxidative stress.

Oxidants have the potential to elicit a broad spectrum of biological effects both in animal models and humans alike including; airway inflammation, lung function decrements, induction of airway hyper-responsiveness and tissue injury, thus making the generation of reactive oxygen species by particulate air pollution one of the prime candidates for the biological mechanisms behind the effects of particulate air pollution.[168, 173, 191]

It is well documented that particles cause pulmonary inflammation and oxidative stress, but the relationship between these effects is less clear. For example, free radicals in high levels give rise to oxidative stress which in turn initiates a series of reactions, one of which is the influx of inflammatory cells. Equally, activated inflammatory cells generate large quantities of free radicals leading to oxidative stress reactions. So which came first – the chicken or the egg?
Ultrafine particles have been shown to be capable of eliciting a variety of toxic effects, but the mechanisms by which they elicit these effects are unclear. MacNee[185] outlines five possible mechanisms which could account for the toxicity of ultrafine particles; 1) particle number, 2) particle surface area, 3) particle surface chemistry, 4) interstitialization of particles and 5) oxidative stress. Numerous studies have been carried out in an attempt to ascertain, which, if any of these possible mechanisms are responsible for the toxic effects of particles. What has resulted is a body of evidence to suggest that these possible mechanisms cannot in fact be separated and that in many cases there are more than one possible mechanism by which particles elicit a response. Several studies have examined the effect of particle number and surface area. Brown et al[174] used inert polystyrene particles to show that size and number were important to the toxic effects of particles. Stone et al[169] hypothesis that the numbers of ultrafines are responsible for an increased inflammatory response, suggesting that the harmful effects of the ultrafines was due, in some way, to the large surface area available for the production of free radicals. Tran et al[176] showed that the onset of inflammation and the impairment of particle clearance was related to the large surface area burden and not lung mass burden. Impairment of a macrophages ability to phagocytose particles is thought to be the result of a phenomenon known as overload. This phenomenon has only yet been shown in the rat and occurs after exposure to high concentrations of airborne particles. The overload phenomenon has been best described with particles such as carbon or titanium dioxide, which are not generally toxic at low levels. Overload is thought to occur when macrophages have phagocytosed a volume of particles equivalent to 60% of their internal volume, at which point macrophages began to show impaired ability to move and carry their particle burden to the start of the mucociliary escalator for clearance. Anything that interferes with the normal process of phagocytosis and macrophage migration to

the mucociliary escalator can lead to the adverse outcome of interstitialization. Interstitialization is an adverse outcome because once in the interstitium, particles cannot be cleared via the normal routes and so they either remain in the interstitium where they chronically stimulate interstitial cells, or they transfer to the lymph nodes. The effects of particles in the lymph nodes are not known but adjuvant effects might be anticipated. Continuing exposure under these conditions leads to effects such as inflammation, increased epithelial permeability, proliferation and retardation of clearance culminating in fibrosis and cancer in the long term.[156, 178]

The surface activity of particles can be important as the large surface area provided by the ultrafine particles may allow adsorption of substances from the environment or from the lung epithelial lining fluid, the result of which may be to increase the reactivity of particles, for example transition metals or bacterial endotoxin. Iron is an example of a transition metal and transition metals are important in the oxidative activity of many different particle types, particularly the ultrafine fraction of PM_{10}. Iron itself has the ability to partake in Fenton chemistry and produce reactive oxygen species (ROS). Reactive oxygen species are capable of causing oxidative damage to macromolecules which subsequently results in such effects as lipid peroxidation, oxidation of amino acid side chains, DNA damage and DNA strand breakage among others. The localized release of transition metals results in the production of free radicals, using the supercoiled plasmid DNA scission assay MacNee & Donaldson showed that PM_{10} was able to generate free radical activity.[178, 182] Stone et al[175] showed, using the same technique, that ultrafine carbon black generated more free radical activity than fine carbon black, by showing that they induced more scissions in supercoiled plasmid DNA. Iron generates free radical activity through the production of the hydroxyl radical. The importance of the hydroxyl radical was shown by MacNee and Donaldson, who used iron chelators to block production of the radical while Stone et al used the hydroxyl radical specific scavenger mannitol which resulted in a decreased inhibitory effect of ultrafine carbon black. Donaldson et al showed a similar effect with TiO_2 particles implying the hydroxyl radical played a role in the impairment of metabolic activity and supporting the theory that particles elicit damage via free radical generation.[177]

Although ultrafine particles have been shown to generate free radicals, some questions remained as to whether this free radical activity was in fact caused by the presence of transition metals or whether some other factor was responsible for the effects; it is possible that ultrafine particles could have an intrinsic toxicity that is independent of transition metals. Studies using ultrafine carbon black provide a possible mechanism by which they elicit free radical activity and promote inflammation, independent of transition metals, which may be applicable to other ultrafine particles. Brown et al used particle leachates prepared in citrate or saline and instilled them into rat lung. No inflammation was evident suggesting that soluble factors or transition metals from the particles were not responsible for the lung inflammation found with particles alone.[170] Based on the premise that the expression of pro-inflammatory mediators could be switched on via intracellular Ca^{2+}, a study by Stone et al[169] described the effects of CB and ufCB on calcium homeostasis in MM6 cells and presented evidence to suggest that ultrafine carbon black increased the resting cytosolic calcium concentration of the MM6 cell line. Further work using the pharmacological agent thapsigargin was carried out in an effort to determine whether the increased cytosolic calcium was due to leakage from the intracellular calcium store located in the endoplasmic reticulum (ER), as a result of apoptosis. Thapsigargin inhibits the ER calcium pump, resulting in release of the ER calcium store and hence an increase in the cytosolic calcium concentration. Release of the ER calcium store stimulates the entry of extracellular calcium via calcium-release-activated calcium (CRAC) channels in the plasma membrane. If ufCB had initiated apoptosis the effects of thapsigargin would have been diminished. Conversely however a 2.6 fold increase in the size of the calcium response was observed in the presence of ufCB. The increased calcium response to the thapsigargin was found to be due to an increase in the influx of extracellular calcium, possibly by CRAC channels. To prove that this enhanced influx of calcium was not simply a phenomenon of the transformed cell line, Stone et al also showed an identical effect on thapsigargin stimulated calcium influx of primary cells obtained from BAL of the rat lung which is >80% alveolar macrophages. Other findings from this study showed that ultrafine and fine latex beads induced comparable results to those observed with ultrafine and fine carbon black in MM6 cells, suggesting that the size, rather than the chemical composition was the reason for the increased calcium influx. It also reaffirmed the possible role of ROS; treatment with antioxidants nacystelin and mannitol inhibited the enhanced response to thapsigargin induced by uf carbon black in MM6 cells, suggesting that ROS are involved in the mechanism by which ultrafine particles induce the opening of plasma membrane Ca^{2+} channels.

In view of the free radical activity associated with ultrafine particles, it would seem likely that there would be evidence of oxidative stress, a result of free radical production. Oxidative stress effects may take the form of direct oxidative damage such as lipid peroxidation or could include the induction of genes that are activated by redox-sensitive transcription factors including a number of pro-inflammatory gene products such as cytokines, cell adhesion molecules, antioxidant enzymes and receptors. [160]

One of the major consequences of exposure to particles and the subsequent oxidative stress is an inflammatory reaction. A critical component of the inflammatory response to particles in the lungs is the release of cytokines from activated macrophages and lung epithelial cells, resulting in neutrophil recruitment. NF-κB is a transcriptional regulator of the REL family that controls the expression of a wide variety of cellular and viral genes including many proinflammatory molecules such as cytokines IL-2, IL-6, IL-8 and TNF-α as well as cell adhesion molecules such as ICAM-1 and E-selectin. Because of the major role NF-κB plays in the regulation of these genes, it also contributes greatly to the control of inflammation. The NF-κB hetero dimmer, comprising p65 and p50 proteins, is found bound to its inhibitor IκB in resting cells. IκB masks the nuclear translocation signal, thus preventing its translocation to the nucleus. Under the influence of oxidative stress IκB is phosphorylates and degraded via the ubiquitin proteosome system and NF-κB translocates to the nucleus to bind to the promoter region of key genes for inflammatory mediators, allowing their transcription.[159, 184]

2.6) Alterations in Blood Rheology as a Result of Exposure to Ultrafine Particles

A unique feature of the pulmonary microcirculation is the close proximity of the distal airspace to the circulating blood, across the alveolar-capillary membrane allowing easy access for the inflammatory mediators in the airspaces to reach the blood. It has been hypothesised that ultrafine particles have the ability to completely by-pass the pulmonary defence systems and pass into the blood circulation. Nemmar et al attempted to investigate whether technetium 99m-labelled ultrafine particles of denatured albumin (99^{m} Tc albumin) pass into the systemic circulation and how quickly after intratracheal administration of the particles in hamsters this occurs.[160] According to their findings the effect proved to be rapid and substantial with radioactivity detected as soon as five minutes after intratracheal instilliation and 2.88% of the administered dose per gram of blood was the proportion found to have left the lungs.

MacNee and Donaldson hypothesised that the inflammation arising in the lungs of persons inhaling particles could impact on the coagulation system via the local production of procoagulant factors in the lungs, or as a result of the effects of mediators released from the lungs can act on the liver to increase the levels of procoagulant factors.[156] Seaton et al proposed that the alveolar inflammation provoked by ultrafine particles, in addition to promoting exacerbations of lung disease, has an additional effect on the coaguability of blood thus increasing the susceptibility of individuals to acute episodes of cardiovascular disease.[187] Several haematological factors including plasma viscosity, fibrinogen, factor VII, and plasminogen activator inhibitor all rise as a consequence of inflammation and are known to be predictive of cardiovascular events. To test Seaton's hypothesis, Peters et al[188] examined blood plasma viscosity data that had been collected in Augsburg as part of the MONICA Augsburg study carried out in 1984-85. They found a two to three fold increase in plasma viscosity above the 95^{th} percentile, which can be considered a risk factor for myocardial infarction. It is thought that this increase in plasma viscosity is caused by particles depositing in the lungs and initiating an inflammatory reaction. Further work on the Augsburg data examined the effects of particles on CRP. CRP is a sensitive parameter for inflammation, tissue damage, and infection and is a risk factor for heart attack. The analysis showed that the effects of particles were more pronounced for CRP than for plasma viscosity.[188]

Heart rate can also be considered an important factor as it is an independent marker for the autonomous control of the heart rate and found that heart rate did in fact increase during the smog episode and was most strongly associated with concentrations of suspended particles, an effect that was particularly pronounced in individuals who also had elevated plasma viscosity.

Gardner and colleagues reported increases in fibrinogen in animals exposed to urban particles, although the mean plasma viscosity for all exposed rats were higher than those of the control rats but none of the differences reached significance due to the variability in responses within the groups.[190] Ghio and colleagues showed similar results in healthy human volunteers exposed to concentrated air particles (CAPs) with significant differences observed in the concentration of blood fibrinogen between air exposed and CAPs exposed subjects.[189] In contrast however, Seaton reported that PM_{10} was negatively associated with plasma fibrinogen levels, in a study designed specifically to investigate the hypothesis that particulate air pollution leads to changes in blood coaguability as a consequence of alterations in clotting factors such as fibrinogen. This study also found significant negative relationships between three day mean personal exposure estimates and haemoglobin, PCV, and red cell count as well as significant negative relationships with platelets and Factor VII.[187] Seaton's results do not support the concept of increased fibrinogen concentrations, at least at pollution levels as low as those measured during the study, there does remain the possibility that higher concentrations of pollution however, may well result in the increase in fibrinogen. Schwartz examined the association between air pollution and several such intermediate biomarkers of cardiovascular risk in a national sample of subjects chosen to be representative of the US population.[191] He found significant and consistent associations between PM_{10} and the three cardiovascular risk factors (platelet counts, plasma fibrinogen and white blood cells) examined. Stout and Crawford have shown seasonal variations in plasma fibrinogen in the elderly and suggested that the greater concentrations in winter could increase the risk of Cardiovascular Disease (CVD).[192] They found that fibrinogen levels were 23% higher in the colder part of the year than in the summer months and that plasma viscosity also varied with season and temperature although the relation was not as strong as with fibrinogen.

Woodhouse found a statistically significant seasonal variation in fibrinogen and Factor VII clotting activity (FVIIc).[193] For fibrinogen the 0.13 g/l difference was less than that found by Stout and Crawford (0.78g/l). Using data from the Northwick Park heart study in which a plasma fibrinogen concentration one standard deviation increased the risk of IHD death by 67% in the first five years of follow-up, Woodhouse estimated that the seasonal difference seen in their study would correspond to a 15% increase in risk, which compared favourably with the 20% increase in the 65-75 year age-group each winter in England and Wales. For FVIIc the seasonal difference equated with a 9% increase in risk.

Prospective studies have shown that fibrinogen predicts the development of cardiovascular disease. The risk in those with high fibrinogen concentrations is greater in younger people than in older people. The predictive effect of plasma fibrinogen on ischaemic heart disease and stroke was independent of other risk factors including serum cholesterol, blood pressure and smoking.

Historically Cardiff City was an industrial city, a bustling seaport exporting coal, steel and iron, and as a result much of the particulate pollution in the city would likely have been derived from the steel works or other associated industries. Nowadays however, industry plays a much smaller part and as a result of improvements to the road networks and the increased usage of motor cars, buses and other heavy vehicles, particulate pollution is now derived, in the main, from vehicular sources.

The international literature has consistently shown that current ambient concentrations of particles have led to a short-term impact on daily mortality and hospital admissions for respiratory and cardiovascular causes. Other effects that have been observed include worsening symptoms among asthmatics and patients with other chronic respiratory diseases and increases in medication use of such particles and long term studies have shown that exposure to fine particles can result in a reduction of life expectancy by up to two years. Biological studies have shown associations between particles and such effects as oxidative tissue damage, alterations in blood coagulation and increased levels of inflammatory markers.

When such evidence is taken altogether, there seems little room for doubt that particulate pollution does indeed pose a threat to the health of the population as a whole. Still the associations remain tenuous and it is also profoundly clear that more research is required before it can be stated with any certainty that particulate pollution is solely responsible for the effects observed, and in fact it is the opinion of many that the effects observed in many studies cannot be attributed solely to particulate pollution and than the best one can hope for is to find a component of air pollution that best represents the effects of air pollution as a whole.

In this study an attempt is made to combine the evidence from the epidemiological field with that from the biological arena in the hopes that by examining the levels of various biomarkers of effect in an epidemiological setting, some conclusions can be drawn as to whether ambient urban concentrations of ultrafine particles affect the concentrations of certain blood biomarkers which are implicated in the causal pathway of cardio-respiratory diseases. As much of the current research and subsequent legislative decisions are focused on the PM_{10} and fine fractions of particulate pollution, the decision was taken to attempt to examine the ultrafine fraction of particulate pollution particularly as it appears that this is the size fraction that is beginning to emerge as being likely to have the most serious consequences for health.

Chapter 3
Methodology

3.1) Section A
A Comparison of the Levels of Biomarkers of Inflammation, Coagulation and Oxidant Damage in Two Populations with Differing Levels of Exposure to Ultrafine Particles

3.2) Rationale
Although the primary research literature provides evidence to suggest that exposure to particles may elicit a response that results in increased morbidity and mortality from respiratory and cardiovascular diseases, some evidence exists that suggests that these effects are not necessarily restricted to susceptible subgroups (i.e. children, elderly and those with pre-existing cardiovascular and/or respiratory illnesses). Many of the effects of air pollution will manifest themselves symptomatically in the vulnerable population resulting in increased hospital admissions and possibly deaths from cardiovascular or respiratory diseases. While a response severe enough to require hospitalisation or even medication may not occur in the healthy population, it is possible that prolonged exposure to ambient levels of particulate pollution increases an individuals' chance of developing respiratory or cardiovascular illnesses that may later require attention. It is not beyond the realms of possibility that inflammatory and oxidative reactions occur in healthy individuals as a result of exposure to particulate pollution. This study is an attempt to investigate whether the healthy population of Cardiff are in fact affected by long term cumulative exposure to particulate air pollution, by seeking to provide evidence of biological effects of exposure to particulate pollution on a healthy population rather than the susceptible populations normally studied.

3.2.1) Selection of Biomarkers of Effect
Several biomarkers of effect were chosen to be examined in relation to the traffic and pollution data collected during the study. Although the literature presents

evidence to show that there are a wide variety of biological factors are affected by particulate pollution, it was not prudent to select and examine every biological factor for which evidence exists. In the main the evidence primarily suggests two specific factors of the biological system that may be affected by exposure to particulate pollution, the blood (particularly blood coagulation/viscosity) and the immune system. These are two very complex systems each with several important functions in human biology and again evidence in the literature appears to imply that certain aspects of each system are affected by exposure to particles and so it seemed appropriate to consider these factors to be the ones worth examining. On examining the literature and discussing the feasibility of testing blood for different biomarkers, a comprehensive range of biomarkers, designed to reflect both the potential effects on the blood system and the immune system were selected.

	Biomarker of Effect
Coagulation	Thrombin Clotting Time
	Prothrombin Clotting Time
	Activated Partial Thromboplastin Time
	Fibrinogen
Oxidant Factors	TBARS
	Carbonyl Concentration
Blood Cell Counts	Reticulocytes
	White Blood Cells
	Neutrophils
	Platelets
Inflammatory Biomarkers	C Reactive Protein
	Interleukin – 6
	Tumour Necrosis Factor - alpha

Table 3.1: List of the Biomarkers chosen for investigation and the biological system they are associated with

Thrombin Clotting Time

Thrombin is a glycoprotein formed by two polypeptide chains consisting of 36 and 259 amino acids. Thrombin has two main functions, to stimulate platelets, causing them to expand and aggregate and to cleave fibrinogen into fibrin monomers. Thrombin clotting time is a measure of the rate of conversion of fibrin to fibrinogen (i.e. a measure of haemostaticlly active fibrinogen).

Prothrombin Clotting Time

Prothrombin, another clotting factor in the coagulation cascade, it is a precursor to thrombin. Prothrombin is proteolytically cleaved to form thrombin as the first step in the coagulation cascade. Prothrombin time is a measure of how long it takes the blood to clot and is an important clotting test as it measures the levels and activity of five important clotting proteins, including Factor VII.

Activated Partial Thromboplastin

Thromboplastin is the protein in the blood responsible for the conversion of prothrombin into thrombin during the coagulation cascade. Activated partial thromboplastin time is a test carried out in conjunction with prothrombin time. It is another measure of how long it takes for blood to clot but it focuses on measuring the clotting time from activation of Factor XII to the formation of the fibrin clot.

Fibrinogen

Fibrinogen is an extracellular, dimeric glycoprotein synthesized by the liver. It is the circulating precursor of the fibrin clot and contributes significantly to blood viscosity, and coagulation, cell to cell adhesion and platelet aggregation. Fibrinogen is an acute phase protein and its levels increase during inflammation and infection.

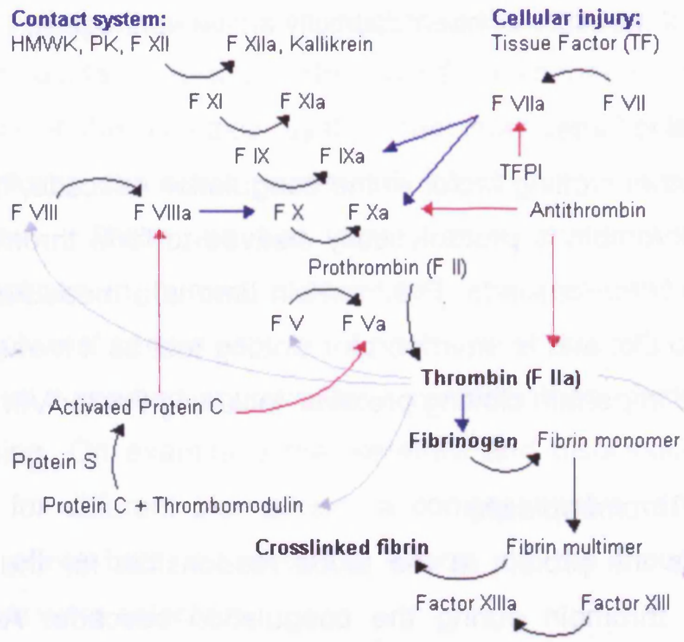


Figure 3.1: Coagulation Cascade –Retrieved From: http://en.wikipedia.org/wiki/Coagulation

Different cell types have different functions in the body; some like platelets play a role in coagulation, while others, such as neutrophils have an important role in the immune response. Counts of several different cell types were made during the study including counts of total red blood cells, total white blood cells, neutrophils and platelets. Red blood cells are responsible for transporting oxygen around the body and are what give blood its red colour, while white blood cells are important in the immune response and can be made quite quickly. The most abundant of the white blood cells are neutrophils.

Neutrophils

Neutrophils play a central role in the early immune response, when they are the predominant cell type infiltrating the tissue. Neutrophils are produced in the bone marrow and during inflammation production increases ten fold from the normal levels of 10^{11} neutrophils per day.

Platelets

Platelets, also known as thrombocytes, are cell fragments which gather at the site of tissue damage to form a clot and prevent blood loss. Platelets are activated by several different activators including thrombin and once activated they release a variety of different coagulation factors including fibrinogen.

There is a large amount of evidence to suggest that one of the most common effects of exposure to particulate pollution is oxidative damage and generation of reactive oxygen species. Oxidative attack of vital cell components by reactive oxygen species has been recognised in the pathogenesis of numerous human diseases including cardiovascular disease. Two markers of oxidant damage were measured during the course of the study they were TBARS and Carbonyl Concentration.

Thiobarbituric Acid Reactive Substances and Protein Carbonyl Conc

Plasma concentrations of thiobarbituric acid reactive substances (TBARS) are sensitive markers of oxidative tissue damage. TBARS concentrations were measured in the medical microbiology department using a fluorometric assay details of which can be found in appendix 1.

Protein carbonyls are formed by a variety of oxidative mechanisms and they are also sensitive indices of oxidative injury. The concentrations of protein carbonyls are measured using ELISA, again in the medical microbiology departments (appendix 1).

A number of different markers of inflammation were examined including C reactive protein, interleukin-6, and tumour necrosis factor alpha.

C reactive protein

C reactive protein is a prototype acute-phase protein, so named because of its ability to precipitate the somatic C-polysaccharide of *Streptococcus pneumoniae*. The serum levels can increase 1,000 fold during an acute phase response. Consisting of five identical polypeptides held together by non-covalent interactions, CRP binds to a wide variety of micro-organisms and activates complement resulting in the deposition of the opsonin C3b on the surface of micro-organisms. CRP is an extremely sensitive systemic marker of inflammation and tissue damage. Plasma CRP is produced by hepatocytes, predominantly under transcriptional control by the cytokine Il-6.

C reactive protein has been used to identify the presence and severity of an inflammatory response. Moderately increased levels above the normal range have been linked with future cardiovascular events in people and measurements of C-reactive protein in the patients with ischemic heart disease provide a novel method for detecting individuals at high risk of plaque rapture. C Reactive Protein concentrations are measured using an in vitro diagnostic assay, details of which can be found in appendix 1.

Interleukin-6 and Tumour Necrosis Factor Alpha (TNF-α)

Interleukin-6 is a cytokine that is secreted by a number of different cell types, including macrophage, T_h2 cells and monocytes to stimulate an immune response. Il-6 has a number of different functions including targeting proliferating B cells to promote a terminal differentiation into plasma cells. The important function of Il-6 for this study however is its ability to target hepatocytes and induce the synthesis of acute phase proteins including CRP and fibrinogen.

TNF-α is another acute phase protein which initiates a cascade of cytokines and increases vascular permeability, thereby recruiting macrophages and neutrophils to the site of infection. TNF-α is secreted by activated macrophages resulting in blood clotting with the purpose of containing the infection.

Both Il-6 and TNF-α concentrations are measured using standard test kits which employ the ELISA method. Details of the procedures can be found in appendix 2.

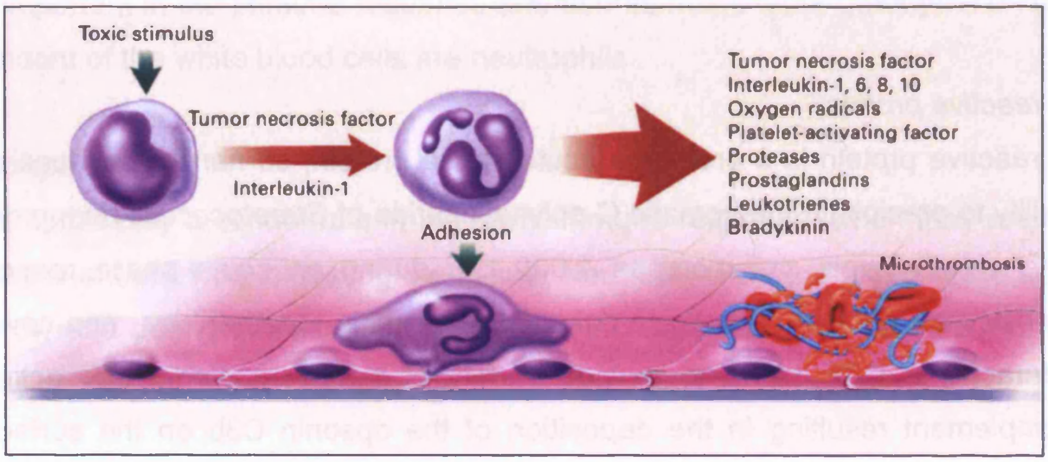


Figure 3.2: Inflammation Cascade – Retrieved From http://www.slz.nl/hora/onderwijs/INFLAM/Inflammation.htm

3.3) An Exposure Comparison Study of Biomarkers of Inflammation, Coagulation and Oxidant Damage

The exposure comparison study consisted of two groups of healthy males of a certain age range, comprising two groups – a so-called exposed group and an unexposed group. The aim was to recruit 150 men, with 75 drawn from the exposed area and 75 drawn from the unexposed area. It was decided that by recruiting 150 men there would be sufficient statistical power in the results while maintaining a realistic chance of recruiting the full number within the limited time-frame. Before beginning recruitment there were several decisions to be made regarding the design of the study and how best to go about recruiting people into the study. It was thought that the recruitment stage was going to be the most difficult part of the work and serious consideration was given as to how best to maximize recruitment.

The main steps in the exposure comparison study:

- Selection of the areas to be included
- Selection of the individuals to be included
- Recruitment of the individuals into the study
- Measurement of particle numbers both indoors and outdoors at participants residences
- Measuring biomarkers of effect in the blood

3.3.1) Area Selection

Selection of the streets to make up the exposed and unexposed areas in the study was based on two main parameters;

- Traffic Flow in the street
- Proximity of residences to the traffic

The residential criterion for the two groups of men was as follows;

Exposed Group:	To reside within 20 metres of urban road with high volume traffic flow
Unexposed Group:	Reside at a distance greater than 0.75km from any urban road with high volumes of traffic flow

Many cities' councils or local government departments routinely collect traffic information, particularly on busy and heavily used roads and ideally such information would have been used to identify the roads of interest for this study. Unfortunately however, at the time of commencing the study traffic information was not routinely recorded in Cardiff and traffic flow data was available for only a small number of streets. What little information there was regarding traffic flow in Cardiff City was obtained from the Transport Department of the Local Authority and provided the starting point for the study.

Street	AM	PM	Daily	Street	AM	PM	Daily
The Philog*	1107	1271	23780	Ty Glas Road*	1330	1394	27240
Mackintosh Place*	731	619	13500	Fidlas Road*	1910	1984	38940
Cowbridge Road West*	2557	2274	48310	Pendwyallt Road*	1014	1098	21120
Ninian Road*	579	900	14790	Sloper Road	1118	1205	23230
Llandaff Road*	973	970	19430	North Road	2350	2541	48910
Bridge Road	1127	1402	25290	Cowbridge Road East*	1206	1032	22380
Moorland Road*	1115	572	16870	Heol Hir North	207	125	3320
Planet Street	398	426	8240	Cherry Orchard Road	727	446	11730

***These streets were included in the exposure comparison study**

Table 3.2: Traffic Data provided by Cardiff Council

The information in the above table was used to identify the streets that would be used in the study to represent the exposed areas as for most streets the volume of traffic was quite high. Only some of the streets identified by the Council as having high volume traffic were suitable for inclusion in the study due mainly to the fact that some did not have a high number of residences. As far as selecting the streets from which participants could be considered unexposed (i.e. the streets with low traffic volume) was concerned no official information was available from any source. In this instance, local knowledge was again used to identify these streets. Cardiff is not unlike other cities in that there are several areas in the city that are primarily residential areas and these were considered to be the areas to be considered for inclusion as unexposed area. Several such areas were identified and in general each area consisted of several small streets or avenues making up a larger estate or community from which to recruit participants. Extra streets thought to have high traffic volume and the streets for the unexposed group were identified using local knowledge of Cardiff City. These extra streets were selected as a result of discussions with various people local to Cardiff and who were likely to know which were the more heavily trafficked areas, for example although the Transport Authority were unable to provide much concrete details, some of the staff involved in collating the initial data were able to suggest other streets which would fit our purpose. Other data had been collected as part of a project conducted in the Department of Architecture in Cardiff University, and although these data were not available to us, the staff could confirm whether streets we selected were suitable for inclusion.

Assigned as Exposed	Assigned as Unexposed		
Whitchurch Road	Nant Y Drope	Crosswells Way	Wavvell Close
Pencisely Road	St Isan Road	St Gildas Road	Forsythia Drive
Manor Way	Gibson Close	Everswell Road	Crystal Wood Road
Western Avenue	Hydrangea Close	Springwood	The Shires
Cathedral Road	Azalea Close	Jasmine Drive	Penmark Green
Wellfield Road	Brython Drive	Dennison Way	Clos Y Cwarra
Park Road	Lon Werdd	Cradoc Close	Lon Y Ffin
Richmond Road	Deepfield Close	Margeurites Way	Vicarage Gardens
Tudor Street	Hollybush Heights	Spring Grove	Mallards Reach
	Deepwood Close	Fieldfare Drive	

Table 3.3: List of all streets identified using local knowledge to be included in the study

Table 3.3 is a list of the extra streets selected for inclusion in the study as exposed and unexposed streets. Despite the fact that no traffic information exists for these streets they were selected on the basis that they are all A or B roads and are main routes either to the city centre or to the motorway which means that they are likely to have quite high volumes of traffic.

For the streets selected as unexposed areas the common feature of all the streets chosen was that they were primarily residential streets and were not generally accessed by anyone other than the residents of the areas. Although identifying the residential areas of Cardiff was not hugely difficult in itself, the danger was that areas selected would be affected by any potentially high traffic volume roads in the vicinity or that the streets themselves would be used as through roads, short-cuts or 'rat-runs' by motorists attempting to avoid getting caught in traffic on the busier streets in the locality. For this reason, where possible, closed communities and cul de sacs were selected for inclusion, although unfortunately this was not always possible.

3.3.2) Criteria for the selection of Individuals

Given the specific aim of this part of the study was to examine the effects of particles on *healthy* people, it was necessary to take several factors into consideration before selecting individuals to take part in the study and as such a list of inclusion and exclusion factors was drawn up prior to commencing the recruitment stage.

These factors were as follows:

Inclusion Criteria: Male

Aged between 50 and 70 years of age

Must have been resident for not less than 5 days prior to giving a blood sample

Exclusion Criteria: Smoker within last 5 years

Household includes a smoker

History of serious disease

History of chronic obstructive pulmonary disease

History of cardiovascular disease

History of chronic inflammatory disease

Infectious disease within six weeks prior to blood sample

Inclusion Criteria

Although undoubtedly the adverse effects of particulate pollution are not exclusive to the male population, the decision to restrict the study to males was taken in an effort to restrict the possibility of variability in the biomarkers between the sexes as it is not known how differently particulate pollution will affect the various biomarkers chosen for this study. Similarly, selecting only males between the ages of 50 and 70 years was again an effort to restrict the variability in the biomarkers, as again little evidence was available regarding the differences in the various biomarkers for different age-groups or between the sexes.

Participants were requested to have been in residence for at least five days prior to giving their blood sample. The effects of chronic exposure rather than acute exposure were of interest in this study and so by requesting that participants be in residence for five days prior to giving a blood sample was an attempt to control for the effects of any acute exposures that may have occurred in areas other than the area of residence and that may affect the levels of biomarkers of effect thus giving false or misleading results.

Exclusion Criteria

Smoking, both direct and passive smoking is known to affect respiratory and cardiovascular functions and so excluding not only smokers, but those who lived with a smoker was an attempt to control for the known confounding effects of smoking.

As the aim of the study was to investigate the effects of chronic exposure to air pollution on the biomarkers of effect in an effort to predict the future risk for developing serious cardiovascular or respiratory illnesses, it was necessary to exclude those people who had a history of chronic obstructive pulmonary disease (COPD) and cardiovascular disease.

As the biomarkers chosen for examination in this study are commonly affected by diseases other than those of interest for this study, it was necessary to exclude people with a history of serious disease, most importantly those with a history of serious inflammatory diseases or complaints such as arthritis.

Although not strictly an exclusion criteria, on the advice of the personnel analysing the blood samples, people were required to allow at least six weeks following an infectious disease such as a cold, before giving a blood sample.

This was to allow all inflammatory biomarkers, which would have increased during the infection, time to recover their normal levels.

3.3.3) Ethical Approval

Before commencing with the recruitment stage of the study it was vital that ethical approval was obtained. Application for ethical approval was submitted to the Bro Taf Local Research Ethics Committee along with a detailed outline of the work to be undertaken. Following a review by the panel, ethical approval was granted.

At this point, with all the criteria for the study in place and ethical approval granted, it was possible to commence with the recruitment stage of the study. One of the stipulations of having the ethical approval granted was that all participants were to be provided with a detailed explanation of what was required of them and that before participation, they were to sign a consent form stating that they had had everything explained to them, been given the opportunity to ask questions and that they were willing to take part in the study (see appendix 1).

3.3.4) Recruitment

The first step to selecting individuals was to acquire the addresses for all residential properties on the streets of interest. This was done using the Royal Mail address finder and addresses were extracted to an Access database to be used to send out letters inviting people to take part in the study. It was decided that a first shot of letters would be sent to try and gauge the level of interest in the study and so a query was created in Access that created a table containing all the addresses for a small number of streets (Mackintosh Place, Ninian Road and Ty-Glas Road) Using the mail merge feature of Microsoft Word a letter inviting people to take part in the study was created for every residential address on the streets of interest and was then sent out to all residences in each of the areas. The letter briefly outlined the intentions of the study and contained some simple questions, designed to ascertain the suitability of people who wished to take part. Residents interested in taking part were required to complete four simple yes/no questions and return the letter to the department in the post paid envelope provided. Returned letters were evaluated and grouped according to a) willingness to take part and b) suitability for participation. If a person fit the criteria as laid out in the letter and was willing to take part, a researcher from the department contacted them with further details about the study and to arrange a suitable time to interview the participant in their own home.

I am willing to have a member of the research team contact me to discuss my taking part in the study	YES ☐	NO ☐
I am male and between the ages of 50 and 70	☐	☐
Are you a smoker	☐	☐
Do you suffer from chest or heart disease, diabetes or arthritis	☐	☐
My name is	-------------------------	
My date of birth is	-------------------------	
My telephone number is	-------------------------	

Table 3.4: Copy of the questions included in the original letter to residents inviting them to take part. For a full copy of the letter refer to Appendix 1.

3.3.5) Measuring the number of particles in the air

Particulate Air Pollution measurements were made using a TSI P-Trak™ condensation particle counter. The P-Trak™ is a small hand held device that measures particles sizes in the range 0.02 to greater than 1 micrometer.

Figure 3.3: The hand-held, ultrafine particle counter (P-Trak™)

The P-Trak™ Ultrafine Particle Counter (UPC) measures ultrafine particle concentrations in real-time and also has the capacity to log this data for analysis at a later date. The units of measurement are numbers of particles per cubic centimetre (pt/cm^3) and the machine is capable of measuring concentrations ranging from zero to 5×10^5 particles/cm^3. The P-Trak™ is capable of recognising single ultrafine particles, and as a result it is far more sensitive than other technologies. Particles are drawn through the P-Trak™ UPC using a built-in pump. Upon entering the instrument, particles pass through a saturator tube where they mix with an alcohol vapour. This particle/alcohol agglomerate then passes into a condenser tube causing the alcohol to condense onto the particles, resulting in the growth of a large droplet. The droplets then pass through a focused laser beam, producing flashes of light which are sensed by a photo-detector. The particle concentration is determined by counting the light flashes. The purpose of condensing the alcohol to the particles is to create large enough droplets to produce enough light scatter as they pass through the laser beam to be detected. The unique feature of the P-Trak™ is its ability to count single particles, thus setting it apart from other air quality monitoring methodologies and instrumentation.

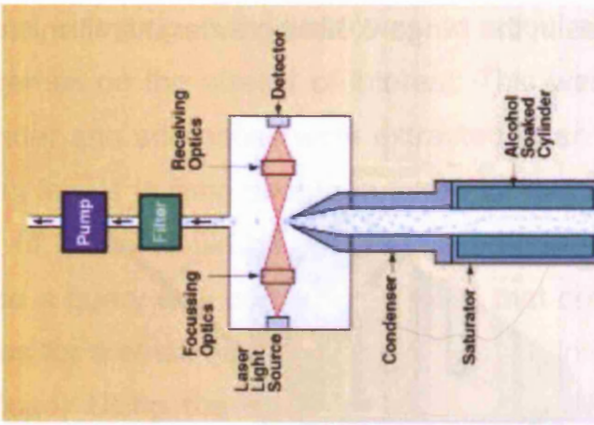


Figure 3.4: Theory of operation of the P-Trak

The P-Trak™ UPC counts ultrafine particles (smaller than 0.1 micrometer in diameter) that often accompany or signal the presence of a pollutant that is the root cause of IAQ complaints and has successfully been used in monitoring indoor air quality and identifying problem areas such as ventilation problems in office buildings. It would appear however that this is the first study in which the P-Trak™ was used in an effort to monitor outdoor air pollution.

During this study particle measurements were taken both indoors and outdoors at each participants home. Measuring the indoor and outdoor levels of ultrafine particles at each participants home served several purposes for the study.

- It allowed comparison of indoor and outdoor levels and thus potentially give an idea as to the amount of indoor particles occurring as a result of the outdoor particles penetrating.
- Comparison of the levels of particulates with the results of the blood tests could provide information regarding the sub-clinical effects of exposure in healthy individuals, if indeed any effects exist.
- Comparison of levels of particulates between street groups may provide some insight to the pattern or distribution of pollution in Cardiff.

Outdoor Samples taken at any time between 8am and 9pm

On average a ten minute sample was collected outdoors and the sample was collected at the participants' house, usually on the footpath outside the garden.

It was thought that ten minute samples would provide enough information on the levels of particulates on the street, particularly if there were more than one participant per street as was the case for most of the streets in the exposed areas. In the unexposed areas it was common to have only one person per streets, however as previously mentioned, the exposed area consisted of a larger number of streets, most of which could be grouped together according to the general area in which they were located. For this reason, again ten minute samples were considered to be enough to provide a picture of the levels of particulates in the different areas.

During the course of the study, extra outdoor measurements were taken at various times of day for different streets as for some streets there were only one or two participants and therefore not enough particle counts to be representative of the levels on the street.

Ideally measurements would have been taken for longer than ten minutes at a time, but given the volume of work that was required and the fact that the intention was to take many measurements in each area it was decided that the measurement times should be as short as possible to be practical yet long enough to provide an accurate reflection of the levels of particles. Examination of the PM_{10} data showed that some variations in the levels tended to occur over the day, with higher levels during morning and evening rush hours and lower levels during the afternoon and at night. Although there is no evidence to support the decision, it was felt that the levels of ultra fine particles were likely to follow the same pattern and so it was decided that ten minute samples, spread over the course of the day, would be sufficient. All measurements for this study were made between 8am and 7pm and therefore it was felt that with the number of measurements to be taken, a range of ten minute samples would provide a reflection of the levels of particles for the particular period in which the measurements were taken.

Time	Mean Number of Particles/cm^3
09:00	29561
10:00	20105
11:00	24428
12:00	25879
13:00	15254
14:00	23105
15:00	18301
16:00	15336
17:00	11965
18:00	27090
19:00	35719

Table 3.5. The mean number of particles measured at each hour throughout the day in the exposed area

It can be seen from the above table that the number of particles measured outdoors in the exposed streets varied only between 11965 at 5pm and 35719 at 7pm a difference of only 23754 particles/cm^3 suggesting that the degree of variation throughout the day is minimal.

			Statistic	Std. Error
Mean Number of particles/cm^3	Mean		20941.00	1422.248
	95% Confidence Interval for Mean	Lower Bound	17940.32	
		Upper Bound	23941.68	
	5% Trimmed Mean		20900.89	
	Median		20236.50	
	Variance		36410203.529	
	Std. Deviation		6034.087	
	Minimum		11664	
	Maximum		30940	
	Range		19276	
	Interquartile Range		10528	
	Skewness		.254	.536
	Kurtosis		-.920	1.038

Table 3.6 Basic Descriptive Statistics for mean outdoor particles in the exposed areas

			Statistic	Std. Error
Mean Number of particles/cm^3	Mean		10906.62	915.363
	95% Confidence Interval for Mean	Lower Bound	9044.30	
		Upper Bound	12768.94	
	5% Trimmed Mean		10525.12	
	Median		9964.00	
	Variance		28488224.668	
	Std. Deviation		5337.436	
	Minimum		2929	
	Maximum		28424	
	Range		25495	
	Interquartile Range		7326	
	Skewness		1.143	.403
	Kurtosis		2.104	.788

Table 3.7 Basic Descriptive Statistics for mean outdoor particles in the unexposed areas

The above tables outline some basic descriptive statistics pertaining to the mean number of particles measured in the exposed (table 3.6) and unexposed (table 3.7) areas, including 95% confidence intervals. From these tables it can be seen that the data collected appear to provide a reasonably accurate reflection of the levels of ultrafine particles for each group.

Indoor Samples taken at any time between 8am and 9pm

Although ten minute samples would have been sufficient, indoor samples tended to be longer by virtue of the fact that the P-Trak™ was allowed to run during the interview with the participants, a time frame ranging from twenty minutes to 1 hour. When taking the indoor measurements, the P-Trak was placed, where possible in a room facing the front of the house, and the street of interest. The room facing the front of the house was the living room or dining room in the majority of houses and therefore unlikely to be affected by the possible other sources of ultra fine particles, particularly those found in a kitchen.

3.3.6) Measuring the levels of Traffic

Some traffic data, collected in 2002, were provided by Cardiff local government but as previously mentioned only limited data were available due to the fact that traffic data are not routinely collected in Cardiff. Unfortunately it was not practical to collect any more traffic data from the streets in the exposed areas and in any case, sufficient data already existed to allow some basic exploratory analysis. This meant that the primary concern for traffic data was the lack of data available for the unexposed streets. Given that the method employed when calculating the traffic volume for other streets was fairly straightforward, an attempt was made to collect some traffic data for a small number of the unexposed streets. Traffic data was collected for one of the streets in the unexposed areas, by counting the number of cars that used the road in the morning and evening rush hours an applying the same formula as that used by Cardiff Transport Authority to calculate a 12 hour average.

Street	AM	PM	Daily
Deepwood Close	14	18	320

Table 3.8: Street for which an attempt was made to collect traffic data using the formula Mean(AM+PM)*10 to calculate a 12 hour traffic count

It was originally intended that this would be done for more than one of the unexposed streets however it proved to be impractical and time-consuming and there fore the decision was taken not to continue with the collection of these data, although this is something that should be examined for any future work.

3.3.7) Collecting blood samples and measuring the levels of biomarkers

The initial plan was to collect blood in the participants own home in an effort to make things easier for the participants. However blood must be drawn by a qualified nurse or phlebotomist and as one was not available to come to participants' homes it was necessary to ask participants to come into the hospital outpatients unit to give their blood samples. When drawing the blood samples, the phlebotomists needed to be aware of the biomarkers of effect to be investigated and were required to use particular vacutainers when drawing the blood required for each test and to specifically store the blood samples depending on the biomarker being tested.

Measurement Required	Vacutainer Type	Volume of Blood Needed	Laboratory
Reticulocytes, FBC & Erythropoeitin	EDTA-Purple Top	5mls	Haematology
Fibrinogen, Factor VII & Full Coags	Blue Top	5mls	Haematology
CRP	Yellow Top	3.5mls	Biochemistry
Carbonyl Conc & TBA Conc	EDTA-Purple Top	5mls	Microbiology
IL-6 & TNF-α	Yellow Top x 2	7mls	Haematology

Table 3.9: Volume of blood to be collected and treatment required to render the sample suitable for analysis

To make this easier on the phlebotomists collecting the blood samples, a system was devised whereby each participant was given a card bearing their details and a set of labels which bore the necessary information regarding the blood samples to be taken and how they were to be treated, as well as the participants unique identification number, initials and date of birth. The phlebotomist attached these labels to the relevant sample during collection and then packaged and sent the samples to the laboratories for analysis, where the samples were analysed by various qualified laboratory scientists. Providing the labels for the phlebotomists meant that the chances of mislabelling a sample was minimised.

The phlebotomist was then to sign and date the card and these cards would be collected by a researcher and kept as a record of participants who had come in to give blood. As each participant in the study was assigned a unique identification number at the outset, and this number was recorded on the cards along with the participants' initials and date of birth, the cards also enabled researchers to keep track of which samples went to the laboratories and as such identify and attempt to trace missing samples.

Another advantage to providing these cards to participants was that those participants who had been visited and interviewed in their homes but had not provided a blood sample were easily identified and could be contacted and asked to come in.

Despite the fact that expenses were to be covered, the fact that people had to come to the hospital to give blood samples seemed to put a few people off and in fact some participants never did give blood even though they were sent reminder letters and a researcher phoned to ask them to give blood resulting in a total response rate for giving a blood sample of 72%.

Analysis of the blood samples for levels of the various biomarkers of interest was carried out in the laboratories at the hospital. The results were then analysed using Microsoft Excel and SPSS.

There were three laboratories involved in the analysis of the blood samples; haematology, biochemistry and microbiology. Haematology was responsible for conducting full blood screens, including testing for factors such as fibrinogen and factor VII, coagulation tests, erythropoietin and testing for levels of the inflammatory biomarkers IL-6 and TNF-α.

3.3.8) Questionnaires

On visiting each participant, a brief questionnaire was administered by the researcher, the aim of which was to acquire some information about the individuals' personal opinion regarding the area in which they lived. The reason for collecting such qualitative data was enable the comparison of the opinions of participants with the actual quantitative data collected during the course of the study. A copy of the questionnaire can be found in appendix two.

3.4) Analysis

3.4.1) Investigating the relationship between particles in the exposed and unexposed areas

Preparing the data for analysis

The first step in preparing the data for analysis was to download the data from the P-Trak to the computer. Analysis of the particulate pollution measurements involved graphing the data using the P-Trak™ software supplied with the equipment. This software was quite basic however, and beyond graphs and simple statistics such as maximum and minimum measurements and mean measurements, nothing much could be done with the data as it stood. As such the data were exported to Excel and SPSS for further analysis.

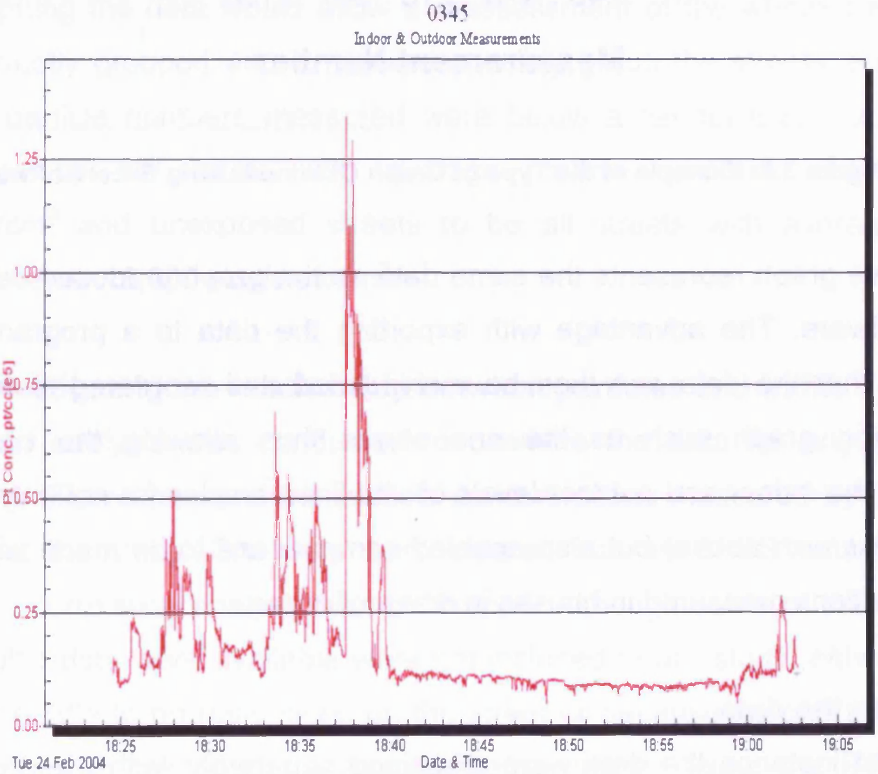


Figure 3.5: Example of the type of graph obtained using the P-trak Software

The above is an example of the type of graph that can be produced using the P-trak software. This particular graph represents the particle concentration measured both indoors and outdoors at the home of one of the study participants. The different locations can easily be identified on the above graph due to the fact that the outdoor measurements show much more variation in the numbers of ultrafine particles whereas the numbers of ultrafine particles measured indoors are much more constant.

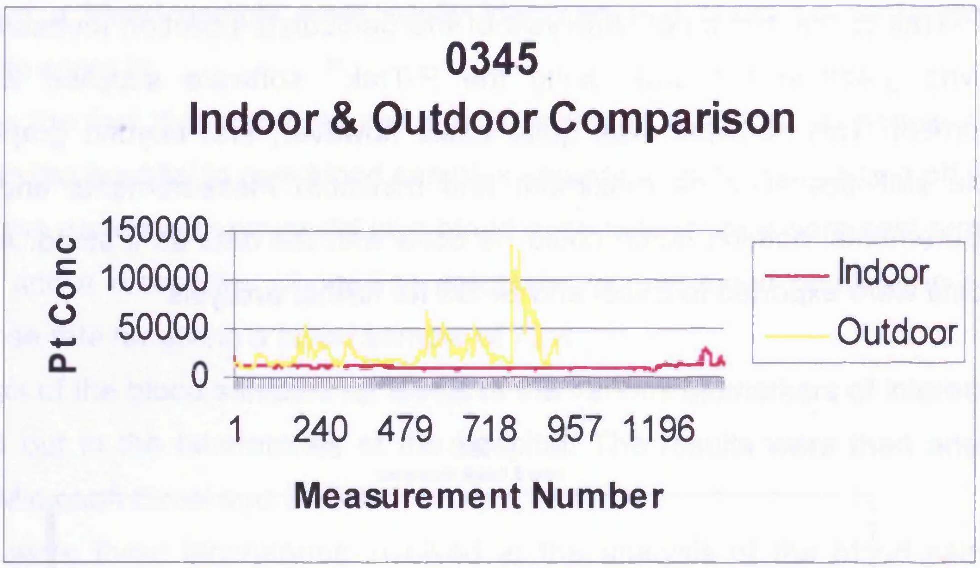


Figure 3.6: Example of the Type of Graph Obtained using Excel Software

The above graph represents the same data as the graph produced using the P-Trak software. The advantage with exporting the data to a program such as Excel is that the data can then be manipulated and reordered to allow for a comparison graph such as the one above thus allowing the comparisons between the indoor and outdoor levels of ultrafine particles for not only individual houses (as with above) but also enabled comparisons to be made with particle concentrations measured in houses in different areas.

Analysing the data

In the first instance the data were examined separately, with the particle data collected at the home of each participant treated as unique and individual data. Graphs comparing the indoor levels and outdoor levels of particles were constructed using Excel and examined for any differences between the indoor and outdoor levels and attempting to identify any similarities between the data collected for each individual in terms of the numbers of particles measured, the degree of variation in the indoor levels and outdoor levels and the range of particle numbers measured.

Secondly all the data were examined together as a whole data set, with all data collected in the exposed areas collated and examined against the data collected in the unexposed areas. Again a series of graphs were constructed using Excel, comparing the average numbers of particles indoors and outdoors for both the exposed and unexposed areas.

Given that a number of the streets were selected using only local knowledge another approach to analysing the particle data was to construct a graph whereby the particle data was graphed from the lowest average measurement to the highest average measurement, the purpose of which was to investigate whether the streets selected as unexposed did indeed have lower levels of particles to the exposed streets. If they did not have the lowest measurements then graphing the data would allow a reassessment of the whether the streets were correctly grouped and to instead possibly group the streets according to whether particle numbers measured were below a certain level (i.e. exposed streets to be all streets with average particle numbers above 15,000 particles/cm^3 and unexposed streets to be all streets with average particle numbers below 15,000 particles/cm^3)

3.4.2) Investigating the relationship between particles and traffic

Correlation analysis was conducted to examine the data for any correlation between traffic volume and the particle concentrations measured by the P-Trak throughout the study. The data were entered into an SPSS data file and a Pearson's correlation analysis was performed. Although some of the streets for which traffic data were available were not included in our study, either because there were little to no residences on the street or because no-one living on the street wished to take part, particulate measurements using the P-Trak were obtained nonetheless, thus allowing correlation analysis on these streets too. The particulate data collected on streets with no participants however was not included when investigating whether the differences in particulate levels between the exposed and unexposed groups resulted in a difference in the levels of blood biomarkers of effect.

3.4.3) Investigating the differences between biomarkers of effect in participants in exposed and unexposed areas

Preparing the data for analysis

The blood samples were analysed in the hospital laboratories using a number of routine tests. The results for all the different biomarkers measured were obtained from the various laboratories and entered into an Excel spreadsheet. Two separate spreadsheets were constructed, one for the results of the first sample and another for the results of the second sample. Once the spreadsheet was complete, a list of all the missing results was compiled and sent to the laboratories in an effort to obtain the missing data.

A database containing all available results was also created in SPSS to allow more sophisticated analyses to be conducted.

The individual reference ranges for each biomarker were obtained from the laboratory staff and the results were examined in relation these reference ranges.

Analysing the data

Analysis of the data involved the construction of scatter plots of the results, separated by exposed and unexposed, for each of the individual biomarkers. These plots were then examined simply to see if any obvious differences existed between the exposed and unexposed areas in terms of the results. The results were then examined in relation to the reference ranges provided by the lab with the intention of investigating whether any apparent difference could be observed in either group in relation to the reference ranges for the individual biomarkers.

The next step in the analysis aimed to identify any statistical differences in exposed and unexposed groups in terms of the levels of the individual biomarkers measured. An obvious approach to the analysis was to compare the mean levels between the exposed and unexposed areas for each individual biomarker of effect. This can only be done however if the data are normally distributed, otherwise the geometric mean should be used. The mean particle number for the each group was calculated simply by collating all data measured for each street within the group and calculating a mean value for the street. The mean values for each street were then used to calculate a single mean value for the group relevant to the street. If not using the mean an alternative approach is to use the median value but if using the median, then different statistical tests must be employed. In order to test whether the data follows a normal distribution or not there are two methods which can be used – the construction of a histogram, or the construction of a Q-Q plot. Histograms were constructed for each of the individual biomarkers and it was found that although much of the data did follow a broadly normal distribution, some results did not indicating that it would be inappropriate to compare the mean measurements in each group for some of the biomarkers and therefore non-parametric tests such as Kruskal-Wallis and Mann-Whitney U tests were the tests of choice for analysis.

3.5) Section B

A study designed to examine whether proximity to different roads types increases ones likelihood of being admitted to hospital with cardiovascular or respiratory complaints.

The premise behind this section of the study was that persons living in areas where they are potentially exposed to high levels of ultra-fine particles as a result of high volumes of traffic might be more likely to be admitted to hospital suffering from either cardiovascular or respiratory illnesses. In an effort to investigate whether this is in fact the situation and as an attempt to develop a possible method of predicting who may be at risk, a study was designed that involved splitting Cardiff into categories based on road type and usage and by using logistic regression analysis and correlation analysis, examining whether living in any of these categories appeared to have any affect on hospital admissions for a variety of respiratory and cardiovascular diseases. This section of the study was carried out in three distinct phases that included;

- defining categories and assigning postcodes to each
- identifying and collecting data on hospital admissions for the ICD codes of interest
- analysis of the data using logistic regression and correlation analysis

Due to the type of analysis to be performed, the most important stage of this study involved the construction of an SPSS data file containing all the necessary information, including information on hospital admissions, road categories, distance groups, base population data.

3.5.1) Definition of the categories

While road type was the primary factor of interest to this section of the study, it was recognised that another important factor that may affect the number of hospital admissions was distance of residences from the areas of highest traffic volume, in this case distance from the city centre, as well as the type of road on which the residence was located. The decision was therefore taken to create four distance categories and then to assign the roads within each 'distance category' to a subcategory based on the road type.

The first step was to define a central point from which all distances could be calculated. Cardiff has historically been an industrial city and as with most coastal industrial cities, the main city centre has built up around, and radiated out from the port location. The port in Cardiff is no longer as active and it was therefore decided that it would not be the most suitable place to have as the central point; as such it was thought that Cardiff Central Station was the best place from which to start the calculations. Cardiff Central Station is located in the city centre, close to the port, and is the main station for both buses and trains in the city, thus making the roads leading towards it some of the busiest and most congested in the city.

Using GIS, three zones of 2km radius and one of 6km radius, using central station as the starting point were created. X was defined as the area up to 2km from the centre point, Y is defined as the area between 2 and 4km from the centre point, Z relates to the area between 4 and 6km and S relates to the area between 6 and 12km from the city centre.

Figure 3.7: Map of Cardiff with Cardiff Central Station and the distance boundaries marked. The image was created using GIS software.

Following this a database was constructed with fields containing postcode, road name, and the distance zone into which the road fell as well as a field into which the category for each individual road was to be entered.

It was decided that the subcategories within each distance zone would consist of separate categories for A roads, B roads, bus routes, cul de sacs and all other properties. Within the first distance zone, 0-2km from central station, it was decided that the A road and B road subcategories would be combined as, once into the city centre, very little differentiation exists between the two road types in terms of their traffic volumes.

The categories were defined as follows:

Category Number	Roads Type and Distance from City Centre
1	A and B roads within 0-2kn km of the city centre
2	Bus routes within 0-2km of the city centre
3	Cul de Sacs within 0-2km of the city centre
4	All other properties/roads within 0-2km of the city centre
5	A roads within 2-4km of the city centre
6	B roads within 2-4km of the city centre
7	Bus routes within 2-4km of the city centre
8	Cul de Sacs within 2-4km of the city centre
9	All other properties/roads within 2-4km of the city centre
10	A roads within 4-6km of the city centre
11	B roads within 4-6km of the city centre
12	Bus routes within 4-6km of the city centre
13	Cul de Sacs within 4-6km of the city centre
14	All other properties/roads within 4-6km of the city centre
15	A roads within 6-12km of the city centre
16	B roads within 6-12km of the city centre
17	Bus Routes within 6-12km of the city centre
18	Cul de Sacs within 6-12km of the city centre
19	All other properties/roads within 6-12km of the city centre

Table 3.10: Road Categories and description of roads within each category

Once the individual categories were defined the next stage involved assigning the roads within each distance category to their relevant subcategory using GIS, Ordnance Survey street maps and bus route maps that were provided by Cardiff Bus. The database was then complete with respect to road data and ready for the addition of the hospital admissions data.

3.5.2) Identifying and collecting data on hospital admissions for ICD codes of interest

The literature review showed that for many studies examining the effects of exposure to particulate pollution involved the use of hospital admissions data. In Wales approximately half a million individuals receive inpatient care each year (i.e. are admitted to hospital). The most comprehensive source of hospital admissions data is the Patient Episode Database for Wales (PEDW) and so it was decided to use this information source to gain information on the numbers of hospital admissions with a diagnosis corresponding to any of the ICD codes of interest.

Information regarding the base population was drawn from the NHS Administrative Register, a database containing information on people registered with Welsh GPs and living in Wales.

In-patient data - PEDW

PEDW contains all inpatient and day case activity undertaken in NHS Wales as well as data on any Welsh residents treated across the border in English trusts. PEDW was established in 1991 and modified in 1997 to align the Welsh inpatient data and day care dataset with England to allow benchmarking and cross-border comparisons. Since 1999, PEDW has become the main source of Welsh comparative data and is being used increasingly to monitor performance within the Welsh NHS.

Each hospital has an electronic system that collates patient details and this data is downloaded at not only at health authority level but also at the regional information service – Health Solutions Wales (HSW) where the data are cleaned and amalgamated into a single data set. The system processes approximately 100,000 episodes of patient care per month, with regular timeliness and data quality reports produced. The database contains demographic information; clinical and administrative details; and diagnostic procedures (ICD 9&10 coded).

NHS Administrative Register (NHS AR)

The NHSAR system is, similarly to the PEDW database, managed by Health Solutions Wales. There are over 3 million people on the system with details such as name, address, postcode and GP recorded for each individual. In addition to details about the individual, details for the individuals' GP and the practice details are also held.

ICD Codes

The International Classification of Diseases is the World Health Organisation's internationally accepted classification of death and disease. It is used to classify diseases and other health problems recorded on many types of records such as death certificates and hospital records, including inpatient records in Wales. Various cardiovascular and respiratory diseases were identified using the International Classification of Diseases, tenth revision (ICD 10).

The ICD codes of interest for this study were:

ICD Code	Diagnosis	ICD Code	Diagnosis
I10	Essential Primary Hypertension	I46	Cardiac Arrest
I11	Hypertensive Heart Disease	I47	Paroxysmal Tachycardia
I12	Hypertensive Renal Disease	I50	Heart Failure
I13	Hypertensive Heart and Renal Disease	I51	Complications and Ill-Defined Descriptions of Heart Disease
I15	Secondary Hypertension	I60	Subarachnoid Haemorrhage
I20	Angina Pectoris	I61	Intracerebral Haemorrhage
I21	Acute Myocardial Infarction	I62	Other Non Traumatic Intracranial Haemorrhage
I25	Chronic Ischemic Heart Disease	I65	Occlusion and Stenosis of Precerebral Arteries, Not Resultig in Cerebral Infarction
I26	Pulmonary Embolism	I66	Occlusion and Stenosis of Cerebral Arteries, Not Resultig in Cerebral Infarction
I27	Other Pulmonary heart Diseases	I67	Other Cerebrovascular Diseases
I28	Other Diseases of Pulmonary Vessels	I68	Cerebrovascular Disorders in Diseases Classified Elsewhere
I42	Cardiomyopathy	I74	Arterial Embolism and Thrombosis
I44	Atrioventricular and left bundle-branch block		
I45	Other Conduction Disorders		

Table 3.11: List of ICD-I Codes (cardiovascular diseases) and the associated primary diagnosis

ICD Code	Diagnosis
J20	Acute Bronchitis
J21	Acute Brochioloitis
J40	Bronchitis Not Specified as Acute or Chronic
J41	Simple and Mucopurulent Chronic Bronchitis
J42	Unspecified Chronic Bronchitis
J43	Emphysema
J45	Asthma
J47	Bronchiectasis
J60	Coalworker's Pneumoconiosis
J61	Pneumoconiosis Due to Asbestos and Other Mineral Fibres
J62	Pneumoconiosis Due to Dust Containing Silica
J63	Pneumoconiosis Due to Other Inorganic Dusts
J64	Unspecified Pneumoconiosis
J66	Airway Diseases Due to Specific Organic Dust
J67	Hypersensitvity Due to Organic Dust
J68	Respiratory Conditions Due to Inhalation of Chemicals, Gases, Fumes and Vapours
J69	Pneumonitis Due to Solids and Liquids
J70	Respiratory Conditions Due to Other External Agents

Table 3.12: List of ICD-J Codes (respiratory diseases) and the associated primary diagnosis

3.5.3) Constructing the database

Construction of the database occurred in three stages; collecting data on the 2002 hospital admissions for each of the categories of interest, collecting the base population data for each category and then combining the two separate database into one SPSS data file and preparing the data for analysis.

Firstly, using the Patient Episode Database, Wales (PEDW), the number of reported episodes for various cardiovascular and respiratory diseases and subsequent hospital admissions in 2002 were identified by their ICD code. Only primary diagnosis was of interest for this study.

The second stage in the building of the database involved identification and addition of the base population data for each category using the National Health Service (NHSAR) database.

Finally an SPSS data file was prepared with all the necessary variable fields and both databases were merged into one and edited in preparation for analysis.

Due to confidentiality issues staff at Health Solutions Wales (HSW) undertook the task of producing the first two parts of the database. HSW were provided with the database previously constructed and containing fields with postcode, road name, distance group and category. Using the postcode information, HSW searched the PEDW database and constructed an excel spreadsheet containing data on the number of admissions to hospital for 2002. The spreadsheet also contained fields, with information regarding the primary diagnosis on admission, admission date, and episode number among the data provided. Most importantly, for every individual admission, HSW matched it with the road category in which the person lived. There were approximately 5,000 cases in the database, although some of the cases were repeat admissions and so not every case represented a unique individual.

Similarly, HSW staff constructed an access database containing information on the base population for each category, again the database contained information on such factors as age, sex, and socioeconomic status. In this data base there were approximately 316,000 cases. Each individual case in this database should represent a unique individual as each case represents a single person, identified by a unique NHS number.

3.6) Analysis

There were several stages involved in the analysis of the data including; basic descriptive statistics, correlation analysis and regression analysis.

The first stage of the analysis took the form of basic descriptive statistics to examine the distributions and patterns that might have existed in the data. Performing such basic statistical methods can also provide and insight into the most appropriate methods to employ when engaging in further, more complicated statistical analysis of the data.

The primary analysis was to take the form of logistic regression and thus it was necessary to create a dichotomous variable. To this end a variable was created in the SPSS data file where an admission to hospital with a diagnosis of interest (ICD-I or ICD-J) was assigned the value '1' and all other cases in the file were assigned the value '2'. This variable, labelled 'admitted for ICD of interest/not admitted' was to be the dependent variable in the regression analysis.

Evidence has suggested that factors such as age, sex and socio-economic status will affect incidence and severity of both cardiovascular and respiratory diseases and so the decision was taken to include these factors in the regression model from the outset. Information on these factors are recorded both in the PEDW database and in the NHSAR database and so including them in the analysis was a straightforward task. The main factor of interest however was the road category. The logistic regression was performed using three different road variables; category, distance and road type. The category variable refers to the original nineteen categories identified and was the variable of most interest; the distance variable refers to the four different distance groups; while the road type variable refers to a variable created where all roads of a similar type were grouped together regardless of distance from the city centre.

Chapter Four

Particulate Pollution from Vehicular Sources may Result in Adverse Health Effects

4.1) Section A

An exposure comparison study of biomarkers of inflammation, coagulation and oxidant damage

This study attempted to examine whether there were any differences in the levels of various biomarkers of effect in people living close to areas of high traffic and people living in areas with very little traffic. As a city Cardiff does not suffer from the same high volumes of traffic as such other UK cities as Birmingham or London. However Cardiff has the advantage that it is a particularly green city offering the opportunity to compare areas of differing traffic volumes, from reasonably high to very low.

This section outlines the results of the analysis investigating any differences in biomarkers of effect between potentially exposed and unexposed groups.

4.1.1) Response Rates

As anticipated, recruitment was to prove the most difficult part of the study. A first batch of approximately 500 letters was sent to residences on a small group of exposed streets (Mackintosh Place and Ninian Road) in an effort to gauge the response from potential participants. In this first batch of letters a detailed information sheet *(refer to appendix 1)* providing full information about the study and specifying what would be required of people should they agree to take part was included. The response rate from this initial batch was quite low with less than 30 responses of which only 4 were eligible for participation. It was felt that perhaps too much information was being provided in the initial stages and that this might well be discouraging people from agreeing to take part. For this reason, the second batch of letters included only the introduction letter outlining the basic intentions of the study and the questions regarding the suitability of participants along with a post-paid envelope in which to return the letter once the questions had been answered. The responses to the second round of letters were more numerous with 70 responses from 500 letters although the number of people eligible to take part remained low.

Although the letters sent out provided no indication to potential participants as to the level of exposure to ambient air particulates, the nature of the study meant that very often it would be obvious to the potential participants which exposure group they belonged to and as a result it was noticed that it was much more difficult to recruit individuals from the unexposed areas than from the exposed areas. It is thought that the reason for this is that individuals in the exposed areas were more likely to perceive the traffic volumes in their areas to be a problem, both socially and with respect to their health and that as such these people would be more likely to respond positively to taking part in the study. Conversely those people living in the unexposed areas may not perceive traffic volume in their areas to be a problem and may therefore be less interested in taking part in the study. The difficulty in recruiting participants from the unexposed areas is outlined in the table below, where it is obvious that for a similar number of letters sent out, the response rate was lower in the unexposed group and subsequently the number of respondents that decided to take part in the study following discussions with a researcher was also lower in the unexposed group.

	Exposed	Unexposed
Number of Letters Sent	4043	4276
Total Response	250	189
Total Eligible Response	90	68
Agreed to Participate	70	51

Table 4.1: Breakdown of responses to recruitment letters sent out to residents living in areas of interest

Although recruitment was a continuous process carried out over three years the number of eligible persons willing to take part in the study did not reach the desired 75 for either group. Unfortunately time did not allow for recruitment to continue beyond March 2005 as there needed to be enough time to time to arrange interviews, prepare and send out information packs, go to interview the

participant, allow them time to attend the outpatients unit to provide the necessary blood samples and for the laboratories to analyse the samples.
The time constraints also meant that for some participants only a single interview, rather than the two originally planned, was possible.

4.1.2) Investigating the relationship between particle numbers in the areas assigned exposed and unexposed

The first step in the analysis was to describe and compare all the individual particulate data collected at the home of each individual and to try and identify any patterns in the individual measurements, both indoors and outdoors, in both the exposed or unexposed areas.
What can be seen from the graphs presented below (fig. 4.1-4.2), as is typical of the majority of measurements, is that in the exposed areas the outdoor levels are generally higher than the indoor levels, usually by ~2,000 particles/cm^3, but this is not always the case in the unexposed areas, where more often there is not such a difference between the indoor and outdoor measurements (fig. 4.3-4.4). Also evident from the graphs is that in the exposed areas the levels of particles outdoors fluctuate far more and to a much greater degree than do the levels of particles outdoors in the unexposed areas. The reason for this is that the P-trak is very sensitive and easily picks up the differing levels of particles generated by the passing vehicles and equally the levels quickly return to what must be assumed to be the normal background levels when there is no traffic. As the roads in the exposed areas have higher volumes of traffic than the roads in the unexposed areas, it seems natural that the levels of particles would fluctuate more in these areas. Also as the profile of the traffic using the roads in the unexposed areas is much different to that of the traffic using the roads in the unexposed areas this results in the much higher peaks in traffic levels, caused by such vehicles as articulated trucks, buses and vans, all of which appear to generate much higher numbers of ultrafine particles. Figures 4.1-4.6 below are a small sample of the type of results obtained, for a complete set of graphs refer to appendix 3.

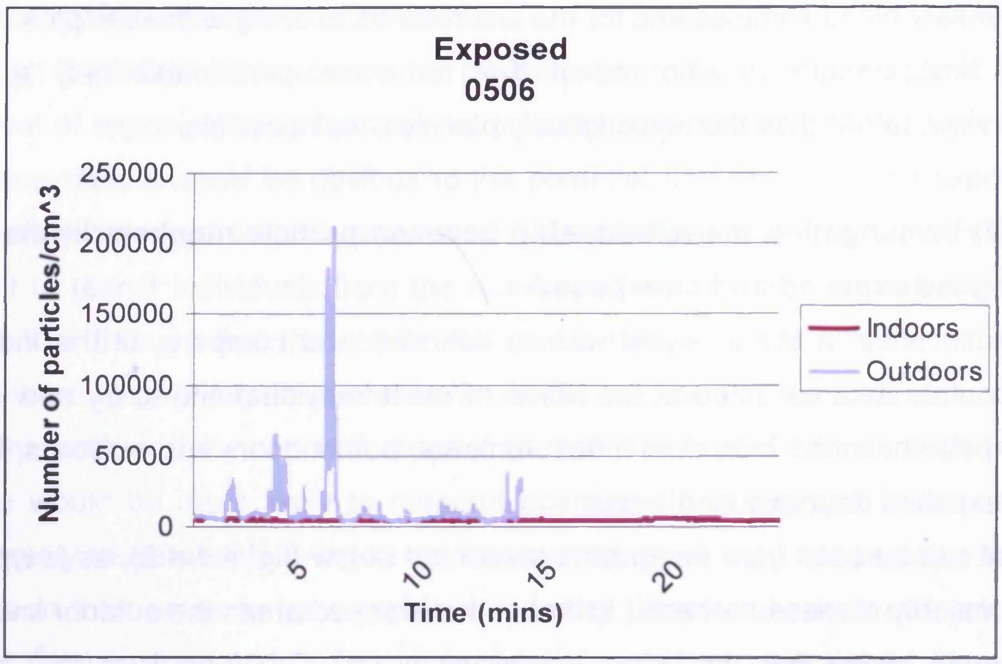


Figure 4.1

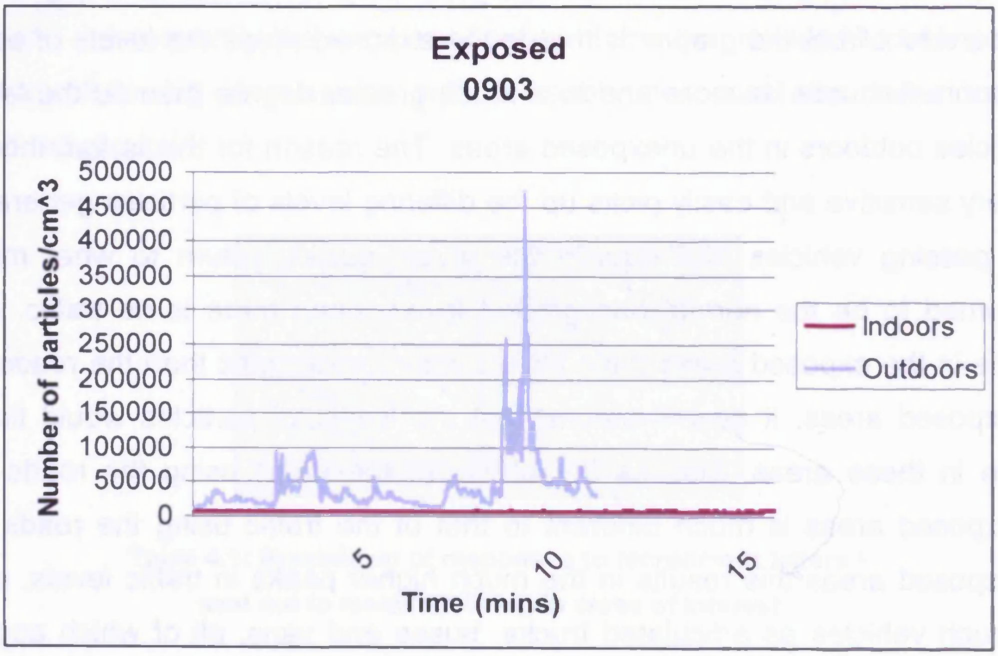


Figure 4.2

Figures 4.1-4.2: Examples of the typical pattern of particle numbers measured in both the exposed areas

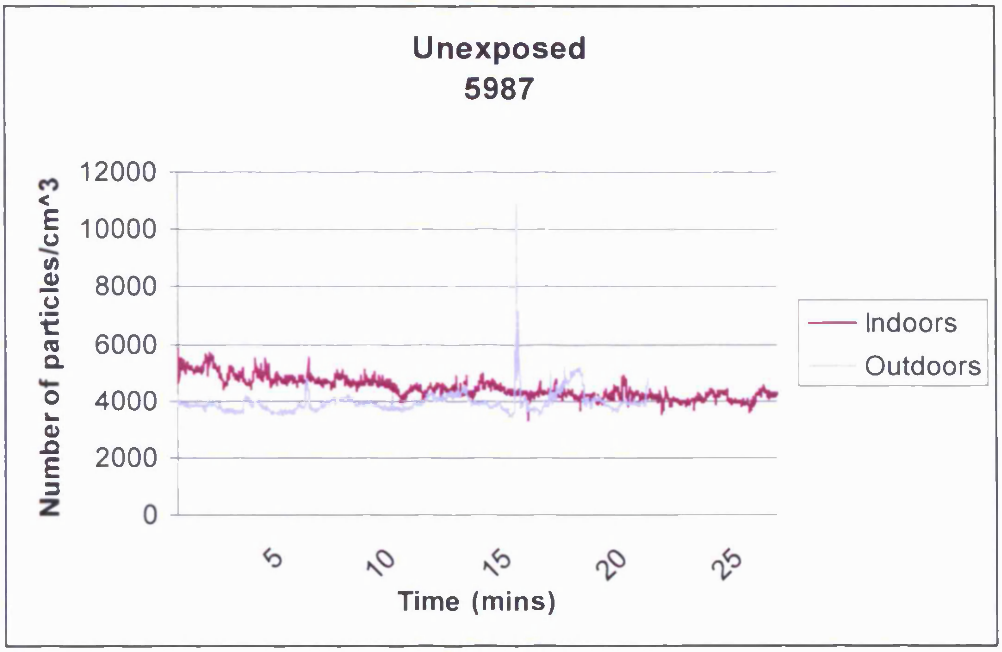


Figure 4.3

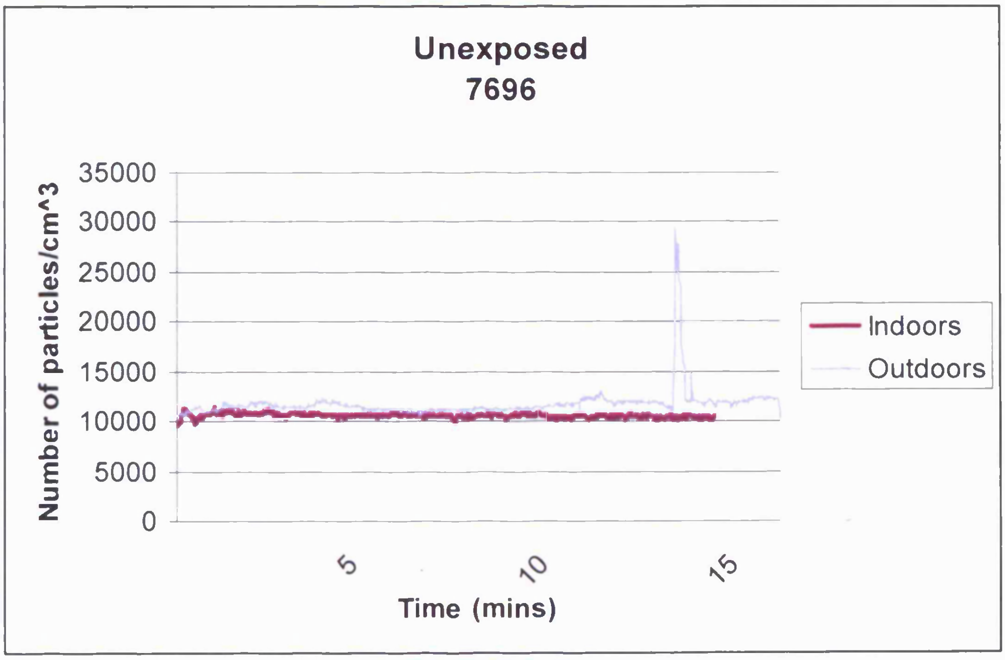


Figure 4.4

Figures 4.3-4.4: Examples of the typical pattern of particle numbers measured in both the unexposed areas

The patterns in particle numbers measured in the unexposed areas, although somewhat similar to those in the exposed areas, tended to show lower numbers of outdoor particles over all to the numbers of particles measured outdoors in the exposed areas. The numbers of particles measured indoors were similar in both groups, thus perhaps suggesting a lack of penetration of ultrafine particles from outdoors.

The most obvious difference between the patterns in the two groups however is the lack of large fluctuations in the numbers of particles measured outdoors in the unexposed areas, compared to the exposed areas. Much of the individual particle number data collected followed a similar pattern when graphed whereby the indoor levels remained relatively stable with no major fluctuations unlike the outdoor measurements which were highly susceptible to large fluctuations due to the sensitivity of the particle counter to sudden changes in particle number and it was noticed often during the course of the study that particle numbers could jump from a few thousand particles to hundreds of thousands of particles when vehicles passed.

Some of the particle number data, when graphed however, showed different patterns to those usually observed. For example figures 4.5 and 4.6 below show fluctuations in the number of indoor particles measured something that would more commonly be associated with the number of particles measured outdoors. In the same way that the P-trak is sensitive to the particles generated by passing traffic outdoors, it is also sensitive to the particles generated by various household tasks such as vacuuming and cooking, both of which can result in the large fluctuations in indoor measurements recorded and shown in the two graphs below.

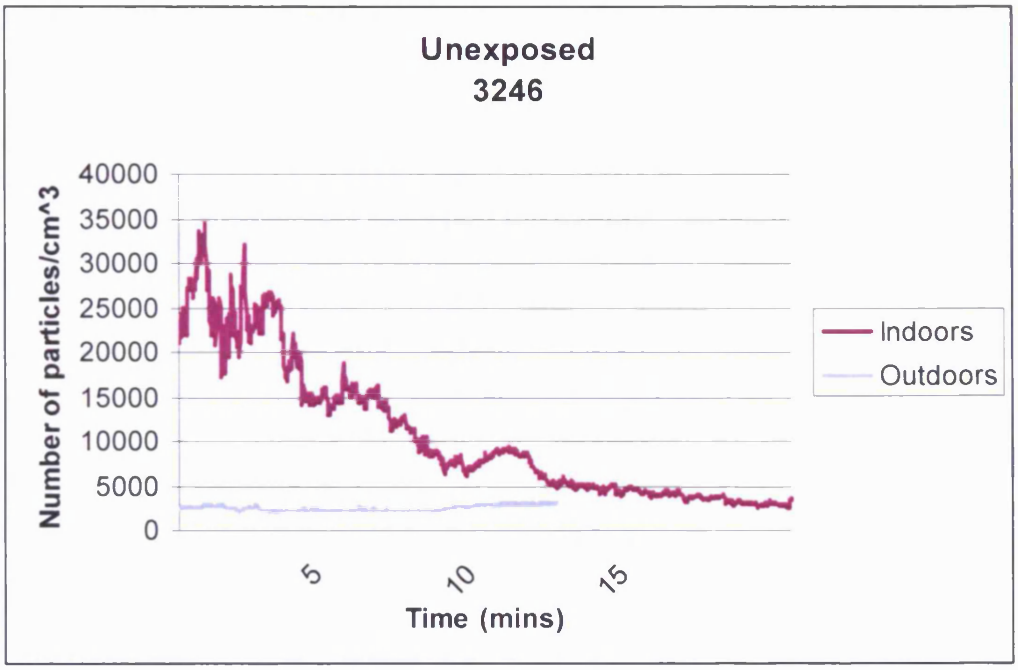


Figure 4.5

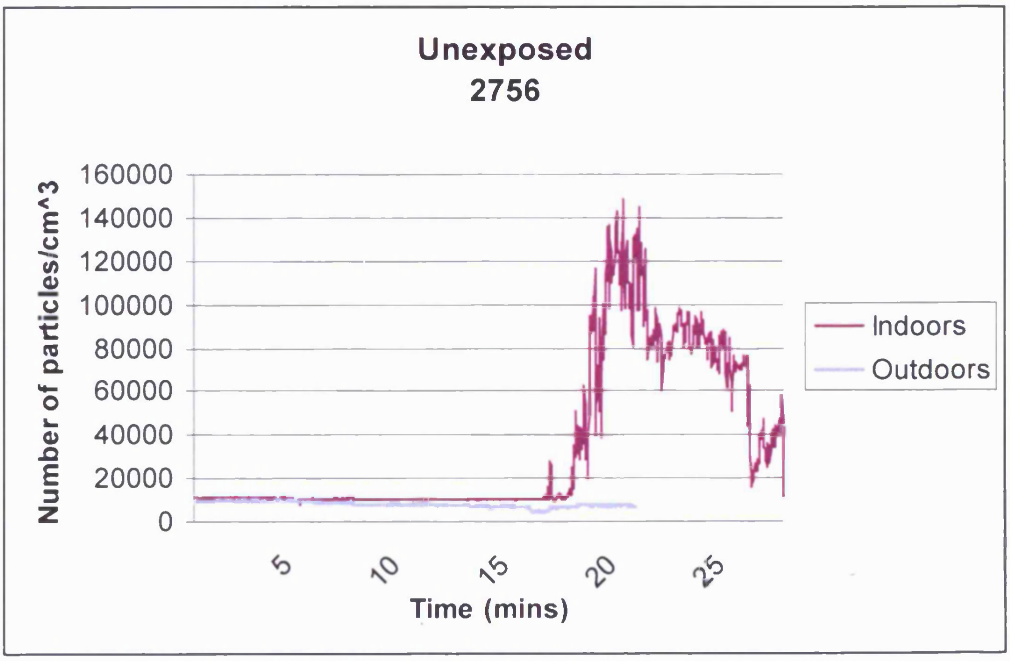


Figure 4.6

Figure 4.5-4.6: Examples of the less typical patterns of particle number measured during the course of the study

The difference in mean particle count between streets assigned as exposed and unexposed

Further examination of the particle data involved examining the difference between the particle numbers measured in the exposed area and unexposed area. This was achieved by comparing the mean particle number measured indoors and outdoors in both the exposed and unexposed areas.

	Indoors	Outdoors
Exposed	11703	20988
Uexposed	12133	10907

Table 4.2: Mean Particle Number/cm^3 for both exposed and unexposed areas

Street	Average Number of Particles/cm^3	Area	Street	Average Number of Particles/cm^3	Area
Penmark Green	2929	2	Denison Way	13098	2
Brython Drive	4504	2	Cowbridge Road West	13742	1
Spring Grove	4788	2	Forsythia Drive	14370	2
Gibson Close	5082	2	Lavender Grove	14385	2
Azalea Close	5411	2	Summer Wood Close	15199	2
Lon Y Fin	5562	2	Llandaff Road	15301	1
Lon Werdd	5702	2	Fieldfare Drive	15457	2
Clos Y Cwarra	6059	2	Tudor Street	16765	1
Deepfield Close	7377	2	Everswell Road	17010	2
Jasmine Drive	7671	2	Margeurites Way	17179	2
Pentwyn Terrace	7714	2	Western Avenue	17566	1
Bridge Road	7809	1	Hill Rise	18008	2
Vicarage Gardens	8498	2	Pencisely Road	18199	1
Nant Y Drope	8627	2	Pendwyallt Road	19616	1
Deepwood Close	8726	2	Mackintosh Place	19810	1
St Isan Road	8832	2	Sloper Road	20319	1
The Shires	9658	2	Hydrangea Close	20358	2
Crystal Wood Road	9717	2	Richmond Road	20663	1
Hollybush Heights	10211	2	Whitchurch Road	21769	1
Cradoc Close	10565	2	North Road	21909	1
Planet Street	10831	1	Cathedral Road	22133	1
Springwood	11356	2	Ty-Glas Road	22324	1
Wellfield Road	11400	2	Manor Way	26617	1
Park Road	11664	1	Cowbridge Road East	27856	1
Wavell Close	11885	2	St Gilda's Road	28424	2
Mallards Reach	12326	2	Ninian Road	29533	1
Moorland Road	12565	1	The Philog	30715	1
Crosswells Way	12737	2	Fidlas Road	30940	1

Table 4.3: Mean outdoor particle number for each street in the study (area 1 = exposed; area 2 = unexposed)

Table 4.2 outlines the mean number of particles/cm^3 measured in the exposed and unexposed areas. The means were calculated using the combined means for each street in the relevant group, the details of which are presented in table 4.3. The mean particle number/cm^3 for each street was calculated using all available measurements for each street.

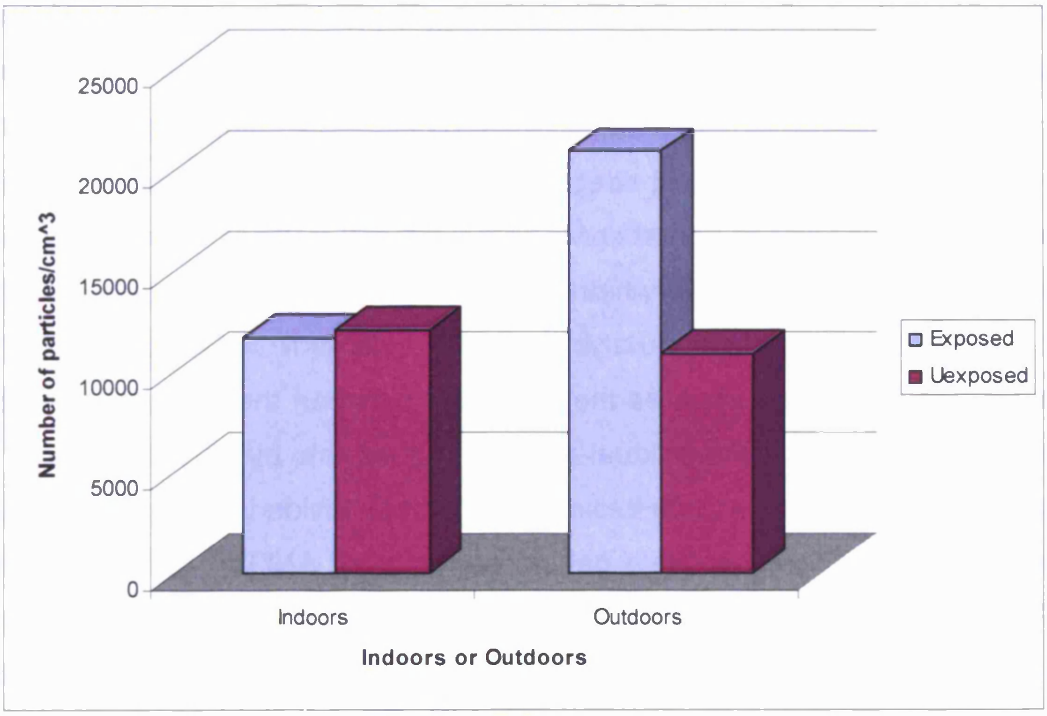


Figure 4.7: Comparison of the mean particle numbers both indoors and outdoors for exposed and unexposed areas

It is clear from the above graph that the mean numbers of particles measured outdoors in the exposed areas is much higher than the mean number measured outdoors in the unexposed areas, in fact the mean number of particles/cm^3 in the unexposed area was almost twice as high as in the unexposed. This is not particularly surprising however as one would expect that if traffic was indeed the main source of ultrafine particles, then areas with high volumes of traffic would be more likely to have higher levels of particulate pollution as a result. The mean number of indoor particles/cm^3 is much similar for both the exposed and unexposed areas. It was previously mentioned that the P-trak is an very sensitive particle counter that measures particles generated by various household activities. These household activities are not likely to be affected by location which means that there are similar sources of particles in the homes in both the exposed and unexposed areas. What is perhaps surprising is the seeming lack of penetration of particles from outside sources in the exposed areas. This may possibly be due to the fact that in the exposed areas participants noted that they keep windows and door shut rather a lot to block the noise from the passing traffic. In so doing they may well also be preventing particles from entering.

Potential for misclassification of streets at the outset

One of the concerns for the study was the lack of traffic data that could be used to help more accurately select streets to be included in each group, which meant that there was a possibility that some of the streets selected may be assigned to the wrong group. To check whether the streets chosen for each area were in fact correctly grouped an Analysis of Variance (ANOVA) was carried out. Analysis of Variance compares the variability between the different groups with the variability within the individual groups. It does this by calculating an F ratio which represents the variance between the groups divided by the variance within the groups. The basic rationale behind carrying out ANOVA tests is to test the null hypothesis that both population means (exposed and unexposed) are equal. A significant F test means that this null hypothesis could be rejected (i.e. that the exposed and unexposed groups differ significantly from each other).

Prior to commencing with the ANOVA however, it is important that the data is examined for any anomalies such as extreme outliers. The simplest way to do this is with a box plot, although there are also a variety of statistical tests which could also be used to do this. A box plot is a graphical representation of the data, with the box representing the portion of the distribution falling between the 25th and 75th percentiles (the lower and upper quartiles). The line across the middle of the box represents the median and the whiskers extend to the largest and smallest values not considered outliers or extreme values. An outlier is a value more than 1.5 box lengths above or below the box and will be flagged by SPSS, usually with a circle, with a number which identifies the outlying value. An extreme value is a value more than 3 box lengths above or below the box and again SPSS will flag these values with an asterisks along with an identifier. ANOVA should not be conducted on any data which has a number of outliers or extreme outliers unless they are removed from the data as extreme values can contribute to a mean value that is not truly representative of the actual distribution of the data. There are two approaches that can be taken should the box plots show any or many extreme outliers, one of which is to exclude these values from the analysis. The second approach and the approach of choice for this study was to compare the data in relation to the median rather than the mean (i.e. to use non-parametric methods), although the decision was taken to conduct comparison of means by way of ANOVA regardless of whether there were any extreme outliers both for the sake of completeness and to allow comparisons between the results using two different approaches.

From the box plot below it can be seen that although there is a single outlier in the unexposed group (value for the case was 28,424 particles/cm^3), there are no extreme values. It is therefore appropriate to continue with ANOVA in this situation.

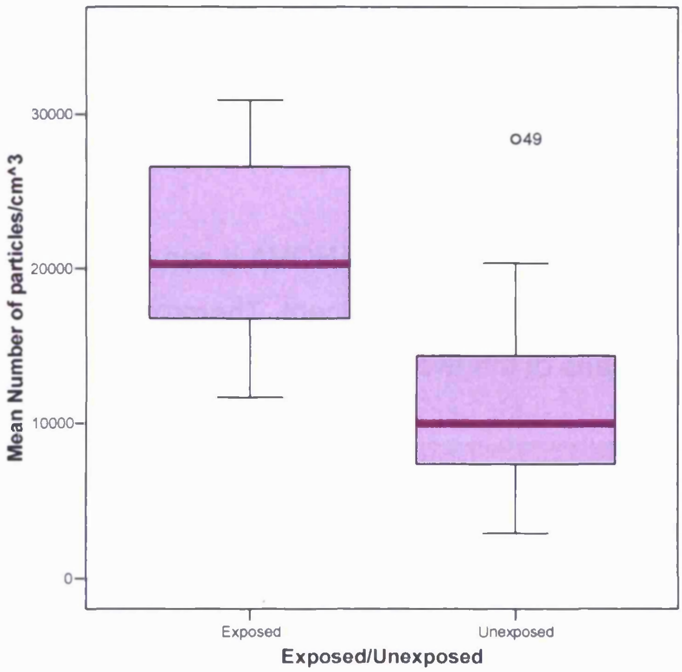


Figure 4.8: Box plot showing the differences between the mean outdoor particle number/cm^3 in the exposed and unexposed areas

Mean particle Concentration

Levene Statistic	df1	df2	Sig.
.647	1	50	.425

Table 4.4: Test of Homogeneity of Variances for the exposed and unexposed groups

The p value (Sig) for the levene statistic is 0.425 and therefore is not significant (significance $p<0.05$). This means that there is no evidence for heterogeneity of variance, in other words there is no significant variance about the means within either group.

ANOVA

Mean particle Concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1196078080.644	1	1196078080.644	38.024	.000
Within Groups	1572774634.029	50	31455492.681		
Total	2768852714.673	51			

Table 4.5: One Way ANOVA comparing the variances within and between the exposed and unexposed groups

In the summary table for the one-way ANOVA it can be seen that the p value for the F ratio is .0005 and therefore significant. Therefore the H_0; there is no difference in the means of the two groups; can be rejected.

Mann-Whitney U test is the non-parametric alternative to the t-test for independent samples but instead of comparing the means of the two groups the Mann-Whitney U Test compares the medians. It converts the scores on the continuous variable to ranks across the two groups and then evaluates whether the ranks for two groups differ significantly. Non parametric tests can be used in place of their parametric counterparts and are particularly useful for dealing with data with extreme outlying values rather than having to exclude them from the analysis altogether.

	Mean Number of particles/cm^3
Mann-Whitney U	59.000
Wilcoxon W	654.000
Z	-4.751
Asymp. Sig. (2-tailed)	.000

Table 4.6: Results of the Mann Whitney test for the exposed and unexposed group

The two important values in the Mann-Whitney U output are the Z value and the significance level (Asymp. Sig. (2-tailed)). The Z value is -4.751 and is highly significant (p=0.0005) which implies that there is a significant difference between the medians of the two groups, exposed and unexposed.

Although from the bar graph there did not appear to be a big difference between the mean numbers of particles/cm^3 measured indoors in the exposed and unexposed groups, ANOVA and Mann-whitney U tests were conducted to confirm this.

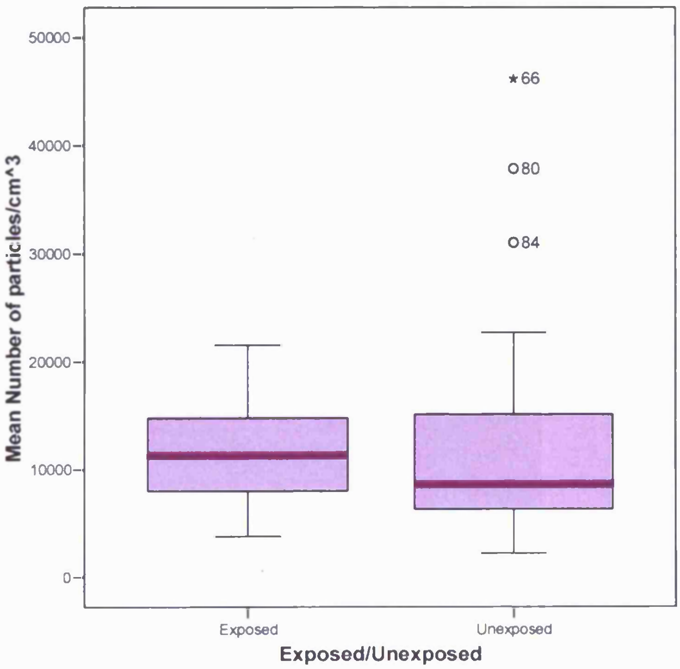


Fig 4.9 Box plot showing the differences in means between the numbers of particles measured indoors in the exposed and unexposed areas

Mean Particle Concentration (Indoors)

Levene Statistic	df1	df2	Sig.
3.297	1	49	.076

Table 4.7 Test of Homogeneity of Variances

Average Particle Concentration (Indoors)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2158349.740	1	2158349.740	.029	.865
Within Groups	3643868011.005	49	74364653.286		
Total	3646026360.745	50			

Table 4.8 Results of the one way ANOVA

The results of the homogeneity of variance test (p=0.076) and of the ANOVA (p=0.865) are not significant and therefore support the assumptions made from the bar graph that the mean number of particles/cm^3 measured indoors in the two groups do not differ significantly. The box plot however shows a number of outlying measurements in the unexposed group. One of these measurements is an extreme outlier (value for the outlying case was 46,175 particles/cm^3) and it is possible that this may affect the outcome of the ANOVA. Again, rather than excluding the data from the analysis, non parametric methods were used to compare the data according to the median rather than the mean.

	Mean Number of particles/cm^3
Mann-Whitney U	249.000
Wilcoxon W	810.000
Z	-.946
Asymp. Sig. (2-tailed)	.344

Table 4.9 Results of the Mann-Whitney U test comparing the mean indoor particle number of the exposed and unexposed groups

The result of the non-parametric Mann-Whitney U test is also not statistically significant (p=0.344) and so supports the ANOVA in terms of the implication that there is not a significant difference between the numbers of particles/cm^3 measured indoors in the exposed and unexposed group. It can therefore be stated that there is no statistically significant difference between the indoor particle counts in the exposed and unexposed groups with respect to either the mean or the median.

The same analysis has also been conducted to compare the differences between the mean indoor and outdoor particle numbers in the exposed group and the differences between the mean indoor and outdoor particle numbers in the unexposed group. As expected there was a significant difference between the mean number of particles indoors and the mean number of particles outdoors in the exposed group (p=0.0005) and there was no statistically significant difference between the mean numbers of particles measured indoors and outdoors in the unexposed area (p=0.532). (More detailed results of this analysis, including box plots can be found in appendix 4).

Investigating the relationship between assigned exposure and measured exposure

Another approach to analysing the data was to plot all the mean particle number measurements for each street against each other in an effort to see if the streets could be more suitably grouped into three or more groups based on the particle number data actually collected.

The first thing to notice when examining the graph below is that although for the most part there are two distinct groups with a exposed and unexposed streets grouped together for the most part, there are some streets where the actual measurements of particles made during the course of the study indicate that the streets may not be in the correct group and could lead to inaccurate results when examining the data with respect to the blood samples. For the streets originally classified as exposed, none of the streets appear to be significantly different from each other with respect to their mean number of outdoor particles but for the streets originally classed as unexposed, there appear to be a number that may have mean particles numbers that are higher than would have been expected. Overall this graph indicates that there may be three or more groups of streets rather than just two and so an attempt was made to split the streets into three groups and reanalyse the data.

	Outdoors
Group 1	7244
Group 2	13483
Group 3	22707

Table 4.10: Mean number of particles/cm^3 for each group

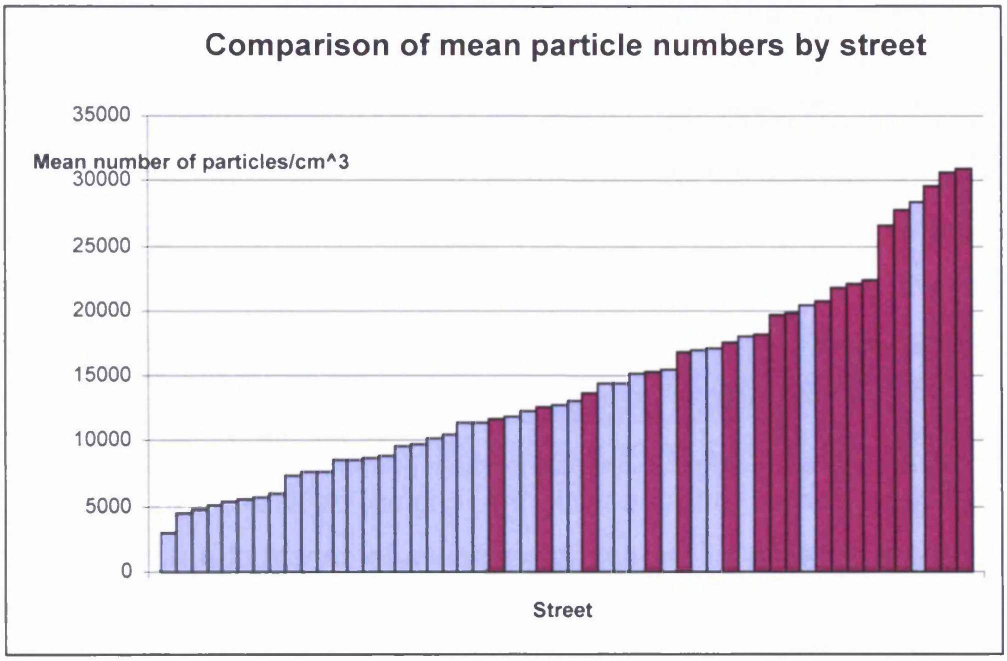


Figure 4.10: Mean number of particles measured outdoors for each individual street in the study – exposed (dark purple) and unexposed (light purple)

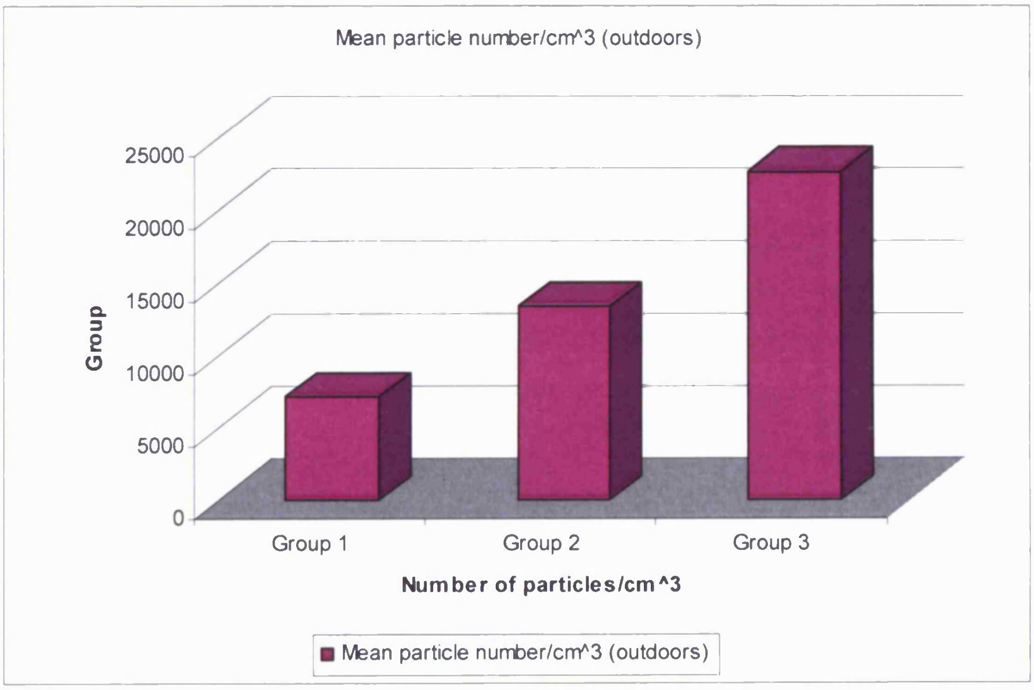


Figure 4.11: Mean number of particles/cm^3 by group

Although the first obvious difference in the particle number measurements occurs at approximately 7,000 particle/cm^3 it was felt that a better upper limit for the first group would be 11,000 particles/cm^3 where another obvious increase in mean particle numbers measured and it is at this number of particles/cm^3 where the first similarities in mean particle number measurements between the exposed and unexposed streets start to appear. The second group included all streets with mean particle number/cm^3 between 11,001 and 17,000 particles and the third group consists of all streets with mean particle numbers/cm^3 of between 17,001 and 50,000 particles.

Using SPSS the streets were recoded according to that criteria and one-way ANOVA was carried out. As with the original ANOVA, the first step when comparing the means of the new groups was to construct a box plot in order to investigate whether there were any serious anomalies such as extreme outliers that would need to be excluded from the analysis. It can be seen from the box plot below that there are no serious anomalies in the data and so the one way ANOVA can be carried out without any changes to the data.

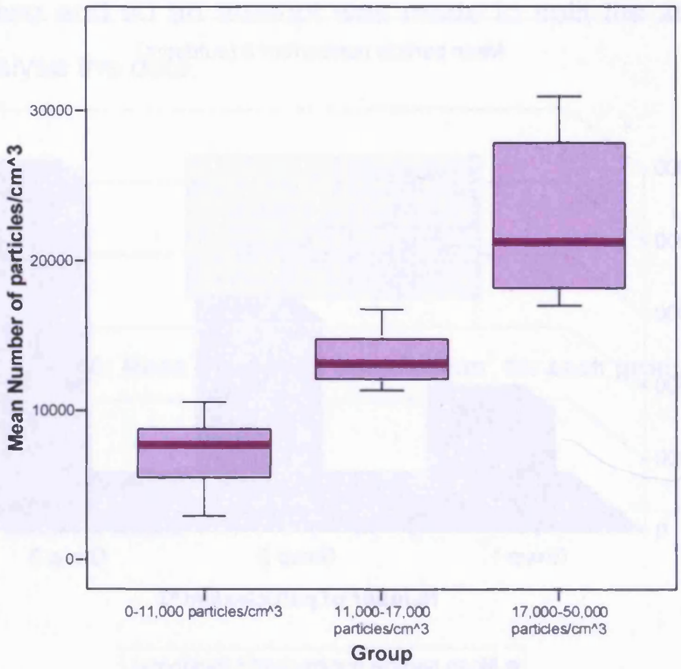


Figure 4.12: Boxplot showing the differences between the three groups defined according to the mean number of particles measured

From the box plot it is easy to see that the levene statistics is likely to be significant, evidenced by the fact that there are clear differences between the groups. For example the spread in group two is much tighter than the spread in group three. There is however no doubt that the medians are different for all three groups which suggests that non parametric tests will need to be used.

Mean particle Concentration

Levene Statistic	df1	df2	Sig.
15.006	2	49	.000

Table 4.11: Test of Homogeneity of Variances for the three groups

Mean particle Concentration

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2227617540.813	2	1113808770.407	100.837	.000
Within Groups	541235173.860	49	11045615.793		
Total	2768852714.673	51			

Table 4.12: Results of the one-way ANOVA for the three groups

In this case the p value (Sig) in the Levene Statistic is less than 0.05 (p=0.0005) and is therefore significant. When the variances in the dependent variables are not equal across the groups the results of the ANOVA table are dubious, even though the results of the ANOVA are highly significant. In this case one should refer to the Robust Tests of Equality of Means table. The two tests used here are the Brown Forsythe and the Welch test, alternatives to the F test and preferable in the case of a significant result in the Levene Statistic.

Welch	90.089	2	30.845	.000
Brown-Forsythe	105.905	2	28.135	.000

Table 4.13: Robust Tests of Equality of Means to substantiate the results of the one-way ANOVA

The significance level for these two tests indicates the significance of the F test. In this case both results are highly significant (p=0.0005) for both which supports the significant F test result.

The results of the ANOVA support the evidence in the box plot that the three groups differ significantly but it is not possible to tell where the differences occur. For this post-hoc tests, in this case Tukey's HSD test, are needed. Tukey's HSD systematically compares pairs of groups and indicates whether there is a significant difference in the means of each pair.

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0-11,000 particles/cm^3	11,000-17,000 particles/cm^3	-6239.491(*)	1147.921	.000	-9013.93	-3465.06
	17,000-50,000 particles/cm^3	-15462.825(*)	1093.158	.000	-18104.90	-12820.75
11,000-17,000 particles/cm^3	0-11,000 particles/cm^3	6239.491(*)	1147.921	.000	3465.06	9013.93
	17,000-50,000 particles/cm^3	-9223.333(*)	1161.903	.000	-12031.56	-6415.10
17,000-50,000 particles/cm^3	0-11,000 particles/cm^3	15462.825(*)	1093.158	.000	12820.75	18104.90
	11,000-17,000 particles/cm^3	9223.333(*)	1161.903	.000	6415.10	12031.56

* The mean difference is significant at the .05 level.

Table 4.14: Results of Tukey's honest significant difference comparing the three groups

Group	N	Subset for alpha = .05		
		1	2	3
0-11,000 particles/cm^3	19	7243.84		
11,000-17,000 particles/cm^3	15		13483.33	
17,000-50,000 particles/cm^3	18			22706.67
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 17.157.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 4.15 Homogenous subsets from Tukey's test

Looking down the table marked 'mean difference' an asterisk next to the value indicates that the difference between the two groups being compared is significant at the 95% level ($p<0.05$). In the case of the three groups all groups were highly statistically significantly different from each other ($p=0.0005$).

Homogenous sub-groups are defined for all post-hoc procedures and presented in the Homogenous Subsets table of the output. In this case Tukey's HSD was the only post-hoc test performed. In the homogenous subsets table for the particle data, three sub-groups are defined indicating that the splitting of the streets according to a range of mean particle numbers was appropriate.

In the event that the result of the Brown-Forsythe and Welch tests were not significant then non-parametric tests should be considered. Non parametric tests assume neither homogeneity nor a normal distribution and are therefore suitable to use when analysing highly skewed data as well as data with a marked heterogeneity of variance. In the case of this data, given that from the box plot it can be seen that there are obvious differences in the medians for each of the three groups it was considered appropriate to conduct non-parametric tests on the data, the results of which would support those obtained using the parametric tests. Non-parametric tests are also less powerful than parametric tests and obtaining a significant result when using a non-parametric test implies that a significant result is likely to be obtained for any test chosen. The Kruskal-Wallis test is the non-parametric alternative to a one-way ANOVA, and was used here to compare the three groups listed above.

	Mean Number of particles/cm^3
Chi-Square	45.189
df	2
Asymp. Sig.	.000

Table 4.16 Results of the Kruskal-Wallis test for differences between the three groups

The results of the Kruskal-Wallis test indicate that the p value associated with a Chi-square value of 45.189 is less than 0.01 and is therefore significant at the 1% level. Since the p value is much smaller than 0.01 (p=0.005), the Kruskal-Wallis test agrees with the parametric test that there is a statistically significant difference between the three groups. What is does not show is whether this where this difference occurs and whether there is a difference between individual pairs of groups or just the group as a whole. It is reasonably obvious from the box plot that the three groups do differ from each other however in order to show investigate whether the difference is significant further analysis is required.

Unlike the Tukey's HSD test in the ANOVA output there is no equivalent post-hoc test to provide information about where the differences occur when using Kruskall-Wallis. In this case Mann-Whitney tests should be used and each group much be compared with the other two in separate analyses.

Groups 1&2

	Mean Number of particles/cm^3
Mann-Whitney U	.000
Wilcoxon W	190.000
Z	-4.943
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000(a)

a Not corrected for ties.

Groups 2&3

	Mean Number of particles/cm^3
Mann-Whitney U	.000
Wilcoxon W	120.000
Z	-4.881
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000(a)

a Not corrected for ties.

Groups 1&3

	Mean Number of particles/cm^3
Mann-Whitney U	.000
Wilcoxon W	190.000
Z	-5.196
Asymp. Sig. (2-tailed)	.000
Exact Sig. [2*(1-tailed Sig.)]	.000(a)

a Not corrected for ties.

Tables 4.17-4.19: Results of the Mann Whitney tests for differences between groups

The tables above show the results of the non-parametric Mann-Whitney tests comparing groups 1&2, 2&3 and 1&3 respectively. The results of the Mann-Whitney tests further support the results in the Tukey's HSD test, with highly significant differences between each of the three pairs of groups (p=0.0005 for all three tests).

If exposure to particulate pollution from motor traffic does indeed cause an effect in relation to the various biomarkers under investigation, then it would be expected that as the particle numbers increase, so too would the effects on the biomarkers. Evidence of causality is strengthened if a dose response relationship can be demonstrated and given that a statistically significant difference between the three exposure groups was observed, it seemed appropriate to investigate the possible presence of such a dose-response relationship with respect to the biomarkers.

4.1.3) Investigating the relationship between particle number and traffic flow

The first step was to construct a table, which contained the street name and available traffic and particle number data. The available traffic data included counts made in the morning and evening and a 12 hour measurement calculated using the morning and evening counts. The pollution data available were mean indoor and outdoor particle counts calculated from the measurements made on the relevant streets during the course of the study. The traffic data provided by Cardiff Transport authority was an AM count and a PM count. A formula that could be applied to the AM and PM measurements to factor them up to a 12 hour count was also provided.

The formula for this is ***mean(AM+PM)*10*** and was applied to the AM and PM counts to give the 12 hour mean traffic count.

Street	AM	PM	12 Hour	Indoor	Outdoor
North Road	2350	2541	48910		21909
Cowbridge Road West	2557	2274	48310	16661	13742
Fidlas Road	1910	1984	38940	13188	30940
Ty Glas Road	1330	1394	27240	8416	22324
Bridge Road	1127	1402	25290		7809
The Philog	1107	1271	23780	21558	30715
Sloper Road	1118	1205	23230		20319
Cowbridge Road East	1206	1032	22380	19163	27856
Pendwyallt Road	1014	1098	21120	8211	19616
Llandaff Road	973	970	19430	14791	15301
Moorland Road	1115	572	16870	7195	12565
Ninian Road	579	900	14790	19045	29533
Mackintosh Place	731	619	13500	9555	19810
Planet Street	398	426	8240		10831

AM=traffic measured at morning rush hour (8-9am)
PM=traffic measured at evening rush hour (4.30-5.30pm)
12 Hour=12 hour trsffic count (Mean(AM+PM)*10
Indoor=mean number of particles measured indoors for each street
Outdoor=mean number of particles measured outdoors for each street

Table 4.20: Traffic counts and mean particle number measurements both indoors and outdoors for all streets

There are four extra streets for which traffic data was available but from where no participants could be obtained. These four streets are therefore not included in the previous analysis although it was felt that it was important to collect outdoor particle counts at these locations so that they could be included in this correlation analysis. The four extra streets are; Sloper Road, North Road, Planet Street and Bridge Road.

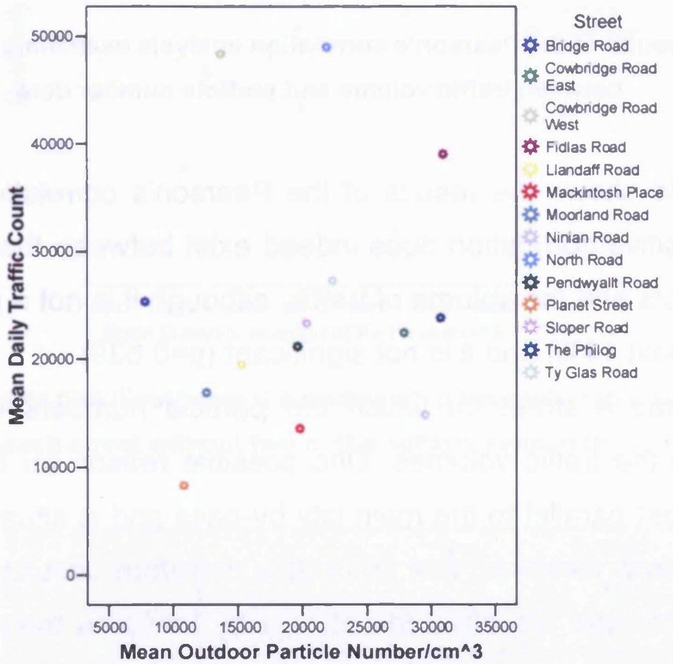


Figure 4.13: Scatter plot illustrating the relationship between traffic count and particle number data for each street

A scatter plot of the mean particle number outdoors against the volume of traffic for the same street shows what appears to be a broadly positive association, with the mean numbers of particles appearing to be higher on the streets where the volume of traffic is higher. This is true for almost all streets other than North Road and Cowbridge Road West, where the mean numbers of particles seem low by comparison to the traffic volume. There could be a number of reasons for this but the most likely explanation is that more measurements of the particle numbers need to be made to give more reflective means.

As there appears to be a potentially positive association between the volume of traffic and the mean number of particles measured outdoors, it seemed appropriate to proceed with more detailed correlation analysis.

		Mean daily traffic count	Mean outdoor particle number/cm^3
Mean daily traffic count	Pearson Correlation	1.000	.137
	Sig. (2-tailed)	.	.639
	N	15	14
Mean outdoor particle number/cm^3	Pearson Correlation	.137	1.000
	Sig. (2-tailed)	.639	.
	N	14	14

Table 4.21: Results of the Pearson's correlation analysis examining the correlations between traffic volume and particle number data

The above table shows the results of the Pearson's correlation test. It can be seen that a positive correlation does indeed exist between the mean number of particles outdoors and the volume of traffic, although it is not a particularly strong correlation (almost 14%) and it is not significant (p=0.639).

Ninian Road was a street for which the particle numbers were higher than expected given the traffic volumes. One possible reason for this is that Ninian Road runs almost parallel to the main city by-pass and is situated less than 500 metres as the crow flies from this route. It is therefore possible that the particle counts on Ninian road are affected not only by traffic on the street but also by traffic using the by-pass. For this reason the correlation analysis was rerun excluding the data from Ninian road.

As already mentioned the two most extreme outliers are North Road and Cowbridge Road West. Unlike the Ninian Road there was no logical explanation for the unexpectedly low particle counts recorded on these to streets and so no reason to exclude them from the analysis.

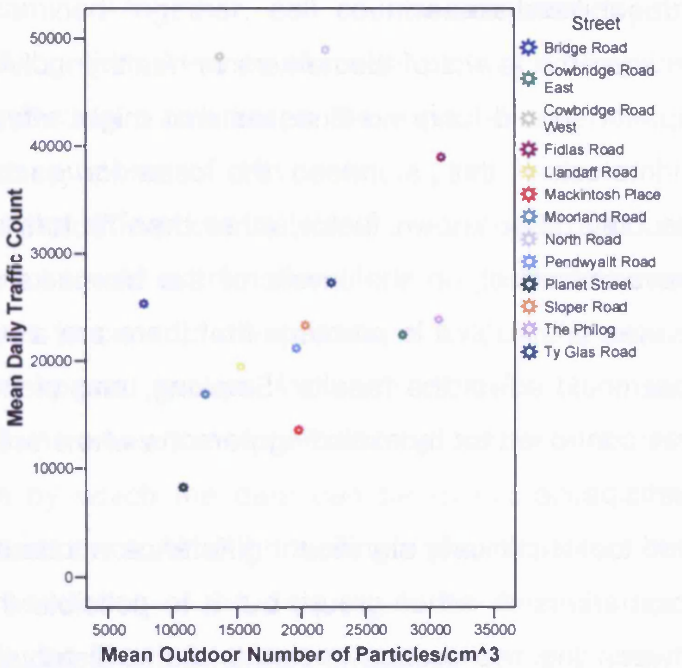


Figure 4.14: Scatter plot illustrating the relationship between traffic count and particle number data for each street without two of the outliers seen in the previous scatter plot

		Daily	Outdoor
Daily	Pearson Correlation	1	.246
	Sig. (2-tailed)		.417
	N	15	13
Outdoor	Pearson Correlation	.246	1
	Sig. (2-tailed)	.417	
	N	13	13

Table 4.22: Results of the Pearson's correlation analysis examining the correlations between traffic volume and particle number data minus two outliers

Excluding Ninian Road from the correlation analysis did in fact increase the correlation to almost 25% although this correlation still did not reach statistical significance (p=0.417).

4.1.4) Investigating the relationship between biomarkers of effect in the exposed and unexposed areas

This study examined the levels of biomarkers in healthy individuals; this means that the participants should have no illnesses that might affect the level of the biomarkers of interest. If this is indeed the case for each participant then theoretically the only other known factor, other than factors such as smoking, which could have an effect on the levels of the biomarkers, is the level of pollution in the area, although it is probable that there are a variety of unknown confounders that could affect the results. Smoking, one of the primary known confounders was controlled for by excluding persons who smoked or lived with a smoker from participation.

It is not expected that a clinically significant difference will be observed between the levels of biomarkers in either group, but it is possible that there may be differences between the two groups in terms of the distribution of the results within the normal range for the biomarker. For example, the normal range in the Haematology laboratories at the Heath hospital is defined using the results of 100 'normal' patients from which the mean values are calculated and the normal range is then defined as +/- two standard deviations and although the levels of the biomarkers in the exposed group may not necessarily be outside the normal range there is a possibility that chronic or prolonged exposure to particulate pollution could increase the levels of the biomarkers towards the top end of the normal range. The results from the two groups (exposed and unexposed) will all be examined in relation to the normal range for the specific biomarker of effect in an effort to examine whether the results in either group do differ from each other within the bounds of the normal range. Initially the intention was to compare the results obtained from first and second visits separately, however the lack of results from second visits meant this was not practical, especially as the majority of the blood samples obtained at second visits came from the exposed areas and as such the number of unexposed results available would limit the analysis that could be conducted and subsequently limit the strength of any conclusions that could be drawn. For this reason the decision was taken that for any participant for which two sets of blood results existed, they would be averaged and a single result for each biomarker would be analysed.

Each of the biomarkers was grouped according to type, i.e. all coagulation factors were examined together, cell counts examined together and oxidant factors examined together. A series of scatter plots and box plots, comparing the levels of each individual biomarker in the exposed and unexposed areas was constructed. The purpose of the scatter plots was to provide a visual representation of the differences between the exposed and unexposed groups while the box plots allowed examination of the range of measurements.

The next step was to conduct comparison of the means analysis between the exposed and unexposed groups for each individual biomarker. To conduct comparison of the means the data must be normally distributed. There are several methods by which the data can be examined to check whether it is normally distributed or not. Although there are statistical tests, it was thought that a graphical representation of the data would be the most appropriate and so histograms displaying the normal curve were constructed and it was found that for most of the biomarkers the data were not normally distributed and so apart from ANOVA, non-parametric tests were used in the analyses.

Table 4.23 outlines the different biomarkers examined and the number of results available in both the unexposed and exposed groups for each one. The minimum, maximum and mean measurements and the standard deviation is also presented. As previously mentioned, low response rates meant that only a small number of blood results were available for each biomarker. The problem of low response rates was compounded by problems in the laboratories which meant that some samples were not analysed correctly or were lost; this is particularly true with respect to thrombin clotting time, prothrombin time and activated partial thromboplastin time, where only 13 samples could be obtained in the unexposed group.

	Exposed					Unexposed				
	N	Minimum	Maximum	Mean	Standard Deviation	N	Minimum	Maximum	Mean	Standard Deviation
Thrombin Clotting Time (Seconds)	34	10.4	13	11.64	0.62	13	10.3	12.6	11.44	0.76
Prothrombin Time (Seconds)	34	11.45	15.4	12.83	1.02	13	11.3	12.9	11.94	0.44
Activated Partial Thromboplastin Time (Seconds)	34	22.3	29.45	26.3	2.093	13	24.5	31	27.53	1.93
Fibrinogen (mg/l)	49	1.8	4	2.92	0.56	36	1.9	5.7	2.96	0.65
TBARS Conc (nmols/l)	51	0.21	1.53	0.84	0.32	33	0.17	1.4	0.74	0.27
Protein Carbonyl Conc (nmols/mg)	52	0.34	2.21	0.79	0.29	35	0.46	2.04	0.98	0.42
Reticulocyte Count ($x10^9/l$)	48	17	151	51.53	27.26	38	15	87	47.68	17.58
Reticulocyte (%)	48	0.4	3.6	1.04	0.58	38	0.3	1.9	0.96	0.34
C Reactive Protein (mg/l)	46	1	9	2.95	1.63	32	1	42	4.43	7.33
White Blood Cell Count	50	4.08	11.25	6.28	1.52	38	4.26	10.49	6.56	1.37
Neutrophils Count	50	1.93	7.22	3.52	1.19	38	2.21	6.04	3.85	1.06
Platelets Count	50	72	448	240.26	60.18	38	147	466	257.63	65.91

Table 4.23: Basic descriptive statistics for each biomarker of effect separated by group

Coagulation factors

In the first place, all the coagulation factors (other than platelets) were examined together. The normal ranges for the coagulation biomarkers are; thrombin clotting time is 9.5-14 seconds, prothrombin clotting time is 11-13.5, activated partial thromboplastin time is 23-33 seconds and for fibrinogen the normal range is 2-4 mg/l.

On examining the scatter plots it is obvious that apart from prothrombin time, none of the samples, exposed or unexposed are outside of their normal ranges, with the exception of a small number of outliers, most noticeable of which the fibrinogen value of 5.7g/l.

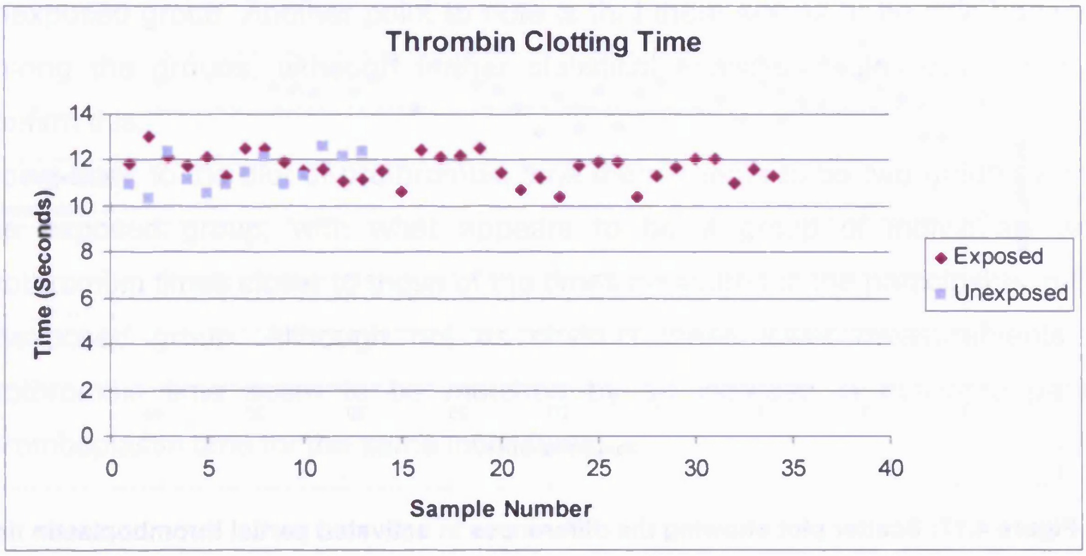


Figure 4.15: Scatter plot showing the differences in thrombin clotting time between the exposed and unexposed groups

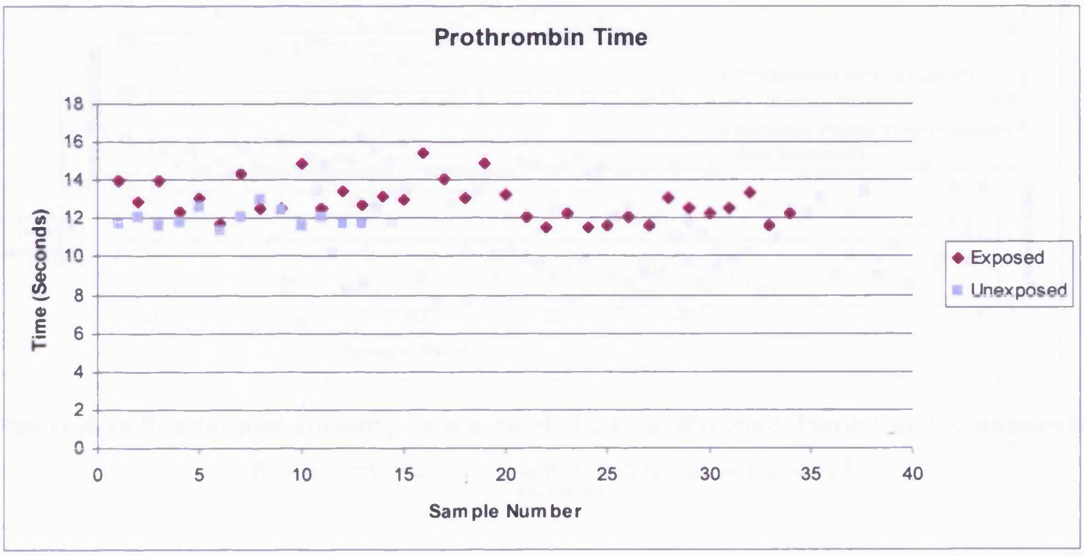


Figure 4.16: Scatter plot showing the differences in prothrombin time between the exposed and unexposed groups

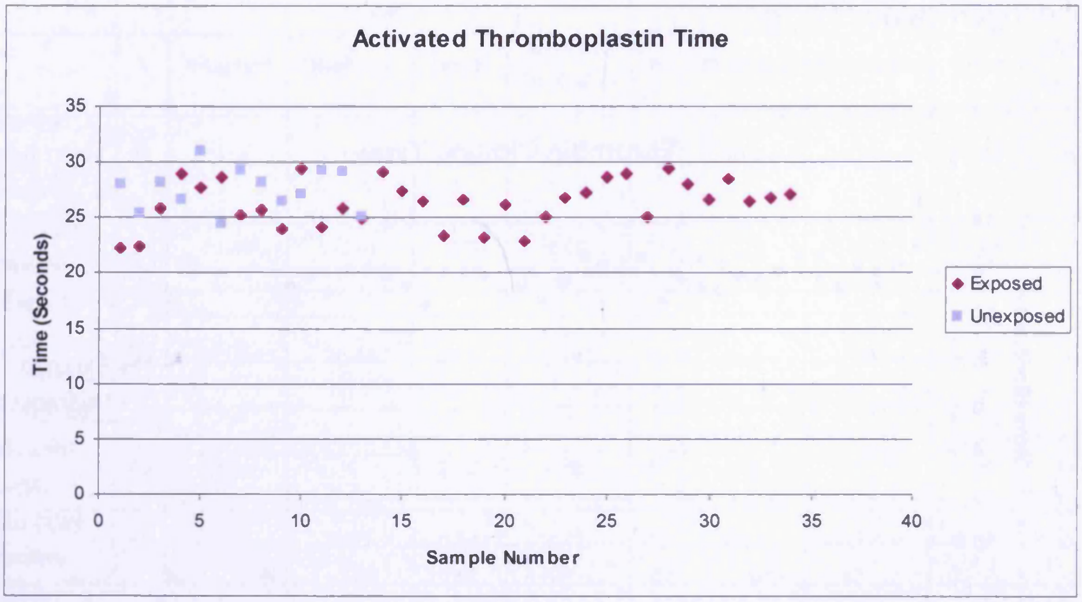


Figure 4.17: Scatter plot showing the differences in activated partial thromboplastin time between the exposed and unexposed groups

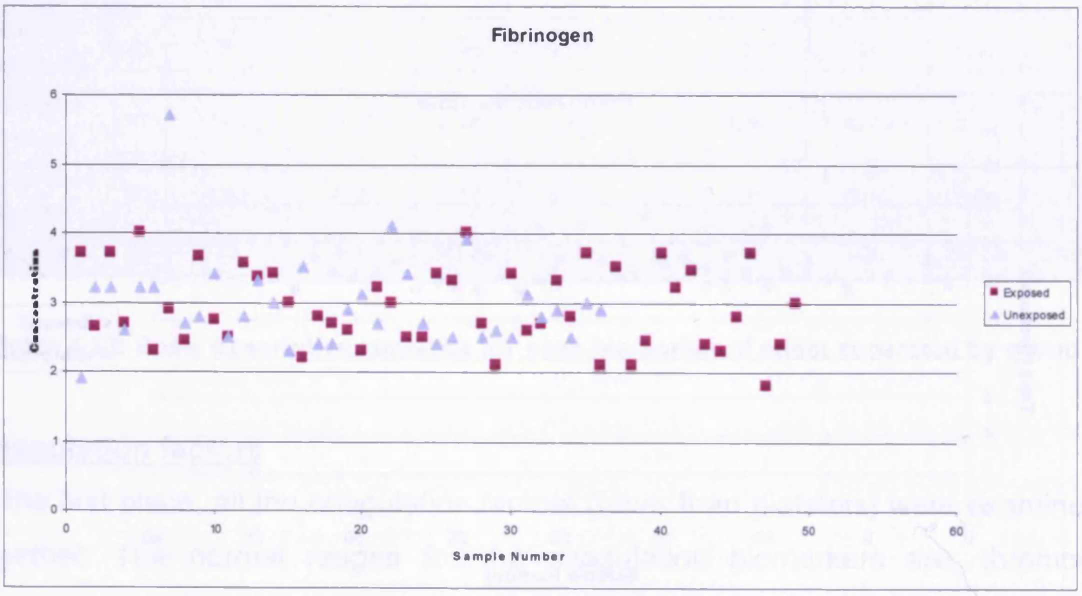


Figure 4.18: Scatter plot showing the differences in fibrinogen concentration in grams per litre (g/l) between the exposed and unexposed groups

There appears to be little difference between the unexposed group and exposed group for any biomarker apart from prothrombin time where the times recorded in the exposed group appears to be higher overall than those recorded in the unexposed group. Another point to note is that there seems to be little variance among the groups, although further statistical analysis would be needed to confirm this.

Going back to the plot of prothrombin time there seems to be two groups within the exposed group, with what appears to be a group of individuals with prothrombin times closer to those of the times measured in the participants in the unexposed group. Although not as obvious these lower measurements of prothrombin time seem to be matched by an increase in activated partial thromboplastin time for the same individuals.

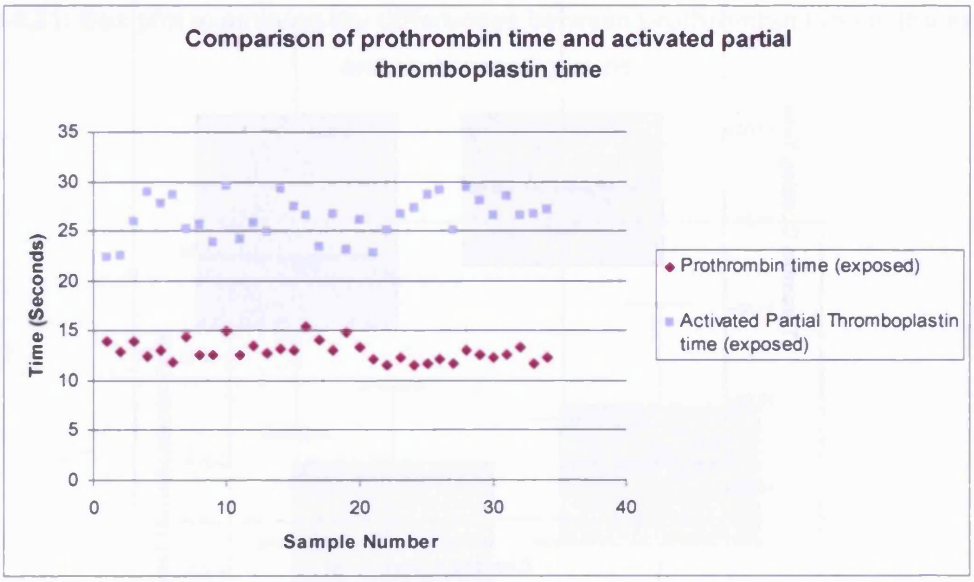


Figure 4.19 Scatter plot showing how activated partial thromboplastin times compare to prothrombin clotting times in the same person

Broadly speaking the activated partial thromboplastin times appear to decrease as the prothrombin times increase. Although correlation analysis between prothrombin time and activated partial thromboplastin time did show a negative correlation, the result was not statistically significant (p=0.200).

		Prothrombin Time	Activated Partial Thromboplastin Time
Prothrombin Time	Pearson Correlation	1	-.190
	Sig. (2-tailed)		.200
	N	47	47
Activated Partial Thromboplastin Time	Pearson Correlation	-.190	1
	Sig. (2-tailed)	.200	
	N	47	47

Table 4.24 Results of the correlation analysis between protrombin and activated partial thromboplastin time

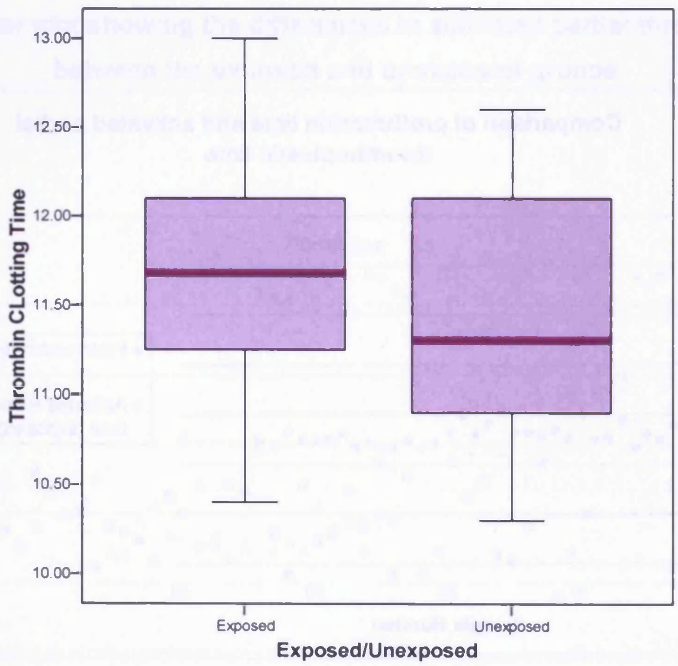


Figure 4.20: Box plot comparing the differences in thrombin clotting time for the exposed and unexposed groups

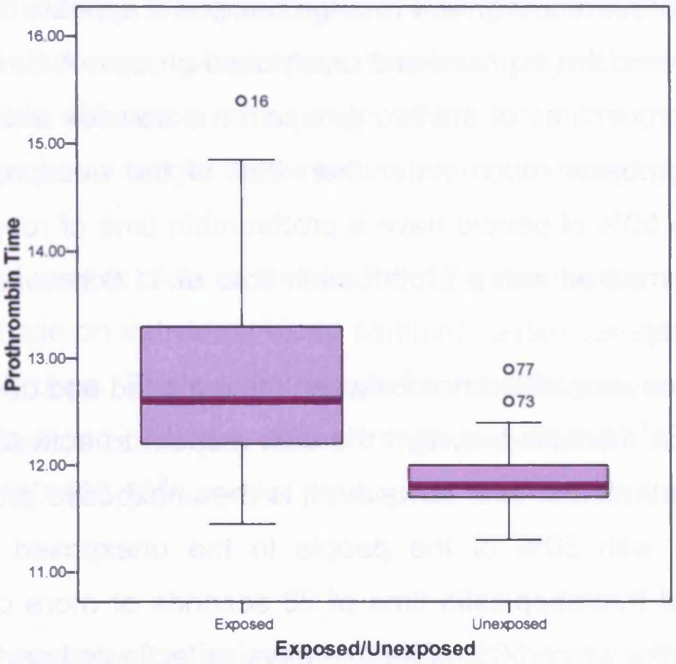


Figure 4.21: Box plot examining the differences between prothrombin time in the exposed and unexposed groups

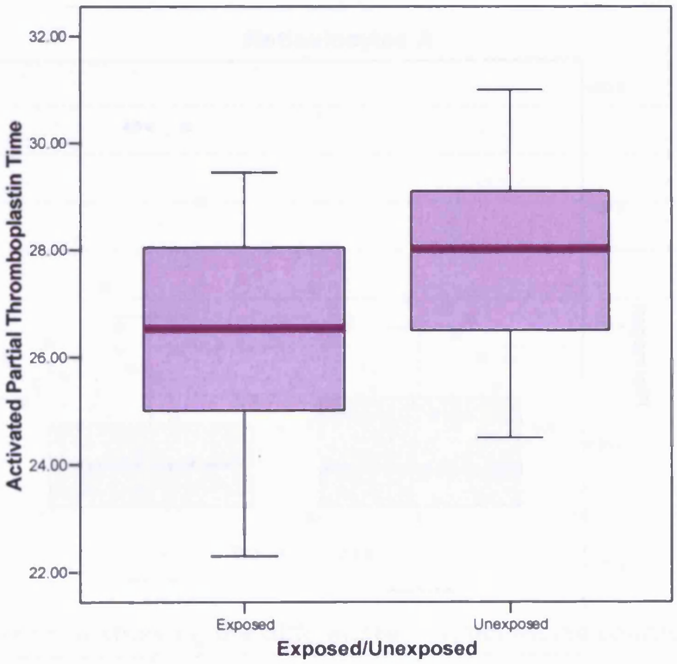


Figure 4.22: Box plot examining the differences between activated partial thromboplastin time in the exposed and unexposed groups

At first glance the biomarker most likely to show an effect between the groups appears to be prothrombin time. From the box plot it appears that there is a large difference between the exposed and unexposed groups. A clear difference in the median prothrombin time of the two groups for is obvious and the spread within the exposed group is much wider than that of the unexposed group. In the exposed group 50% of people have a prothrombin time of roughly 12.6 seconds or above as compared with a prothrombin time of 11.8 seconds or longer in the unexposed group.

The one other obvious difference between the exposed and unexposed groups is the difference in medians between the with respect to activated thromboplastin time. Unlike prothrombin time however, it is the unexposed group which has the higher median with 50% of the people in the unexposed group having an activated partial thromboplastin time of 28 seconds or more compared with the exposed group for which 50% of people have an activated partial thromboplastin time of approximately 26.5 seconds or more. Given how the individual measurements appeared to mirror each other in the scatter plots this difference between the groups is not entirely unexpected and may be something worth investigating further.

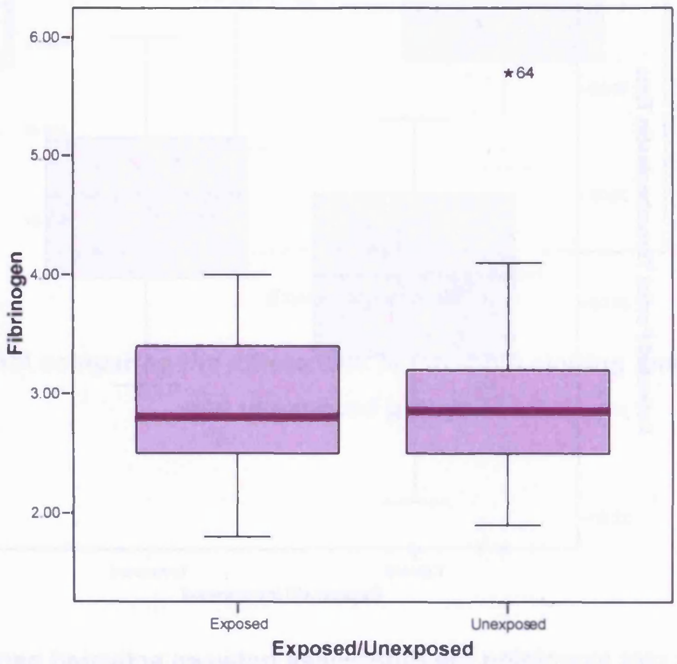


Figure 4.23: Box plot showing the differences in fibrinogen concentrations for the exposed and unexposed groups

With respect to fibrinogen concentration, little difference appears to exist between the exposed and unexposed group. Both groups have virtually identical medians and with the exception of one extreme outlying value in the unexposed group, there is little difference in the distributions within the groups, other than the exposed group has a longer upper tail than the unexposed group.

The next group of scatter plots examines the difference in the various cell count measurements made on individual blood samples, again separated into exposed and unexposed groups. The normal range for reticulocytes is 20-80x10^9/l and 0.5-2%. For white blood cells the normal range is 4-10.5x10^9/l; for platelets the normal range is 150-400 10^9/l and for neutrophils the normal range is 2-8 10^9/l.

Reticulocytes

As the second measure of reticulocytes represents the percentage of red blood cells that are reticulocytes per litre of blood, it is expected that little difference in the patterns in the both the scatter plots and box plots will be seen. This is indeed the case as can be seen from the graphs presented below.

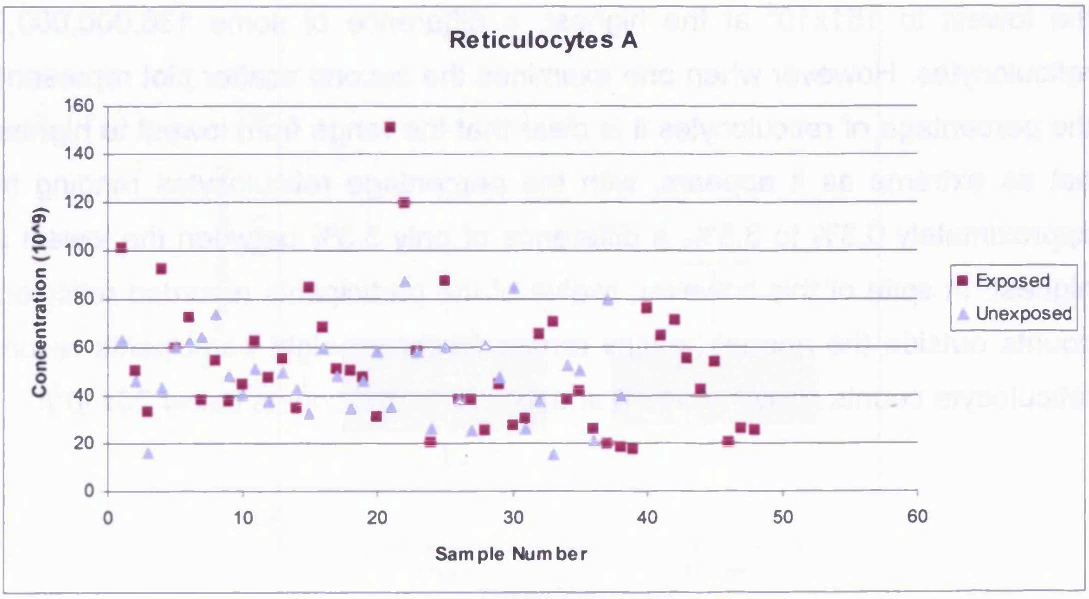


Figure 4.24: Scatter plot showing the differences in reticulocyte counts (x10^9/l) between the exposed and unexposed groups

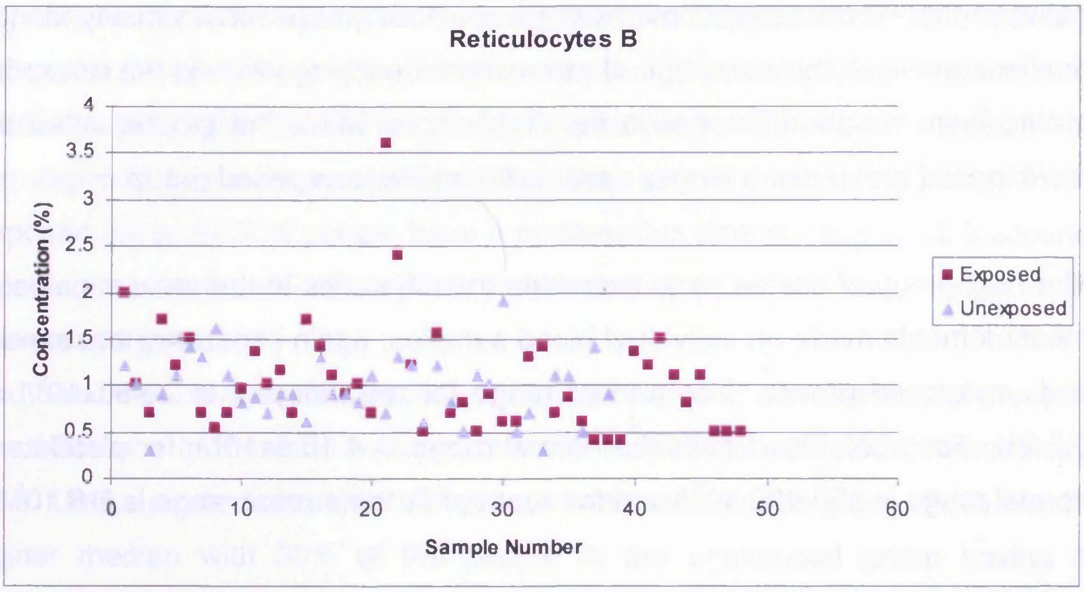


Figure 4.25: Scatter plot showing the differences in reticulocyte counts (%) between the exposed and unexposed groups

First appearances are deceiving when it comes to interpreting these scatter plots, from the examining the plot representing reticulocyte counts it would appear that a large degree of variance exists with a count ranging from $15x10^9$ at the lowest to $151x10^9$ at the highest, a difference of some 136,000,000,000 reticulocytes. However when one examines the second scatter plot representing the percentage of reticulocytes it is clear that the range from lowest to highest is not as extreme as it appears, with the percentage reticulocytes ranging from approximately 0.3% to 3.6%, a difference of only 3.3% between the lowest and highest. In spite of this however, twelve of the participants recorded reticulocyte counts outside the normal healthy range. Of these, eight participants recorded reticulocyte counts above $80x10^9/l$ and four recorded counts below $20x10^9/l$.

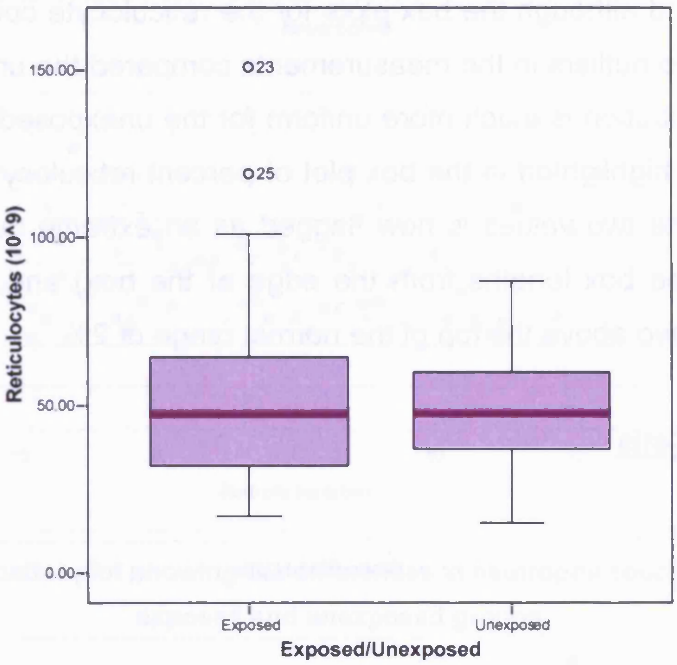


Figure 4.26: Box plot showing the differences in reticulocyte counts ($x10^9/l$) between the exposed and unexposed groups

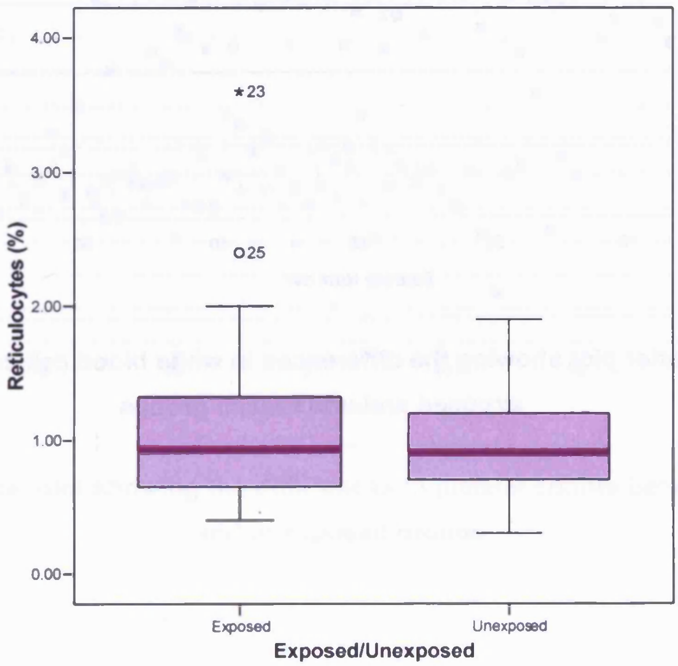


Figure 4.27: Box plot showing the differences in reticulocyte counts (%) between the exposed and unexposed groups

What is obvious from the box plots is that the medians are almost identical for both groups and although the box plots for the reticulocyte counts show a wider spread with two outliers in the measurements compared the unexposed group in which the distribution is much more uniform for the unexposed group. The same two cases are highlighted in the box plot of percent reticulocytes but in this plot the larger of the two values is now flagged as an extreme outlier (the value is more than three box lengths from the edge of the box) and these two values were the only two above the top of the normal range of 2%.

Other Blood Cells

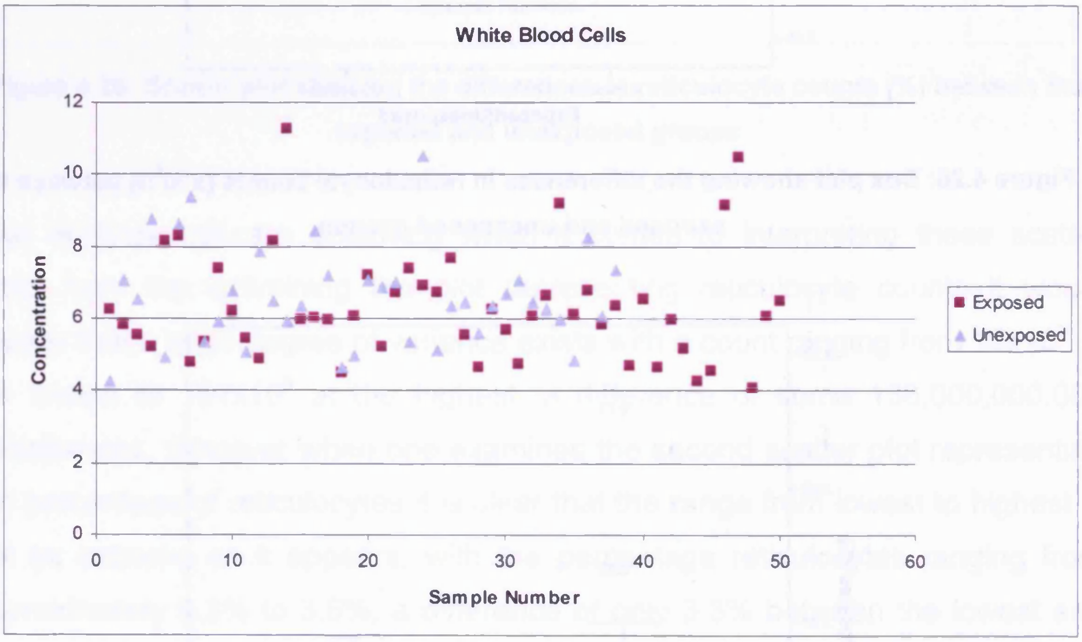


Figure 4.28: Scatter plot showing the differences in white blood cell counts between the exposed and unexposed groups

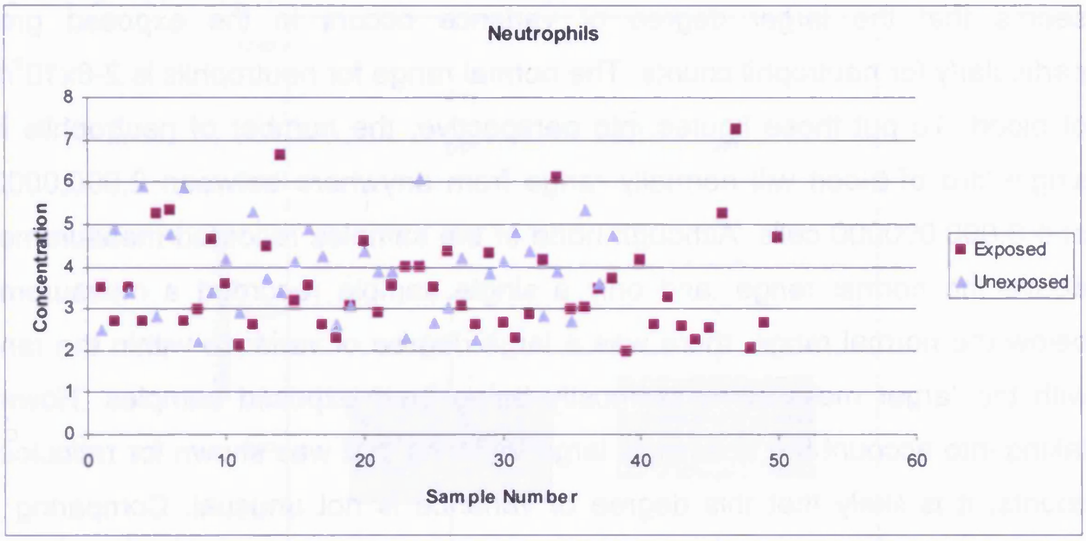


Figure 4.29: Scatter plot showing the differences in neutrophil counts between the exposed and unexposed groups

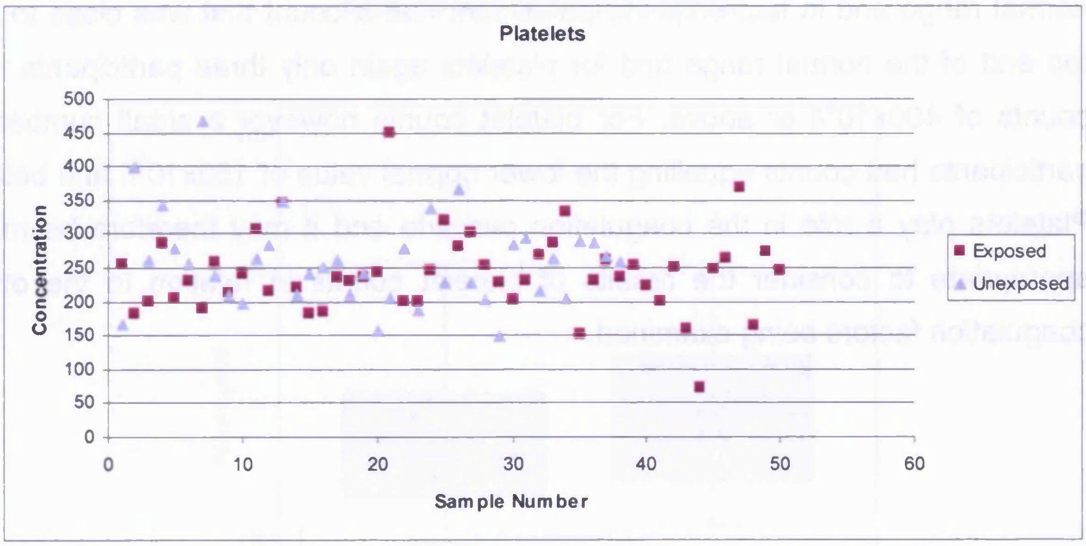


Figure 4.30: Scatter plot showing the differences in platelet counts between the exposed and unexposed groups

There appears to be a large degree of variance within the two groups although it seems that the larger degree of variance occurs in the exposed group, particularly for neutrophil counts. The normal range for neutrophils is 2-8x10^9/litre of blood. To put those figures into perspective, the number of neutrophils in a single litre of blood will normally range from anywhere between 2,000,000000 and 8,000,000000 cells. Although none of the samples recorded measurements above the normal range, and only a single sample recorded a measurement below the normal range, there was a large degree of variance within the range, with the larger measurements mostly being from exposed samples. However taking into account the seemingly large variance that was shown for reticulocyte counts, it is likely that this degree of variance is not unusual. Comparing the measured data with the values for the normal ranges for each cell type, it can be seen that only three of the participants had white blood cell counts outside of the normal range, none of which were much higher than the normal range. With respect to neutrophil counts, none of the participants had counts outside of the normal range and in fact only one participant had a count that was close to the top end of the normal range and for platelets again only three participants had counts of 400x10^9/l or above. For platelet counts however a small number of participants had counts equalling the lower normal value of 150x10^9/l and below. Platelets play a role in the coagulation cascade and it may therefore be more appropriate to consider the results of platelet counts in relation to the other coagulation factors being examined.

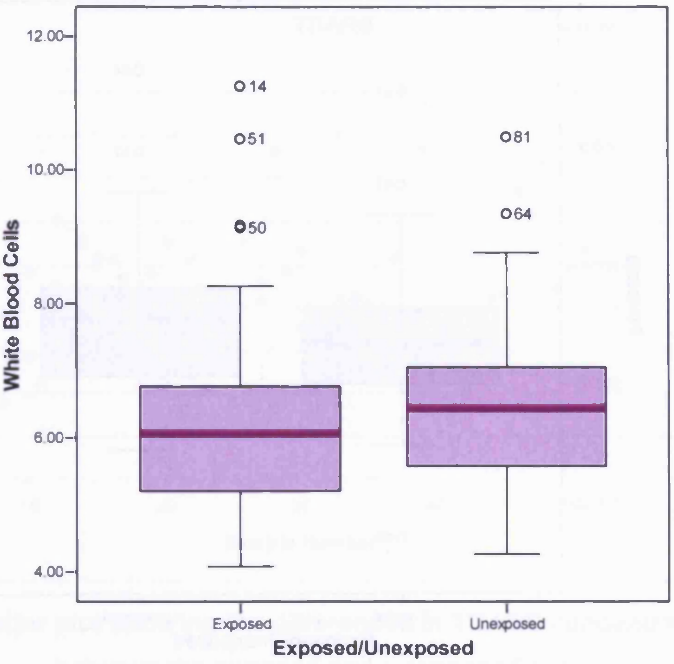


Figure 4.31: Box plot showing the differences in white blood cell counts between the exposed and unexposed groups

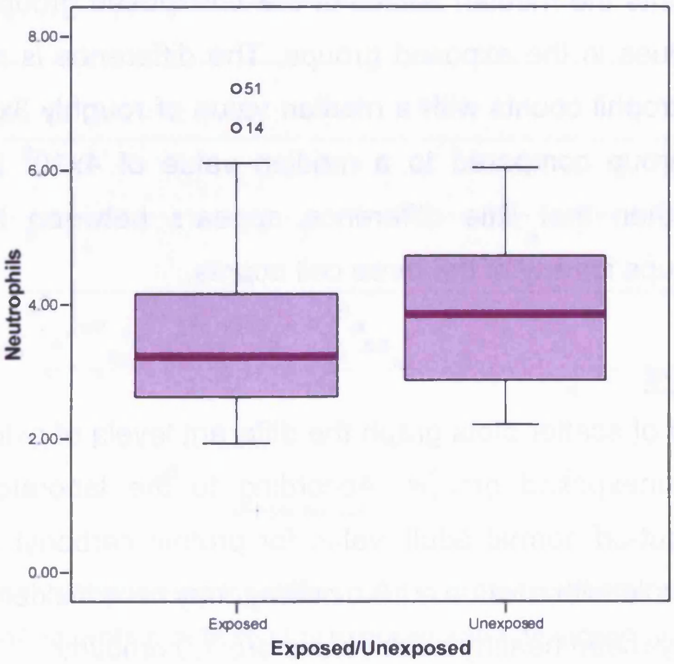


Figure 4.32: Box plot showing the differences in neutrophil counts between the exposed and unexposed groups

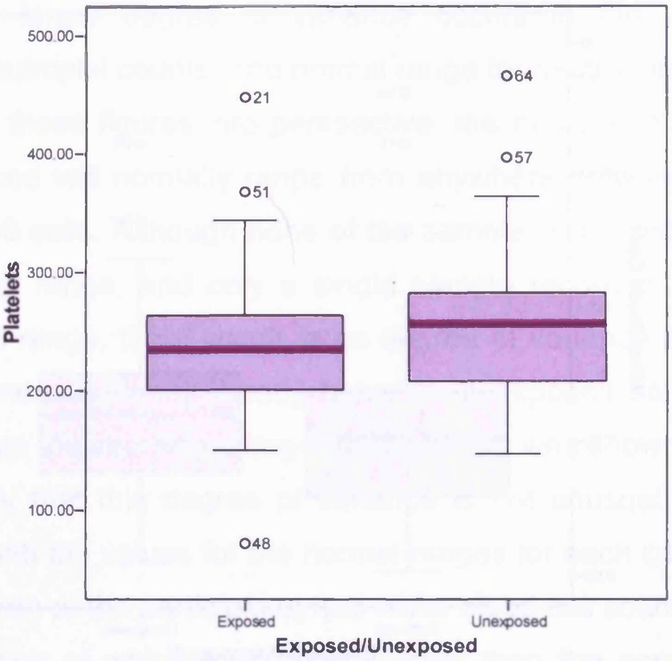


Figure 4.33: Box plot showing the differences in platelet counts between the exposed and unexposed groups

For all cell counts the median values in the unexposed groups are higher than the median values in the exposed groups. The difference is most obvious with respect to neutrophil counts with a median value of roughly $3x10^9$ neutrophils in the exposed group compared to a median value of $4x10^9$ in the unexposed group. Other than that little difference appears between the exposed and unexposed groups for any of the three cell counts.

Oxidant Factors

The third group of scatter plots graph the different levels of oxidant factors in the exposed and unexposed groups. According to the laboratory analysing the samples, the 'cut-off normal adult' value for protein carbonyl is <=1.0 nmol/mg and so any sample with a value >1.0 nmol/mg may be considered significant. For the TBAR assay mean healthy adult values are 1.0 nmol/ml.

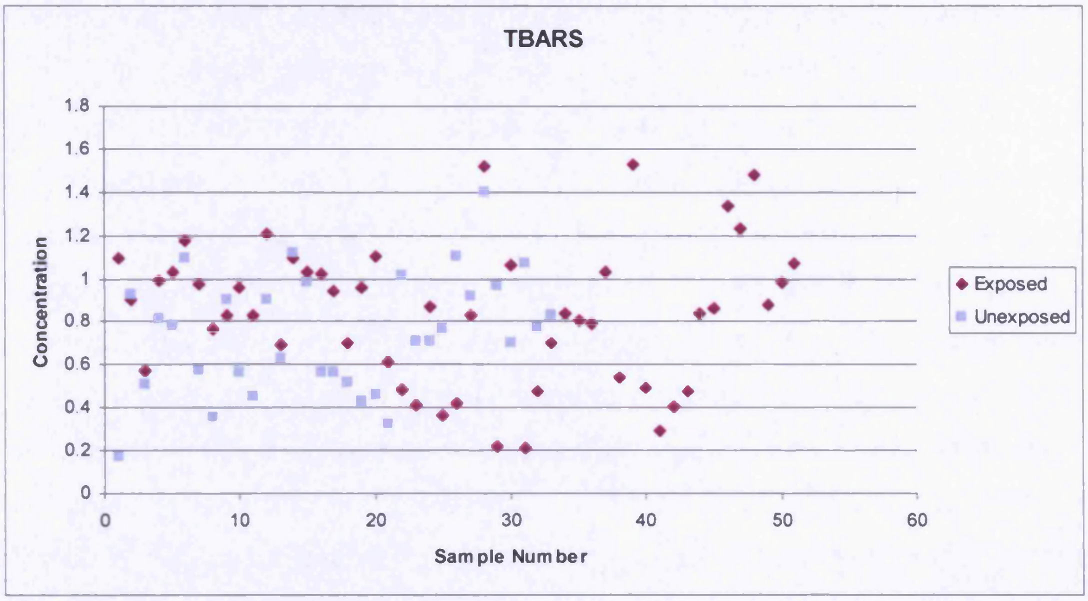


Figure 4.34: Scatter plot showing the differences in TBARS concentrations in nmols/l between the exposed and unexposed groups

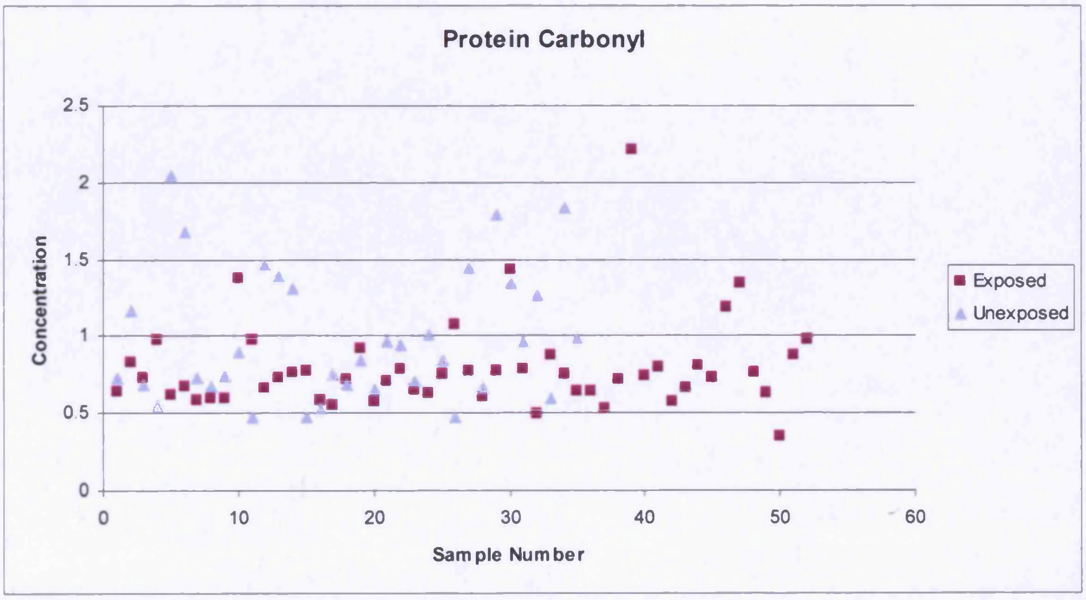


Figure 4.35: Scatter plot showing the differences in protein carbonyl concentrations in nmols/mg protein between the exposed and unexposed groups

From examining the scatter plots it would appear that for TBARS the levels in the exposed group tend to be slightly higher whereas for carbonyl concentration it appears that the levels in the unexposed group are the higher. For both biomarkers there appears to be a high degree of scatter although for carbonyl concentration this scatter appears to occur more in the unexposed group whereas for TBARS concentration it is evident in both the exposed an unexposed group. For TBARS concentrations, twenty two individuals recorded a concentration above 1.0 nmol/ml and of the twenty two, sixteen were participants living in the exposed area. Conversely with respect to protein carbonyl concentration although sixteen participants recorded concentrations above 1.0 nmol/mg of protein, the majority of them were for participants living in the unexposed area. Although for TBA there is a high degree of scatter, much of the values are below the normal healthy value of 1.0 nmol/ml of serum and the samples with values above this healthy level consist primarily of samples from the exposed area.

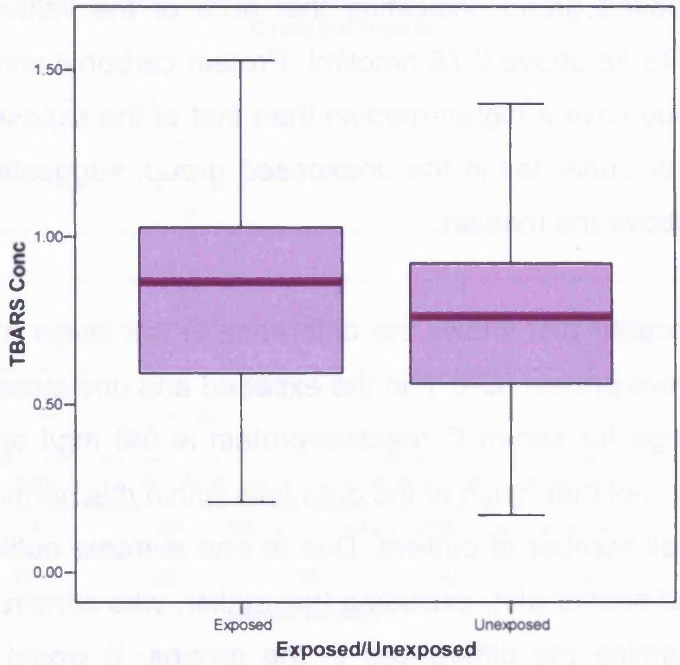


Figure 4.36: Box plot showing the differences in TBARS concentration between the exposed and unexposed groups

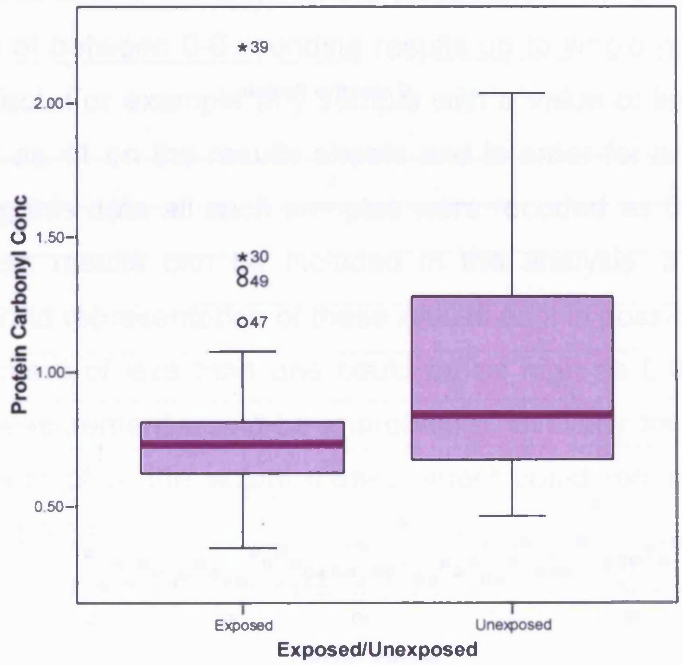


Figure 4.37: Box plot showing the differences in protein carbonyl concentration between the exposed and unexposed groups

There is a slightly higher median value in the exposed group when compared with the unexposed group, indicating that 50% of the values in the exposed group for TBARS lie above 0.75 nmol/ml. Protein carbonyl concentrations in the unexposed group have a higher median than that of the exposed group and also there is a longer upper tail in the unexposed group, suggesting the majority of the values lie above the median.

The following scatter plot shows the difference in the levels of the acute phase protein, C-reactive protein (CRP), in the exposed and unexposed blood samples. The normal range for serum C reactive protein is 0-6 mg/l and it can be seen from the scatter plot that much of the data falls within this normal range, although there are a small number of outliers. Due to one extreme outlier in the exposed group, a second scatter plot, excluding this outlier, was constructed thus making it easier to examine the differences in the groups. It would appear from the scatter plot that little difference in C reactive protein concentrations exist between the two groups.

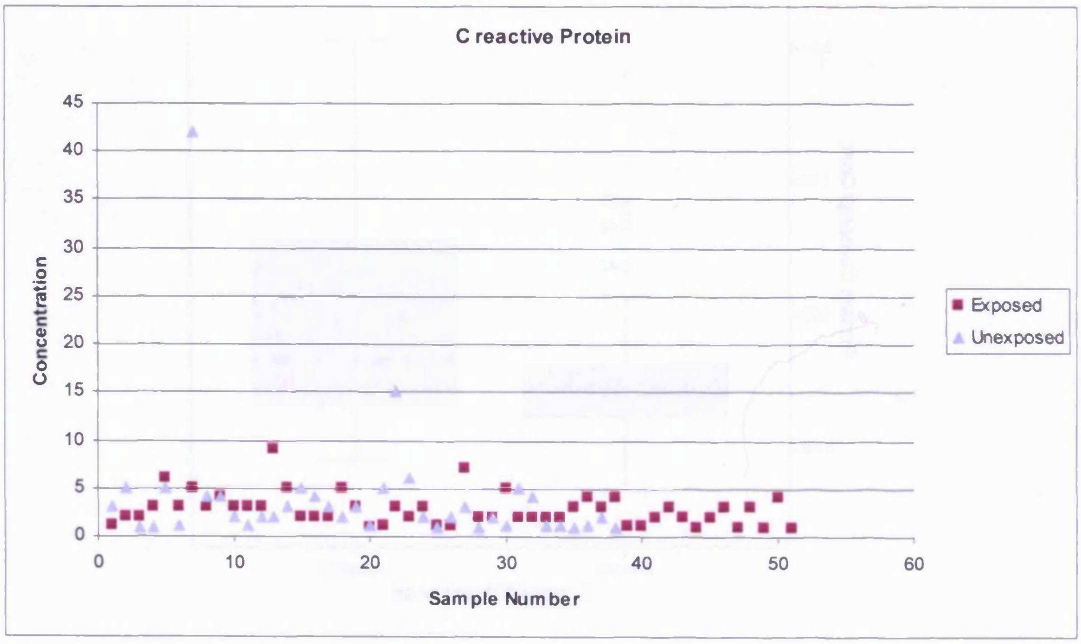


Figure 4.38: Scatter plot showing the differences in C reactive protein concentrations in mg/l between the exposed and unexposed groups

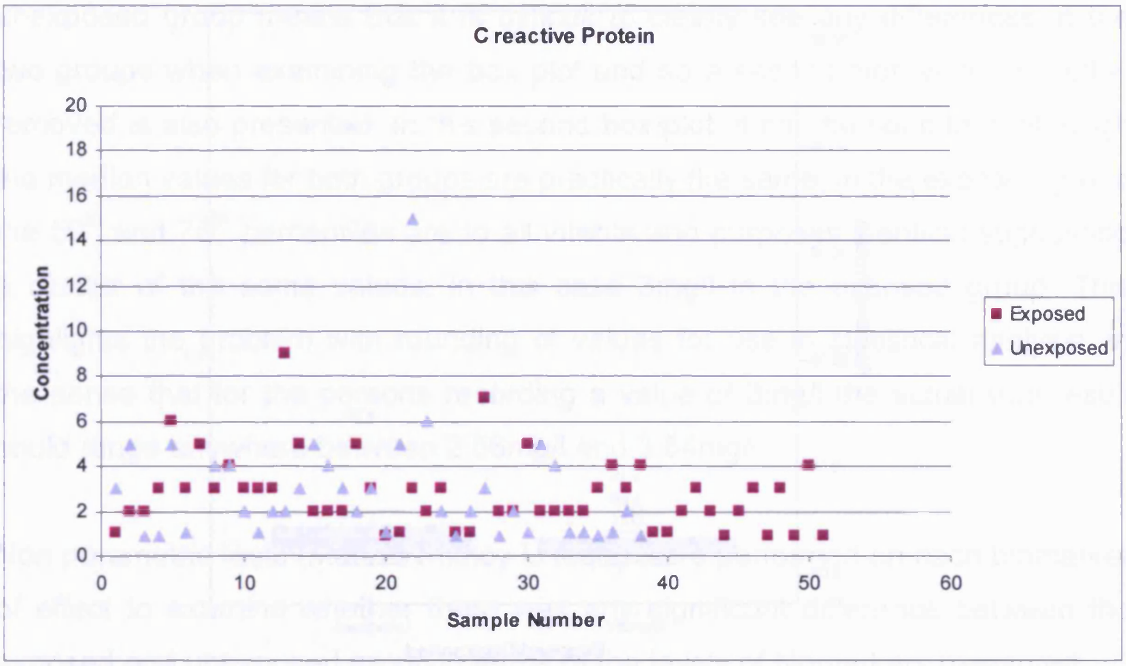


Figure 4.39: Scatter plot showing the differences in C reactive protein concentrations in mg/l between the exposed and unexposed groups minus the one extreme outlying result

A concern with this data is the fact that the values are all whole numbers. With a reference range of between 0-6 rounding results up to whole numbers could be masking any effect. For example any sample with a value of less than one was simply recorded as <1 on the results sheets and in order for any analysis to be carried out using this data all such samples were recoded as 0.9. Although this means that these results can be included in the analysis, it does not allow provide an accurate representation of these results as it is possible that a sample with a measurement of less than one could be as high as 0.9 or as low as 0 (although a 0 measurement would be improbable). Similarly for a sample with a CRP measurement of 3, the actual measurement could range from anywhere between 2.56 and 3.54.

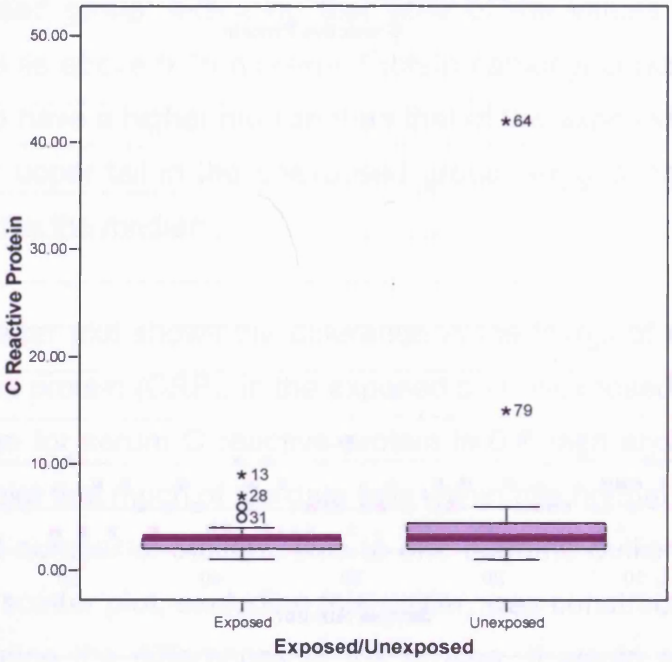


Figure 4.40: Box plot showing the differences in C reactive protein between the exposed and unexposed groups

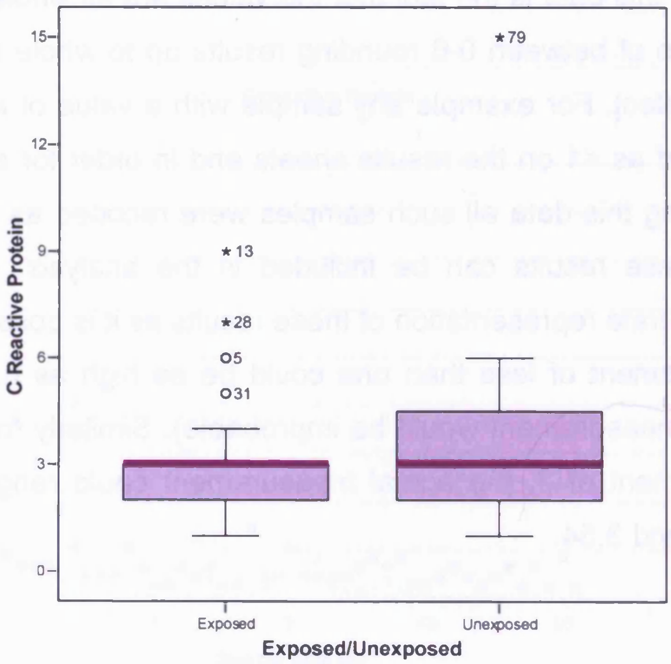


Figure 4.41: Box plot showing the differences in C reactive protein concentration between the exposed and unexposed groups minus the one extreme outlier

Similarly to the scatter plots, the presence of a single extreme outlier in the unexposed group means that it is difficult to clearly see any differences in the two groups when examining the box plot and so a second plot, with the outlier removed is also presented. In this second box plot, it can be seen that although the median values for both groups are practically the same, in the exposed group the 50th and 75th percentiles are to all intents and purposes identical suggesting a cluster of the same values, in this case 3mg/l in the exposed group. This highlights the problem with rounding of values for use in statistical analysis, in the sense that for the persons recording a value of 3mg/l the actual true result could range anywhere between 2.56mg/l and 3.54mg/l.

Non parametric tests (Mann-Whitney U tests) were performed on each biomarker of effect to examine whether there was any significant difference between the exposed and unexposed group in terms of the levels of biomarkers measured.

Biomarker	Z	Asymp Sig 2-Tailed
Thrombin Clotting Time	-0.833	0.405
Prothrombin Time	-2.907	0.004
Activated Partial Trhomboplastin	-1.582	0.114
TBARS Conc	-1.401	0.161
Protein Carbonyl Conc	-1.909	0.056
Reticulocytes (10^9)	-0.170	0.865
Reticulocytes (%)	-0.048	0.962
Fibrinogen	-0.031	0.975
C Reactive Protein	-0.224	0.823
White Blood Cells	-1.382	0.167
Neutrophils	-1.664	0.096
Platelets	-1.293	0.196

Table 4.25: Results of the Mann-Whitney U test comparing the levels of each biomarker in the exposed and unexposed areas

For all biomarkers except Prothrombin time (p=0.004) there was no statistically significant difference between the levels of biomarkers measured in the exposed and unexposed groups. The difference between the two groups with respect to protein carbonyl concentration came close to being significant (p=0.056). A lack of samples for which results could be obtained may be one reason for the lack of significant differences between the groups. It is also possible that in fact no difference exists in the levels of the various biomarkers of effect, but without larger numbers of participants, particularly in the unexposed group, it is difficult to infer anything solid from the results.

Due to the fact that for TBARS concentrations, twenty two individuals recorded a result above the normal value of 1.0 nmol/ml it was felt that further investigation was warranted, in the form of X^2 test. The null hypothesis was that there is no difference in the proportion of people above the cut off value in the exposed and non-exposed groups.

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	19.479(a)	20	.491
Likelihood Ratio	23.009	20	.288
Linear-by-Linear Association	.467	1	.494
N of Valid Cases	22		

a 42 cells (100.0%) have expected count less than 5. The minimum expected count is .27.

Table 4.26: Results of the Chi Square test comparing TBARS concentration in the exposed and unexposed groups.

A statistically insignificant result (p=0.491) suggests that the null hypothesis cannot be rejected and that there does not appear to be any difference in the proportion of persons in the exposed and unexposed groups who recorded a value above the normal value of 1.0 nmol/ml.

4.1.5) Examining the possible presence of a dose-response relationship between numbers of particles measured and levels of biomarkers observed

The results for all the individual participant blood markers were grouped according to the mean particle number measured on their street. The three groups were; 1=0-11,000 particles/cm^3, 11,000-17,000 particles/cm^3 and 17,000 to 50,000 particles/cm^3. Firstly a series of box plots was constructed for each biomarker, comparing the three groups

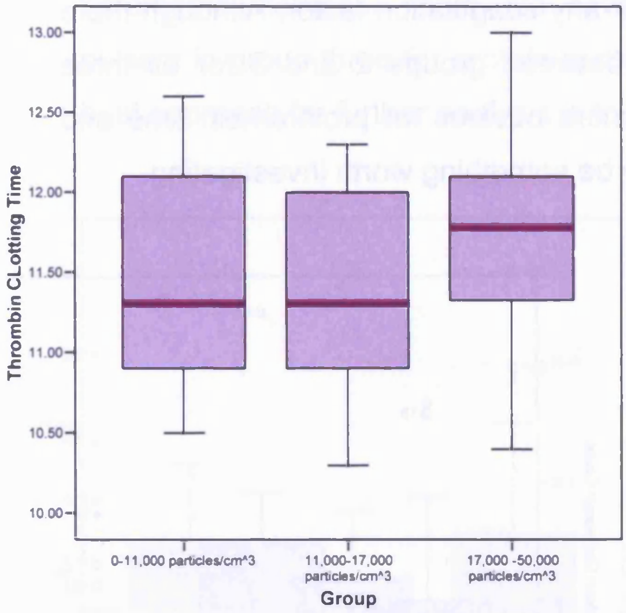


Figure 4.42: Thrombin Clotting Time (Seconds)

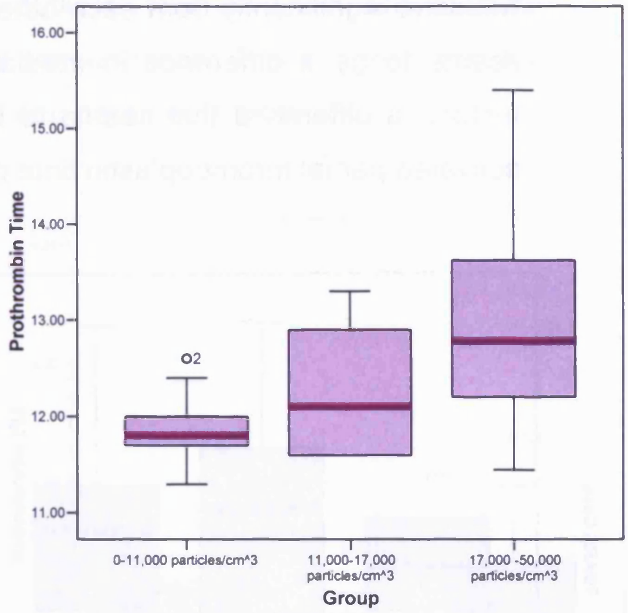


Figure 4.43: Prothrombin time (Seconds)

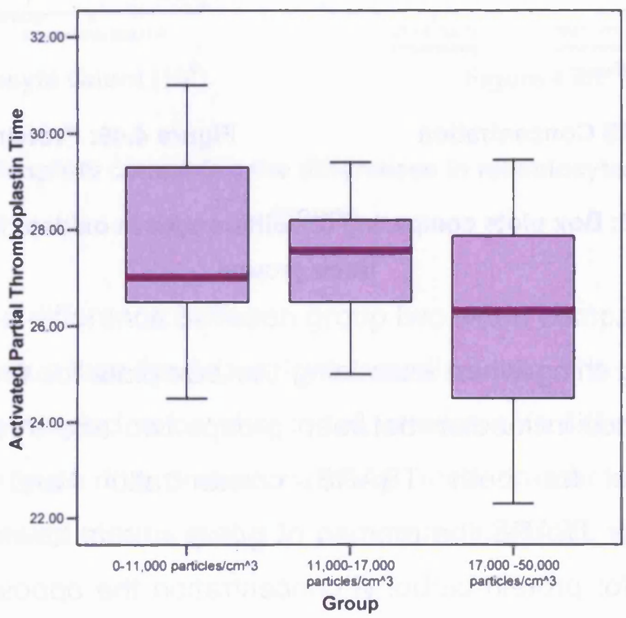


Figure 4.44: Activated partial Thromboplastin Time (Seconds)

Figures 4.42-4.44: Box plots comparing the differences in coagulation factors between the three groups

Examining the box plots it is unlikely that any of the groups of coagulation factors will differ significantly from each other for any coagulation factor. Although there seems to be a difference in medians between groups 2 and 3 for all three factors, a difference that seems to be more obvious for prothrombin time and activated partial thromboplastin time may be something worth investigating.

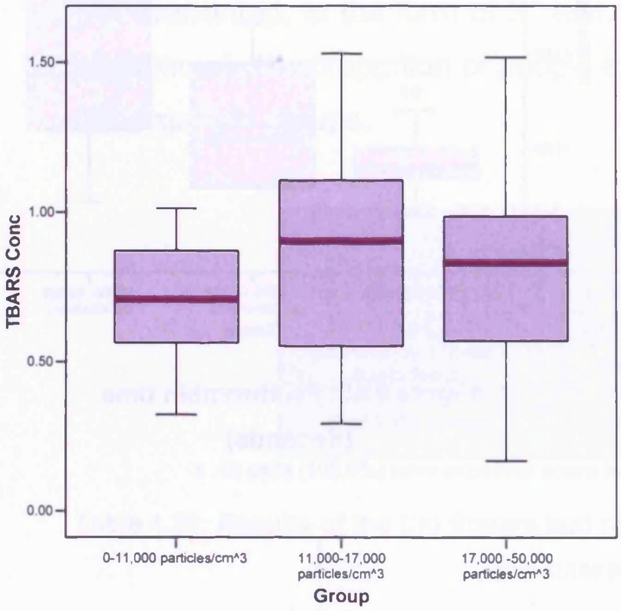


Figure 4.45: TBARS Concentration

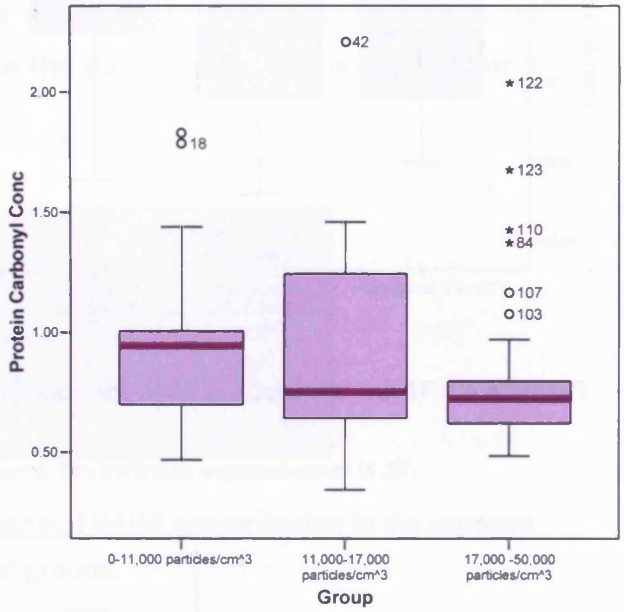


Figure 4.46: Protein Carbonyl Concentration

Figures 4.45-4.46: Box plots comparing the differences in oxidant factors between the three groups

The most striking thing when examining the box plots for the oxidant factors is the large difference in median between groups two and three when compared with group one for both TBARS concentration and protein carbonyl concentration. For TBARS the median of group one is lower than for the other two groups and for protein carbonyl concentration the opposite is true, with the median of group one being greater than groups two and three. In both cases the medians of groups two and three were similar and with respect to TBARS concentration is would appear that the chance of a measurement below the median is greater in both groups. For protein carbonyl concentration there appears to be an increased likelihood of a measurement above the median in group two. Although the presence of several extreme outlying values across the groups would indicate that further analysis should employ the use of non-parametric methods. There are several outliers, four of which are extreme outliers in group three for protein carbonyl concentration. This suggests that the best approach for further analysis is to use non parametric methods.

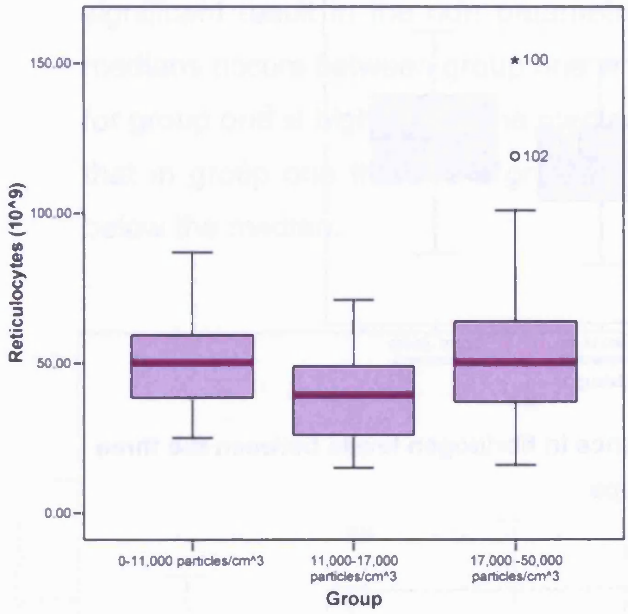


Figure 4.47: Reticulocyte Count (10^9)

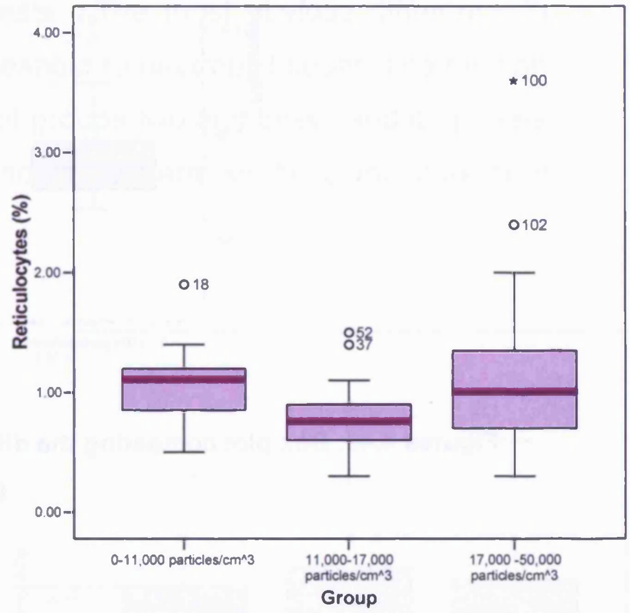
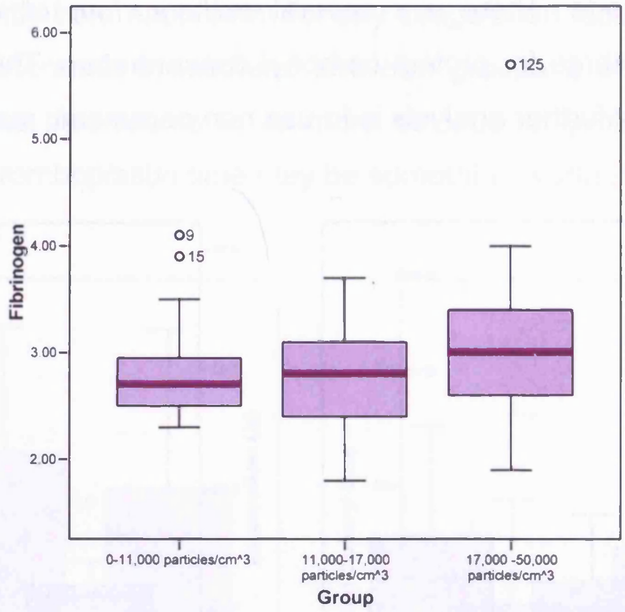


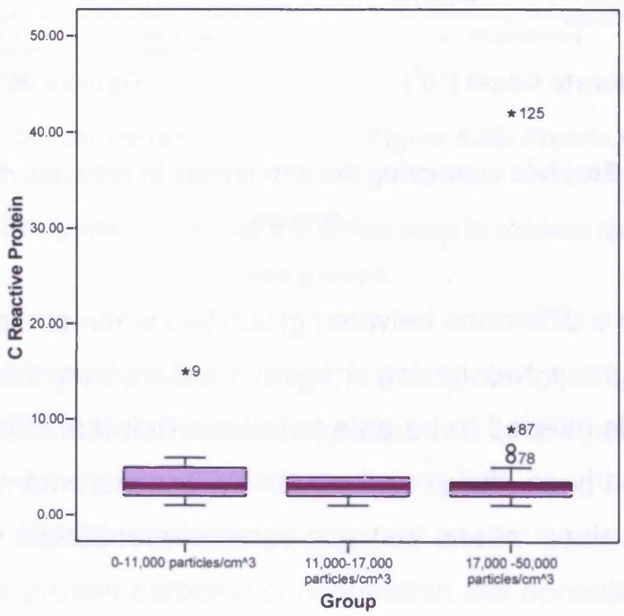
Figure 4.48: Reticulocytes (%)

Figures 4.47-4.48: Boxplots comparing the differences in reticulocytes between the three groups

There looks to be a difference between group two when compared to groups one and three, with group two having a lower median than the other two groups. Further analysis is needed to be able to tell whether this difference is significant or not and similarly to the protein carbonyl measurements the presence of several outlying values means that non-parametric methods will be the methods of choice.



Figures 4.49: Box plot comparing the difference in fibrinogen levels between the three groups



Figures 4.50: Box plot comparing the difference in concentration of C reactive protein between the three groups

There does not appear to be any real difference in medians between the three groups for either fibrinogen concentration or c reactive protein concentration. It is not expected that further analysis will reveal any significant differences between either the groups as a whole or between any pair of groups for either biomarker.

Some slight difference in the medians can be seen in the following box plots although there is nothing to suggest that these differences would produce a significant result in the non parametric tests. The most obvious difference in medians occurs between group one with respect to neutrophil count. The median for group one is higher than the medians of groups two and three, and it appears that in group one there is a greater chance that there will be a measurement below the median.

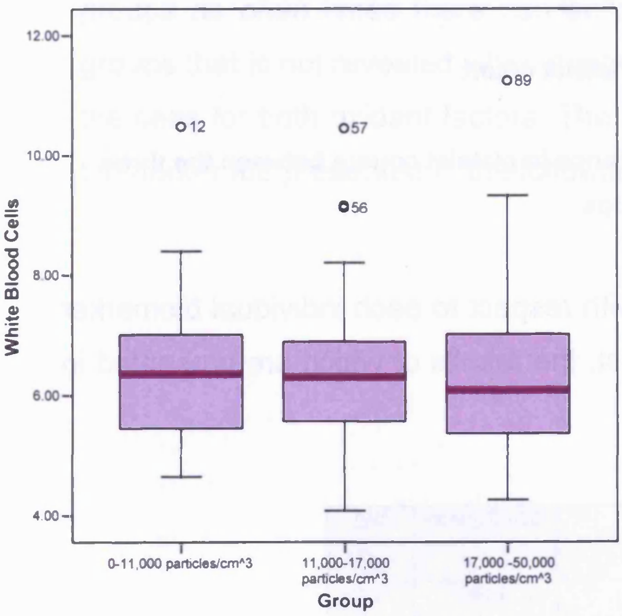


Figure 4.51: White Blood Cell Count

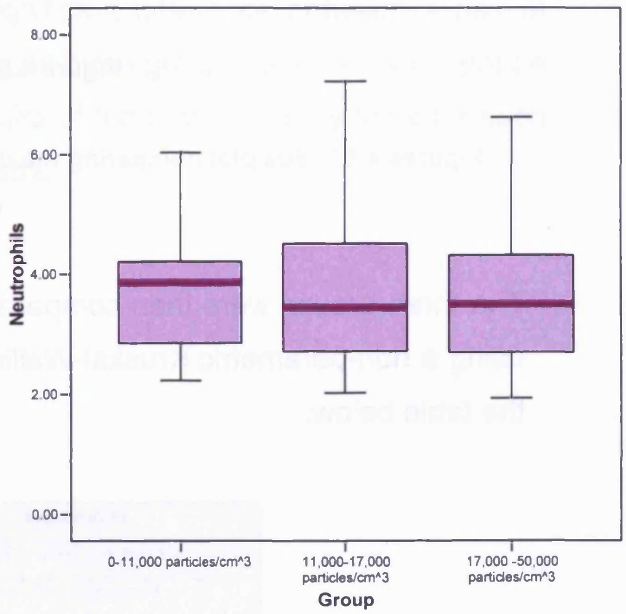


Figure 4.52: Neutrophil Count

Figures 4.51-4.52: Box plots comparing the differences in white blood cell and neutrophil counts between the three groups

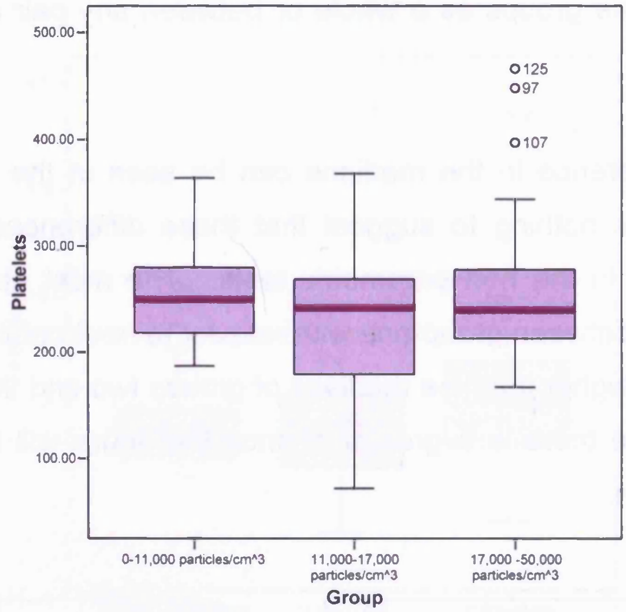


Figure 4.53: Platelet count

Figures 4.53: Box plot comparing the difference in platelet counts between the three groups

The three groups were then compared with respect to each individual biomarker using a non-parametric Kruskal-Wallis test, the results of which are presented in the table below.

Biomarker	Chi-Square	Sig
Thrombin Clotting Time	2.911	0.233
Prothrombin Time	9.180	0.010
Activated Partial Thromboplastin Time	3.640	0.162
TBARS	3.310	0.191
Protein Carbonyl Conc	3.378	0.185
Retics (10^9)	5.836	0.054
Retics(%)	6.291	0.043
Fibrinogen	1.981	0.371
C Reactive Protein	1.018	0.601
White Blood Cells	0.271	0.873
Neutrophils	0.359	0.836
Platelets	1.001	0.606

Table 4.27: Results of the Kruskal-Wallis tests examining the difference between the three groups

As was thought on examining the box plots no significant difference was seen between any of the groups for many individual biomarkers of effect. Only prothrombin time (p=0.01), and % reticulocutyes (p=0.043) showed a significant difference between the groups, with reticulocyte number the only other biomarker to come close to reaching significance (p=0.054). Despite the obvious difference in medians between group one and groups two and three for both oxidant factors (TBARS concentration and protein carbonyl concentration) no statistically significant difference was observed in the results. Although the box plots do not indicate the presence of any significant difference in medians for many of the biomarkers and this was supported by the results of the Kruskal-Wallis test, mann-whitney tests were conducted on each biomarker for different pairs of groups as often times there can be a significant difference between a pair of groups that is not revealed when analysing all three groups together, as might be the case for both oxidant factors. The results of the mann-whitney tests for each biomarker are presented in the following table.

Biomarker	Groups Compared	Z	Asymp. Sig. (2-tailed)	Exact Sig. (2x1-tailed)
Thrombin Time	1-2	-0.4110	0.682	0.720
	2-3	-1.559	0.119	0.125
	1-3	-1.045	0.296	0.302
Prothrombin Time	1-2	-0.864	0.387	0.400
	2-3	-1.727	0.084	0.088
	1-3	-2.853	0.004	0.003
Activated Partial Thromboplastin Time	1-2	-0.164	0.870	0.905
	2-3	-1.392	0.164	0.172
	1-3	-1.611	0.107	0.108
TBARS Conc	1-2	-1.588	0.112	0.116
	2-3	-1.2998	0.194	
	1-3	-1.033	0.302	
Protein Carbonyl Conc	1-2	-0.767	0.443	
	2-3	-0.965	0.334	
	1-3	-1.795	0.072	
Reticulocytes (10^9)	1-2	-2.080	0.038	
	2-3	-2.197	0.028	
	1-3	-1.32	0.895	
Reticulocytes (%)	1-2	-2.328	0.020	
	2-3	-2.196	0.028	
	1-3	-0.059	0.953	
Fibrinogen	1-2	-0.068	0.946	0.957
	2-3	-1.172	0.241	
	1-3	-1.097	0.273	
C reactive Protein	1-2	-1.073	0.283	0.298
	2-3	-0.280	0.779	
	1-3	-0.766	0.444	
White Blood Cells	1-2	-0.147	0.883	
	2-3	-0.315	0.753	
	1-3	-0.500	0.617	
Neutrophils	1-2	-0.294	0.769	
	2-3	-0.271	0.786	
	1-3	-0.595	0.552	
Platelets	1-2	-0.893	0.372	
	2-3	-0.674	0.500	
	1-3	-0.595	0.552	

Table 4.28: Results of the Mann-Whitney U tests examining the differences between individual pairs of biomarkers

Comaparing the individual groups revealed a significant difference between groups one and three for prothrombin time (p=0.004) but no significant differences between either groups one and two or two and three.
A significant difference was observed between groups one and two and groups two and three for reticulocyte count (p=0.038 and p=0.028 respectively) reflecting the fact that the median of group two appeared to be different from the medians of the other two groups on examination of the box plots.
There were no significant differences between any of the pairs of groups for any other biomarker and this was not an unexpected result in light of the information gleaned from examining the box plots apart from the oxidant factors for which it did appear that there may have been a difference between group one and the other two groups.

Two other biomarkers of interest were measured during the course of the study; IL-6 and TNF-α, both of which are markers of inflammation. Problems obtaining the test kits from suppliers however meant that the results for these two biomarkers were not available at the time of writing hence preventing them from being included in the results chapter. The data for these two biomarkers did however become available at a very late stage and it was felt that it was important that the data were not wasted. Therefore the results were analysed and are presented in a separate appendix (appendix 5).

4.1.6) Using the questionnaire data to examine participants perceptions

Each participant was asked a series of open-ended questions designed to gauge their opinions about the traffic in their own immediate areas.

The total number of completed questionnaires for each group is outlined in the table below.

	Exposed	Unexposed
Number of questionnaires	70	51

Table 4.29: Number of questionnaires available for analysis for each group

The first question put to the participants was a simple yes/no question about whether they felt traffic was a problem in their immediate area. The answers are outlined in the table below.

Exposed		Unexposed	
Yes	No	Yes	No
n=53	n=17	n=3	n=48
76%	24%	6%	94%

Table 4.30: Breakdown of how participants answered question one

Perhaps a little surprisingly, roughly 24% of participants living in the exposed areas claimed not to feel that traffic was a problem, compared with roughly 6% of the participants in the unexposed areas who felt that traffic was a problem in their immediate area.

Next the participants were asked to clarify their answers and give reasons why they answered the way they did.

At this point it became clear that although 24% of people in the exposed area claimed that traffic was not a problem in their immediate area, the reason was not because traffic was not heavy but more because the participants were not concerned by it because it did not affect their lives on a day to day basis. Of the 17 participants who claimed traffic was not a problem, all participants admitted to there being heavy traffic in there immediate area but the reasons for not viewing the traffic as a problem varied between not thinking that the high traffic volume resulted in much pollution, to not having a car so not being affected by the heavy traffic, to having chosen to live on a street with heavy traffic because the advantages of such centralised locations outweigh the disadvantages of living with heavy traffic.

94% of the participants in the unexposed areas claimed that traffic was not a problem and the most common reason given was that there was very little traffic other than residential traffic and the odd delivery vehicle using the roads because, being primarily residential areas, there was little reason for other traffic to use the roads and quite often these roads were cul de sacs, which meant that there was no way they could be used as alternative short-cuts or 'rat-runs' to the busier roads.

Of the participants who considered traffic to be a problem in their immediate area one of the most common comments was that the noise and the noxious fumes were the biggest problems for people, particularly when the windows were open. Backed-up traffic, difficulty accessing their homes from the road and the danger such volumes of fast moving traffic presents to the residents, were all cited as reasons for considering traffic a problem. For the three participants in the unexposed areas who considered traffic a problem the reason for this was the use of the road as a 'rat-run' for access, as a method to avoiding heavy traffic on main roads.

The next question put to participants was inquiring as to whether there were particular times of the day when the traffic was noticeably worse than at others. They were given five options; AM only, PM only, both AM and PM, all day and no noticeable heavy traffic.

		AM	PM	Both	All Day	None
Exposed	Problem	2	2	42	7	0
		9.5%	9.5%	79%	13%	0%
	Not	1	0	15	1	1
		6.5%	0%	83.5%	5.5%	5.5%
Unexposed	Problem	0	0	3	0	0
		0%	0%	100%	0%	0%
	Not	0	1	11	0	35
		0%	1%	23%	0%	76%

Table 4.31: Breakdown of how participants answered question two

From the table above it can be seen that in all cases the most common time of day for people to notice increased traffic are the morning and evening rush-hours. In the unexposed group the participants who felt that traffic was a problem all felt that it was particularly bad both in the morning and in the evening. For the three participants that felt traffic was a problem in their area this would correspond well with their previous comments that their roads were used as 'rat-runs' and short-cuts to avoid traffic on the main routes. For the other 8 people who claimed to notice worse traffic in the morning and evening, they all stated that it was simply residential traffic leaving for work or schools in the morning and returning in the evening. Most of the participants in the unexposed areas however asserted that there were no noticeable differences in the volume of traffic using the roads on which they lived.

The third question put to the participants was regarding the type of traffic that most commonly used the roads. Again they were given a series of options and these were; mostly cars, mostly heavy vehicles including whether the road is a bus route or not, or both.

		Mostly Cars	Heavy Vehicles (including buses)	Combination of Both	Neither
Exposed	Not a problem	8	0	9	0
	Problem	26	0	27	0
Unexposed	Not a Problem	13	0	0	35
	Problem	3	0	0	0

Table 4.32: Breakdown of how participants answered question three

For people living in the exposed area who felt that traffic was not a problem, roughly 50% said that the type of traffic was mostly cars while the other 50% claimed it was a combination of both cars and heavy vehicles. Of the 50% saying that both cars and heavy vehicles were common on the road, they stated that other than cars, the road was a regular bus routes and that several buses an hour were using the road each day. Again, for the people who believed that traffic was a problem in their area, the split was roughly 50% although in this case, many of the people that felt the traffic was mostly cars claimed that other vehicles such as buses and good vehicles used the road – just not particularly often.

In the unexposed area the majority of the people did not feel that there was enough traffic in the area to warrant answering the question. And even those who did answer said that the traffic in the area was mostly cars and that it was only residential vehicles.

4.2) Section B

Does proximity to certain road types increase one's risk of being admitted to hospital with respiratory and cardiovascular complaints?

4.2.1) Investigation of the different factors known to affect the odds of admission

As mentioned previously the aim of this section of the study was to attempt to determine whether people living on certain categories of road had increased odds of being admitted to hospital with cardiovascular or respiratory complaints. The study was populated using data from the PEDW and NHSAR databases, provided by HSW. The dataset consisted of a base population of more than 300,000 people and the number of people within the base population who were admitted to hospital with a diagnosis corresponding to an ICD code of interest. An initial interrogation of the data was conducted prior to commencing with the primary regression analysis. The purpose of this initial analysis was to examine the data in an attempt to identify anything of interest that may help to explain the results of the regression analysis, as well as to identify factors which may reasonable be included in the analysis as independent covariates. Basic demographic data can be found in Appendix Six.

Age

As was expected admissions for respiratory and cardiovascular illnesses were very low in the younger population, with the exception of the two youngest groups 0-1 and 2-5 years. There is a sharp increase in admissions between the age-groups 31-40 and 41-50 and this sharp increase continues through the remaining age groups until it peaks in ages 71-80 years. There is a dramatic decrease in numbers between the age-groups 71-80 and 81-90 years and the numbers of admissions drop of again in the final age-group >90 years. Age group rather than age was the variable of choice to be included as a categorical variable in the regression model. The choice to include age-group as a categorical rather than continuous variable was prompted by the fact that when graphed, age-group did not follow the necessary pattern to allow for it's inclusion as a continuous variable.

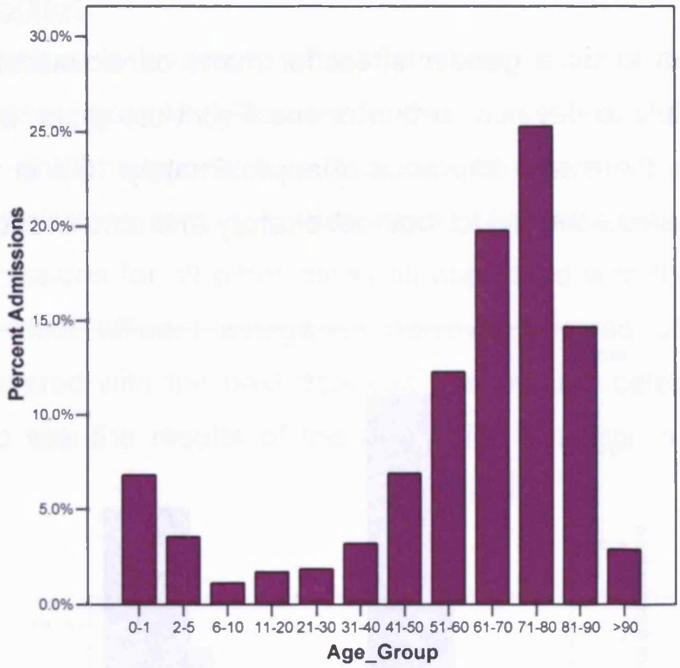


Figure 4.54: Bar Graph of Hospital Admissions By Age Group

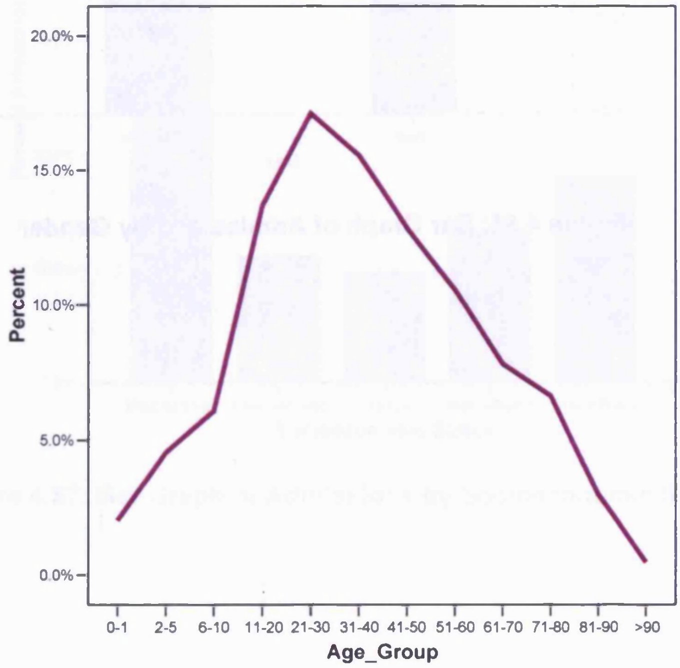


Figure 4.55: Line graph showing the population distribution by age group

Gender

There is known to be a gender effect for many cardiovascular diseases, with males more likely to develop certain illness. From the graph it can be seen that although small, there is a difference of approximately 10% in the percentage of males and females admitted for both respiratory and cardiovascular diseases.

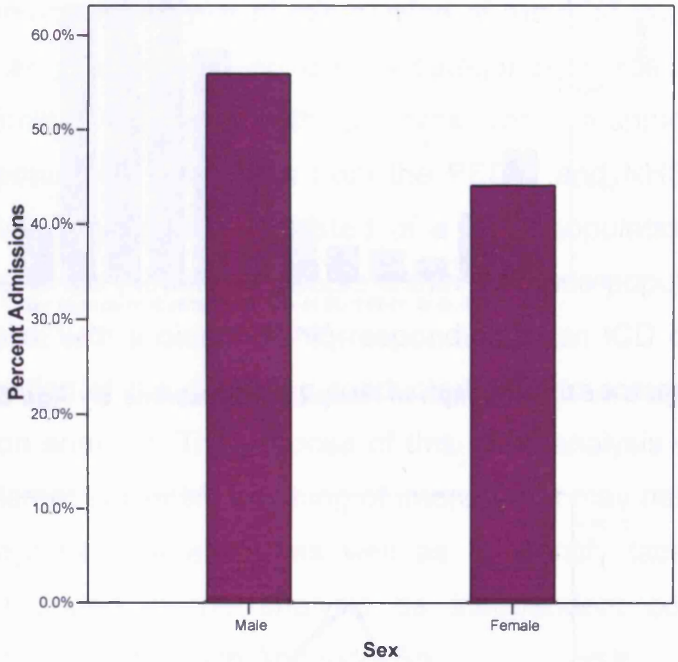


Figure 4.56: Bar Graph of Admissions by Gender

Socioeconomic Status

Socioeconomic status is known to affect admission rates with persons in the more deprived areas more likely to be admitted than those in the more affluent areas. The Bar graph supports this to a certain extent with a much higher percentage of admissions coming from the most deprived areas, with a massive decrease in admissions for all other areas as compared with the most deprived area. The two most affluent categories however showed slightly increased admissions compared with the next deprived and median categories and it will be interesting to see the results of the regression analysis to see if this is a genuine effect.

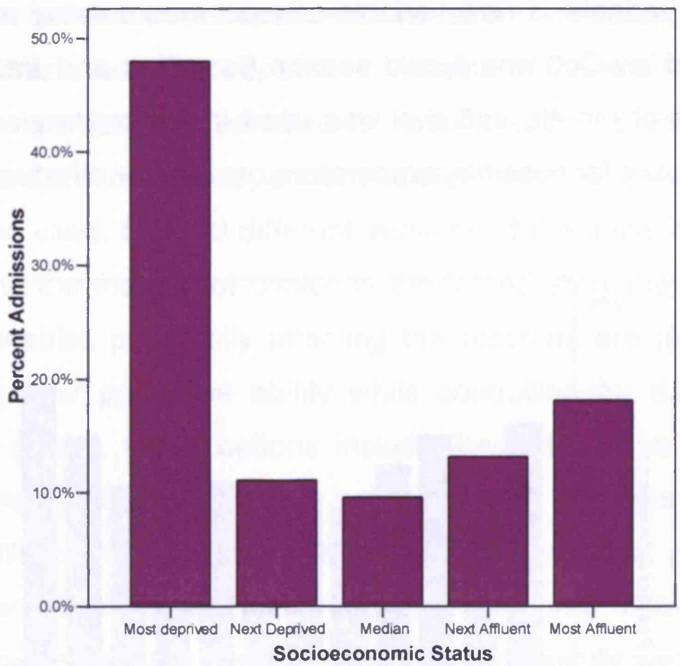


Figure 4.57: Bar Graph of Admissions by Socioeconomic Status

Admission Date

Examination of the bar chart reveals that the winter months (November to January) show slightly higher numbers of admissions than do the other months although, there is a decrease in admissions in February when the percentage of admissions is more similar to that of the summer months than the winter; admissions in March and April however seem to be on a par with the winter months. There is a slight variation in the numbers admitted between May and October but overall the numbers are very similar for all these months.

As there seemed to be little difference in the numbers admitted each month for May to October (inclusive) and similarly for November to April (inclusive) with the exception of February, and because there were various difficulties with putting month in as a variable, a new variable season, was created and consisted of a warm season (May-Oct) and a cold season (Nov-Apr) and was entered into the model in place of month, and this was used in the regression analysis as the variable to account for possible temperature or weather effects on admissions.

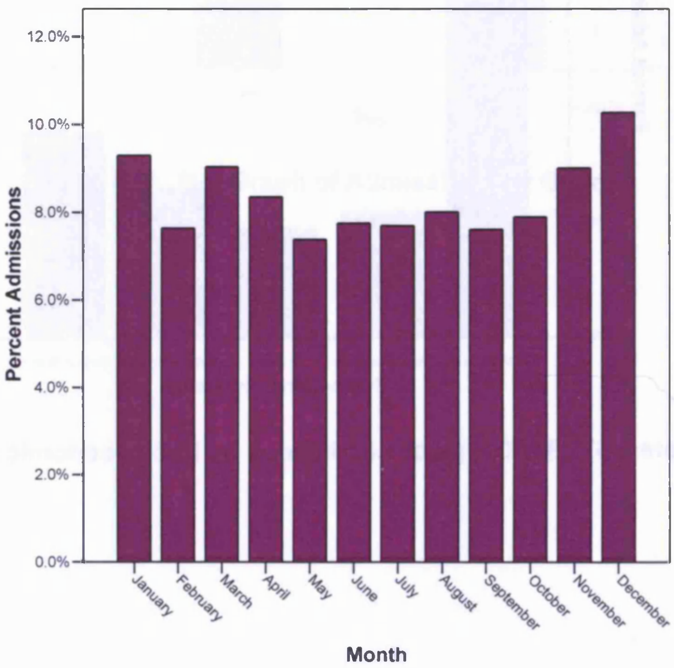


Figure 4.58: Bar Graph of admissions by month

Another factor which is thought to perhaps affect the number of admissions is GP practice code. Ideally information regarding the practice each case was registered with would have been obtained and used in the analysis to examine whether there was any association with admissions. Due to confidentiality issues however this information could not be made available for the current study and could not therefore be included. It is hoped however, that for any future work on this data set, practice codes can be obtained.

4.2.2) Conducting the logistic regression analysis

The first step in the regression analysis was to conduct the logistic regression whereby the dependent variable of interest was whether an individual was admitted to hospital with a diagnosis of interest or not. Logistic Regression allows one to test models in an attempt to predict outcomes with two or more categories. The predictor (independent variable) variables can be either categorical or continuous. The dependent variable of interest in this study was whether or not a person was admitted to hospital with a primary diagnosis of interest i.e. it is a dichotomous dependent variable, for this reason binary logistic regression will be used. Several different versions of the dependent variable will be examined and the method of choice is the forced entry method whereby all the predictor variables potentially affecting the outcome are tested in a single block to assess their predictive ability while controlling for each of the other variables in the model. Other options include forward and backward stepwise procedures, allowing one to select a large group of potential predictors from which SPSS will choose the combination of predictors that provide the best predictive power. The table below outlines the three different dependent variables to be examined in a logistic regression analysis as well as the different predictor variables to be included in each run of analysis. The advantage to using the forced entry method over the other methods is the fact that any and all variables thought to affect the outcome (i.e. thought to be associated with hospital admissions) are entered at the outset of the analysis and in this way one is less likely to over-estimate the associations with the particular variable of interest.

Dependent Variables	Independent Variables
Admitted (1) or not admitted (0) with a primary diagnosis corresponding to any ICD code of interest	Age Group Sex Socioeconomic Status Season of Admission
	Road Category *then* Distance Group *then* Road Type
Admitted (1) or not admitted (0) with a primary diagnosis corresponding to any ICD-I code of interest (Cardiovascular Illness)	Age Group Sex Socioeconomic Status Season of Admission
	Road Category *then* Distance Group *Then* Road Type
Admitted (1) or not admitted (0) with a primary diagnosis corresponding to any ICD-J code of interest (Respiratory Illnesses)	Age Group Sex Socioeconomic Status Season of Admission
	Road Category *then* Distance Group *then* Road type

Table 4.33 Outline of the different variables to be analysed by regression analysis

Although the categories age-group, socioeconomic status sex and month of admission were included in each of the regression analyses, the primary variables of interest were category, distance and road type. These three variables had to be analysed in separate models due to the similarities in the variables and the potential for masking the effect of one by having the other in the model. When entering the variables into the model there is the choice to enter them as categorical variables. Most of the variables to be included in this model are already, by their nature categorical and so must be entered as such.

For example socioeconomic status has five possible categories 1 being the most deprived and 5 being the most affluent.
As this study was primarily concerned with a the effects of exposure to particulate pollution from motor traffic in persons aged 50 and above, the decision was taken to group age into decades, apart from the very young as children between the ages 0-1 and 1-5 which are age groups considered to be a unique and if not dealt with appropriately from the outset they may affect the overall outcome. Age group will also be entered into the model as a categorical variable, for reasons already explained.
For consistency and ease of interpretation, all variables in the model are examined in relation to the first category and each of the variables are coded such that the first category is the worst category, i.e. for socioeconomic status category one is the most deprived group, while for road category, category one consists of all persons with a residential postcode on an A/B road within 0-2km of the city centre, the roads suspected of being the most heavily trafficked. For age-group, the base group was children aged 0-1 years and for sex the base category was males and for seasons the base category was the cold season.
Although there is a large amount of different output provided from the logistic regression, the table of most interest is the 'Variables in the Equation' table. This table is the one which provides the information regarding the importance of each of the predictor variables included in the analysis. The test used is the Wald test, the results of which are presented in the column marked 'Wald'. The column marked 'Sig.' presents the p values for the test and as with most statistical outcomes a p value less than 0.05 indicates a result significant at the 5% level. The other important information is contained in the column marked 'B'. The B values are the values used to calculate the probability of a case falling into one or other categories. The B value can provide information about the direction of the relationship; for example a negative B value indicates that an increase in the independent variable score will result in a decreased probability of the case recording a score of 1 in the dependent variable.

Results of logistic regression analysis for all admissions of interest

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			7196.047	11	.000	
	Age_Group(1)	-1.481	.079	350.368	1	.000	.227
	Age_Group(2)	-2.934	.123	572.484	1	.000	.053
	Age_Group(3)	-3.325	.103	1035.063	1	.000	.036
	Age_Group(4)	-3.488	.101	1196.089	1	.000	.031
	Age_Group(5)	-2.835	.082	1190.922	1	.000	.059
	Age_Group(6)	-1.841	.065	790.443	1	.000	.159
	Age_Group(7)	-1.056	.058	328.339	1	.000	.348
	Age_Group(8)	-.264	.054	23.650	1	.000	.768
	Age_Group(9)	.193	.053	13.246	1	.000	1.213
	Age_Group(10)	.500	.057	76.155	1	.000	1.649
	Age_Group(11)	.790	.088	80.784	1	.000	2.203
	Sex(1)	-.385	.025	240.804	1	.000	.680
	SES_1			156.461	4	.000	
	SES_1(1)	.105	.048	4.915	1	.027	1.111
	SES_1(2)	-.120	.045	7.032	1	.008	.887
	SES_1(3)	-.189	.042	20.514	1	.000	.827
	SES_1(4)	-.412	.037	121.692	1	.000	.662
	Season(1)	-.141	.024	33.284	1	.000	.869
	Category_1			132.220	18	.000	
	Category_1(1)	.639	.130	24.006	1	.000	1.895
	Category_1(2)	.493	.128	14.726	1	.000	1.637
	Category_1(3)	.392	.118	10.954	1	.001	1.480
	Category_1(4)	.544	.159	11.663	1	.001	1.723
	Category_1(5)	.002	.276	.000	1	.995	1.002
	Category_1(6)	.624	.122	26.308	1	.000	1.867
	Category_1(7)	.222	.128	3.014	1	.083	1.249
	Category_1(8)	.363	.118	9.492	1	.002	1.438
	Category_1(9)	.870	.171	25.868	1	.000	2.388
	Category_1(10)	.712	.170	17.526	1	.000	2.038
	Category_1(11)	.715	.124	33.312	1	.000	2.044
	Category_1(12)	.386	.122	9.930	1	.002	1.470
	Category_1(13)	.528	.117	20.373	1	.000	1.695
	Category_1(14)	.446	.125	12.700	1	.000	1.562
	Category_1(15)	1.085	.185	34.244	1	.000	2.959
	Category_1(16)	.464	.139	11.211	1	.001	1.591
	Category_1(17)	.524	.121	18.812	1	.000	1.688
	Category_1(18)	.436	.120	13.092	1	.000	1.546
	Constant	-3.415	.122	789.210	1	.000	.033

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Category_1.

Table 4.34: Results of logistic regression with road category as the independent variable of interest

From the table (table 4.34) it can be seen that the particular variable of interest, the road category variable is significantly associated with hospital admissions with a diagnosis of interest ($p<0.0005$). A full list of road categories can be found on page 124. Age group and socioeconomic status were also significantly associated with hospital admissions, as were both sex and season. There was a decrease in odds (i.e. a protective effect) for all age groups up to and including 61-70 years when compared with children 0-1 years and all were significant ($p=0.0005$). For age groups 71-80 years, 81-90 years and >90 years, there was a statistically significant increase in odds, increasing from an odds ratio of 1.213 for 71-80 years to an odds ratio of 2.203 for >90 years.

With respect to season and sex there was a significantly protective effect associated with both these variables in favour of the warm season and of females (OR=0.869; $p=0.0005$ and OR=0.680; $p=0.0005$ respectively).

Scanning down the results for each of the individual road categories, it can be seen that for all categories of road there is an increase in the odds ratio, implying there is an increased odds that a person living in any of these categories will be admitted to hospital with a primary diagnosis corresponding to an ICD code of interest for persons compared with those living in category 1 (A&B roads within 0-2km of the city centre). The largest of these is increases were for category 15 which corresponds to persons living on 'A' roads between six and twelve kilometres. (OR=2.959; $p=0.0005$). An odds ratio of 2.959 corresponds with a 195% increase in odds for a person living in this category being admitted to hospital over a person living in category one. Other road categories that showed similarly high increases in odds were 'A' roads (OR=2.388; $p=0.0005$), 'B' roads (OR=2.038; $p=0.0005$) and bus routes (OR=2.044; $p=0.0005$) between 4 and 6 km of the city centre. This is somewhat surprising as the originally it was thought that the further away from the city centre and heavy traffic flow a person lived, the less likely they were to be at risk for hospital admissions.

In the following regression analysis, the independent variable 'category' has been substituted with the independent variable 'distance'. This 'distance' variable refers only to the distance of a road from the central point – central station; and does not make any distinction between road type. The base group, to which all others are compared, is the distance group 0-2km from the city centre. The basis behind selecting distance from a city centre as a variable of interest came from the theory that the further away from the city centre, the less concentrated the traffic flow and the further away from busy roads houses were situated therefore possibly reducing the levels of ultra fine particles to which individuals are exposed.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			7300.369	11	.000	
	Age_Group(1)	-1.481	.079	350.803	1	.000	.227
	Age_Group(2)	-2.934	.123	572.624	1	.000	.053
	Age_Group(3)	-3.321	.103	1032.698	1	.000	.036
	Age_Group(4)	-3.485	.101	1195.276	1	.000	.031
	Age_Group(5)	-2.835	.082	1191.618	1	.000	.059
	Age_Group(6)	-1.837	.065	788.275	1	.000	.159
	Age_Group(7)	-1.055	.058	328.737	1	.000	.348
	Age_Group(8)	-.262	.054	23.333	1	.000	.769
	Age_Group(9)	.196	.053	13.691	1	.000	1.216
	Age_Group(10)	.517	.057	81.946	1	.000	1.677
	Age_Group(11)	.823	.088	88.319	1	.000	2.276
	Sex(1)	-.384	.025	239.287	1	.000	.681
	SES_1			159.435	4	.000	
	SES_1(1)	.052	.045	1.330	1	.249	1.054
	SES_1(2)	-.120	.044	7.334	1	.007	.887
	SES_1(3)	-.203	.040	25.518	1	.000	.816
	SES_1(4)	-.426	.037	134.747	1	.000	.653
	Season(1)	-.141	.024	33.479	1	.000	.868
	Distance			17.852	3	.000	
	Distance(1)	-.007	.040	.034	1	.853	.993
	Distance(2)	.129	.040	10.161	1	.001	1.137
	Distance(3)	.072	.042	2.976	1	.085	1.075
	Constant	-2.999	.055	2937.992	1	.000	.050

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Distance.

Table 4.35: Results of the binary logistic regression with all admissions as the dependent variable and where distance is the independent variable of interest

This time the distance category is significantly associated with admission to hospital with a diagnosis of interest (p=0.0005). Similarly to the first regression analysis, age group, season, sex and socioeconomic status were all significantly associated with admission to hospital for a diagnosis of interest. The pattern of odds for age group was the same as in the previous analysis with decreased odds for the first eight age groups and increased odds for the last three. There were some small changes to the odds ratios in the older age groups, but again all were significant with a p value of 0.0005 for all age groups. The situation was similar for socioeconomic status, sex and season as in the previous analysis.

Examining the results of the distance variable more closely it can be seen that with respect to the persons living within 2-4km of the city centre there is a very slight protective effect associated with living at this distance as compared with those living within 0-2km of the city centre although this association is not significant (OR=0.993; p=0.835). The group 4-6km showed an increased odds of admission to hospital for a diagnosis of interest when compared with the group 0-2km, as did the group 6-12km, although only the odds ratio for the distance group 4-6km was statistically significant (OR=1.137; p=0.001).

The independent variable 'distance' was replaced with the independent variable 'road type' for further analysis. The 'road type' variable made no distinction between the distance of the road from central station and simply grouped the roads according to whether they were an 'A' road, 'B' Road, a Cul de Sac and any other, and the base road type, to which all others are compared, is 'A' roads. Again there was little change in the results for the independent variables age group, socioeconomic status, sex and season all of which were significantly associated with hospital admissions for a diagnosis of interest.

The 'road type' type variable was also significantly associated with hospital admissions for a diagnosis of interest with a p value less than 0.0005. Closer examination also shows a significantly increased odds for two road types, 'B' roads and bus routes, when compared with 'A' roads, with increased odds of almost 30% (p=0.0005) and 21% (p=0.0005) respectively. For persons living in Cul de Sacs the effect was protective, with an odds ratio of 0.983 although this was not significant (p=0.720) and for all other properties there was an increased risk of approximately 4% although again it was not significant (p=0.427).

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			7214.080	11	.000	
	Age_Group(1)	-1.480	.079	349.864	1	.000	.228
	Age_Group(2)	-2.932	.123	571.842	1	.000	.053
	Age_Group(3)	-3.327	.103	1037.083	1	.000	.036
	Age_Group(4)	-3.505	.101	1211.445	1	.000	.030
	Age_Group(5)	-2.842	.082	1197.913	1	.000	.058
	Age_Group(6)	-1.845	.065	794.791	1	.000	.158
	Age_Group(7)	-1.061	.058	332.252	1	.000	.346
	Age_Group(8)	-.272	.054	25.117	1	.000	.762
	Age_Group(9)	.185	.053	12.249	1	.000	1.203
	Age_Group(10)	.495	.057	74.902	1	.000	1.640
	Age_Group(11)	.778	.088	78.837	1	.000	2.177
	Sex(1)	-.381	.025	236.362	1	.000	.683
	SES_1			160.344	4	.000	
	SES_1(1)	.159	.041	14.797	1	.000	1.172
	SES_1(2)	-.116	.043	7.075	1	.008	.891
	SES_1(3)	-.156	.039	16.121	1	.000	.856
	SES_1(4)	-.364	.034	114.217	1	.000	.695
	Season(1)	-.141	.024	33.461	1	.000	.868
	Road_Type			49.752	4	.000	
	Road_Type(1)	.260	.068	14.465	1	.000	1.296
	Road_Type(2)	.191	.050	14.458	1	.000	1.210
	Road_Type(3)	-.017	.047	.129	1	.720	.983
	Road_Type(4)	.036	.046	.631	1	.427	1.037
	Constant	-3.015	.065	2158.806	1	.000	.049

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Road_Type.

Table 4.36: Results of the binary logistic regression with all admissions as the dependent variable and where road type is the independent variable of interest

Results of the logistic regression analysis with hospital admissions split by ICD code

The next step in the regression analysis was to investigate whether there was any difference between the different diagnoses. To do this, the admission data were recoded into new variables according to whether the primary diagnosis corresponded to a cardiovascular illness (ICD-I) or a respiratory illness (ICD-J). The regression analysis was then rerun for all the different independent variables, but with admitted with a cardiovascular diagnosis or not as the dependent variable, and then again with the dependent variable changed to represent those admitted with a respiratory diagnosis or not.

ICD I (cardiovascular diseases)

In the following group of analysis the dependent variable admitted/not admitted for any diagnosis of interest has been replaced by the dependent variable admitted/not admitted for any diagnosis with an ICD I code of interest. In the first run of the regression analysis, the primary independent variable of interest was again road category. Again the age group variable was significant (p=0.0005), with a protective effect for all ages from 2 years to 20 years although none of the odds were significant, after which the odds increased for all age groups from 21-30 (OR=1.347; p=0.686) to >90 years (OR=242.606; p=0.0005). Sex again showed a reduced odds indicating a protective effect in favour of females and socioeconomic status showed reduced odds through the categories from the median to the most affluent group again indicating a protective effect for these three groups. Road category was again significant as a variable (p=0.0005) and scanning down the individual categories it can be seen that most individual categories also had significantly increased odds ratios when compared with category one (A&B roads within 0-2km of the city centre). A roads and bus routes within both the 2-4 and 4-6km category showed the largest increases in odds and all were significant. Although B roads also showed increases in odds in the same two distance categories as well as in the distance group 6-12km, but only the Odds ratio for B roads within 4-6km and 6-12km were significant (OR=1.063; p=0.877, OR=1.905; p=0.01 and OR=2.230; p=0.006). Overall all categories showed increased odds when compared with category one (A&B roads within 0-2km of the city centre) and the majority of these were significant.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			2680.285	11	.000	
	Age_Group(1)	-.804	1.000	.646	1	.421	.448
	Age_Group(2)	-12.409	202.277	.004	1	.951	.000
	Age_Group(3)	-1.200	.866	1.919	1	.166	.301
	Age_Group(4)	.298	.739	.163	1	.686	1.347
	Age_Group(5)	1.231	.721	2.919	1	.088	3.425
	Age_Group(6)	3.113	.710	19.240	1	.000	22.490
	Age_Group(7)	4.134	.708	34.060	1	.000	62.418
	Age_Group(8)	4.863	.708	47.183	1	.000	129.408
	Age_Group(9)	5.201	.708	53.982	1	.000	181.450
	Age_Group(10)	5.308	.709	56.107	1	.000	202.031
	Age_Group(11)	5.491	.716	58.885	1	.000	242.606
	Sex(1)	-.614	.035	310.257	1	.000	.541
	SES_1			46.511	4	.000	
	SES_1(1)	.098	.066	2.222	1	.136	1.103
	SES_1(2)	-.127	.064	4.028	1	.045	.880
	SES_1(3)	-.124	.056	4.865	1	.027	.884
	SES_1(4)	-.285	.050	32.951	1	.000	.752
	Season(1)	.052	.034	2.393	1	.122	1.053
	Category_1			84.333	18	.000	
	Category_1(1)	.526	.193	7.406	1	.007	1.692
	Category_1(2)	.452	.189	5.711	1	.017	1.572
	Category_1(3)	.390	.173	5.068	1	.024	1.477
	Category_1(4)	.777	.220	12.468	1	.000	2.176
	Category_1(5)	.061	.392	.024	1	.877	1.063
	Category_1(6)	.882	.175	25.471	1	.000	2.416
	Category_1(7)	.251	.186	1.825	1	.177	1.286
	Category_1(8)	.497	.171	8.430	1	.004	1.643
	Category_1(9)	.852	.244	12.196	1	.000	2.345
	Category_1(10)	.644	.249	6.707	1	.010	1.905
	Category_1(11)	.766	.180	18.201	1	.000	2.151
	Category_1(12)	.593	.176	11.339	1	.001	1.810
	Category_1(13)	.600	.170	12.417	1	.000	1.821
	Category_1(14)	.569	.179	10.081	1	.001	1.767
	Category_1(15)	.802	.291	7.587	1	.006	2.230
	Category_1(16)	.489	.198	6.076	1	.014	1.631
	Category_1(17)	.691	.175	15.651	1	.000	1.997
	Category_1(18)	.543	.175	9.676	1	.002	1.721
	Constant	-9.060	.726	155.750	1	.000	.000

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Category_1.

Table 4.37: Results of the binary logistic regression with ICD-I admissions as the dependent variable and where road category is the independent variable of interest

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			2699.790	11	.000	
	Age_Group(1)	-.805	1.000	.649	1	.421	.447
	Age_Group(2)	-12.412	202.451	.004	1	.951	.000
	Age_Group(3)	-1.197	.866	1.911	1	.167	.302
	Age_Group(4)	.299	.739	.164	1	.686	1.348
	Age_Group(5)	1.229	.721	2.908	1	.088	3.418
	Age_Group(6)	3.113	.710	19.238	1	.000	22.485
	Age_Group(7)	4.131	.708	34.014	1	.000	62.241
	Age_Group(8)	4.858	.708	47.089	1	.000	128.768
	Age_Group(9)	5.196	.708	53.874	1	.000	180.482
	Age_Group(10)	5.313	.709	56.219	1	.000	203.050
	Age_Group(11)	5.518	.716	59.474	1	.000	249.203
	Sex(1)	-.611	.035	308.105	1	.000	.543
	SES_1			48.965	4	.000	
	SES_1(1)	.085	.063	1.819	1	.177	1.089
	SES_1(2)	-.144	.062	5.350	1	.021	.866
	SES_1(3)	-.125	.054	5.422	1	.020	.882
	SES_1(4)	-.291	.049	35.716	1	.000	.747
	Season(1)	.052	.034	2.370	1	.124	1.053
	Distance			18.611	3	.000	
	Distance(1)	.179	.057	9.769	1	.002	1.196
	Distance(2)	.247	.058	17.904	1	.000	1.280
	Distance(3)	.208	.060	12.043	1	.001	1.231
	Constant	-8.665	.709	149.576	1	.000	.000

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Distance.

Table 4.38: Results of the binary logistic regression with ICD-I admissions as the dependent variable and where distance is the independent variable of interest

Running the logistic again and replacing the independent variable 'category' with the variable 'distance' resulted in a similar result to the previous analysis.

The distance category was significant at the 5% level with a p value of 0.0005, and practically identical to the p value obtained when using hospital admissions for any ICD of interest as the dependent variable. In that run the distance category was also significant at the 5% level with a p value of 0.0005.

Once again looking at the individual categories, it can be seen that for all individual categories of distance there is an increased odds ratio when compared with the distance category 0-2km. The largest increase in odds was for the distance category 4-6km for which the odds ratio was 1.280 (p=0.0005), which corresponds to an increase in odds of being admitted to hospital of 28% over persons living in the category 0-2km.

Substituting the ‘distance’ category for the ‘road type’ category in the regression analysis resulted in road type being a significant predictor of admissions with a primary diagnosis corresponding to any ICD-I of interest (p=0.0005). Unlike previous analysis, the individual road types were not significantly associated with an increased odds for admission with a diagnosis corresponding to ICD-I when compared with the base road type (‘A’ roads), apart from bus routes (OR=1.223; p=0.003). A slight protective effect was noticed for the category cul de sacs (OR = 0.988) and a small increase in odds for the category all other properties although neither were significant.

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			2676.310	11	.000	
	Age_Group(1)	-.803	1.000	.645	1	.422	.448
	Age_Group(2)	-12.409	202.478	.004	1	.951	.000
	Age_Group(3)	-1.204	.866	1.932	1	.165	.300
	Age_Group(4)	.277	.739	.141	1	.708	1.319
	Age_Group(5)	1.220	.721	2.867	1	.090	3.388
	Age_Group(6)	3.104	.710	19.131	1	.000	22.292
	Age_Group(7)	4.124	.708	33.905	1	.000	61.830
	Age_Group(8)	4.846	.708	46.859	1	.000	127.248
	Age_Group(9)	5.184	.708	53.639	1	.000	178.452
	Age_Group(10)	5.294	.709	55.809	1	.000	199.165
	Age_Group(11)	5.478	.716	58.616	1	.000	239.468
	Sex(1)	-.606	.035	303.388	1	.000	.545
	SES_1			43.053	4	.000	
	SES_1(1)	.201	.058	12.207	1	.000	1.223
	SES_1(2)	-.094	.061	2.353	1	.125	.910
	SES_1(3)	-.037	.052	.497	1	.481	.964
	SES_1(4)	-.198	.045	19.391	1	.000	.820
	Season(1)	.052	.034	2.375	1	.123	1.053
	Road_Type			20.648	4	.000	
	Road_Type(1)	.049	.100	.238	1	.626	1.050
	Road_Type(2)	.201	.067	8.950	1	.003	1.223
	Road_Type(3)	-.012	.063	.038	1	.845	.988
	Road_Type(4)	.031	.061	.254	1	.614	1.031
	Constant	-8.571	.710	145.839	1	.000	.000

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Road_Type.

Table 4.39: Results of the binary logistic regression with ICD-I admissions as the dependent variable and where road type is the independent variable of interest

ICD J (Respiratory Diseases)

The final run of regression analyses substituted the previous dependent variable for the dependent variable 'admitted with a primary diagnosis corresponding to an ICD-J of interest'. Again the first run had road category as the primary independent variable of interest.

Road category was significant with a p value of 0.0005 for the whole category. Scanning down the table and examining each category individual reveals that several individual categories were significantly associated with increased hospital admissions with a primary diagnosis corresponding to and ICD-J code of interest. The largest increase in odds was for people living in cul de sacs within 6-12km of the city centre (OR=3.229; p=0.001). Living in cul de sacs was also showed significantly increased odds in both the distance categories 0-2km and 4-6km from the city centre (OR=1.728; p=0.022 and OR=1.836; p=0.011 respectively). Other categories with significantly increased odds include bus routes within 0-2km and 4-6km of the city centre and B roads within 4-6km and 6-12km.

All other independent variables apart from sex were significantly associated with hospital admissions. There was a slight increase in odds ratio for the variable sex (OR=1.005) but with a p value of 0.995 it is reasonable to say there is no gender effect, i.e. there is no difference between males and females in terms of their chances of being admitted to hospital with a respiratory illness. In terms of the variable season there was a significant protective effect (OR=0.539; p=0.0005) in favour of the warm season. For socioeconomic status there was a slight increase in odds from the first group (most deprived) to the second (next deprived) but this was not significant. For all other groups there was a protective effect that increased through the groups (i.e. odds ratio decreased) and all were significant.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			3028.600	11	.000	
	Age_Group(1)	-1.487	.080	343.546	1	.000	.226
	Age_Group(2)	-2.903	.123	558.507	1	.000	.055
	Age_Group(3)	-3.368	.107	998.837	1	.000	.034
	Age_Group(4)	-3.822	.116	1081.498	1	.000	.022
	Age_Group(5)	-3.360	.101	1106.739	1	.000	.035
	Age_Group(6)	-3.264	.107	936.658	1	.000	.038
	Age_Group(7)	-3.420	.124	756.177	1	.000	.033
	Age_Group(8)	-2.723	.105	677.874	1	.000	.066
	Age_Group(9)	-2.202	.092	568.090	1	.000	.111
	Age_Group(10)	-2.293	.133	297.341	1	.000	.101
	Age_Group(11)	-2.527	.358	49.832	1	.000	.080
	Sex(1)	.005	.050	.009	1	.922	1.005
	SES_1			103.857	4	.000	
	SES_1(1)	.138	.092	2.224	1	.136	1.147
	SES_1(2)	-.293	.096	9.307	1	.002	.746
	SES_1(3)	-.601	.101	35.489	1	.000	.548
	SES_1(4)	-.700	.085	67.826	1	.000	.497
	Season(1)	-.618	.052	141.367	1	.000	.539
	Category_1			62.462	18	.000	
	Category_1(1)	.504	.251	4.033	1	.045	1.656
	Category_1(2)	.547	.239	5.228	1	.022	1.728
	Category_1(3)	.264	.227	1.356	1	.244	1.302
	Category_1(4)	.029	.362	.006	1	.937	1.029
	Category_1(5)	.315	.464	.461	1	.497	1.371
	Category_1(6)	.092	.246	.140	1	.708	1.097
	Category_1(7)	.278	.243	1.313	1	.252	1.321
	Category_1(8)	.143	.229	.389	1	.533	1.153
	Category_1(9)	-.357	.547	.427	1	.514	.700
	Category_1(10)	.862	.335	6.631	1	.010	2.368
	Category_1(11)	.608	.240	6.399	1	.011	1.836
	Category_1(12)	.063	.239	.070	1	.791	1.065
	Category_1(13)	.451	.224	4.032	1	.045	1.569
	Category_1(14)	.019	.263	.005	1	.941	1.020
	Category_1(15)	1.172	.366	10.253	1	.001	3.229
	Category_1(16)	.603	.269	5.033	1	.025	1.827
	Category_1(17)	.308	.231	1.780	1	.182	1.361
	Category_1(18)	.336	.231	2.107	1	.147	1.399
	Constant	-3.172	.220	207.469	1	.000	.042

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Category_1.

Table 4.40 Results of the binary logistic regression with ICD-J admissions as the dependent variable and where road category is the independent variable of interest

The distance variable was a significant variable with a p value of 0.015. The distance category 2-4km showed a protective effect compared with the distance category 0-2km which was significant (OR=0.843; p=0.03) while the other two distance categories showed very slightly increased odds compared with the category 0-2km but these were insignificant. Results for the other variables were again similar to those obtained in the previous run when examining the effect of road category.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			3039.583	11	.000	
	Age_Group(1)	-1.487	.080	343.666	1	.000	.226
	Age_Group(2)	-2.902	.123	558.457	1	.000	.055
	Age_Group(3)	-3.365	.106	998.414	1	.000	.035
	Age_Group(4)	-3.829	.116	1088.184	1	.000	.022
	Age_Group(5)	-3.361	.101	1108.594	1	.000	.035
	Age_Group(6)	-3.260	.107	936.032	1	.000	.038
	Age_Group(7)	-3.420	.124	757.274	1	.000	.033
	Age_Group(8)	-2.713	.104	675.384	1	.000	.066
	Age_Group(9)	-2.192	.092	566.381	1	.000	.112
	Age_Group(10)	-2.274	.133	294.154	1	.000	.103
	Age_Group(11)	-2.505	.358	49.062	1	.000	.082
	Sex(1)	.004	.050	.007	1	.931	1.004
	SES_1			117.754	4	.000	
	SES_1(1)	.011	.086	.017	1	.895	1.011
	SES_1(2)	-.276	.095	8.496	1	.004	.759
	SES_1(3)	-.677	.098	47.777	1	.000	.508
	SES_1(4)	-.754	.083	82.009	1	.000	.470
	Season(1)	-.618	.052	141.700	1	.000	.539
	Distance			10.483	3	.015	
	Distance(1)	-.171	.079	4.718	1	.030	.843
	Distance(2)	.067	.077	.742	1	.389	1.069
	Distance(3)	.007	.079	.007	1	.933	1.007
	Constant	-2.829	.073	1508.282	1	.000	.059

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Distance.

Table 4.41: Results of the binary logistic regression with ICD-J admissions as the dependent variable and where distance is the independent variable of interest

When considering road type, B road, bus route and cul de sac categories showed an increase in odds when compared with the A road category with an increase of in odds of 85% (p=0.0005); 57% (p=0.0005) and 26% (p=0.047) respectively. The category all other properties also showed a significant increase in odds of almost 40% (p=0.0005). Again the result obtained for the other variables did not differ hugely from those obtained when investigating the associations with either road category or distance, with no apparent gender effect and protective effects for both socioeconomic status and season.

Variables in the Equation

		B	S.E.	Wald	df	Sig.	Odds
Step 1(a)	Age_Group			3070.828	11	.000	
	Age_Group(1)	-1.486	.080	343.360	1	.000	.226
	Age_Group(2)	-2.903	.123	558.654	1	.000	.055
	Age_Group(3)	-3.375	.106	1005.115	1	.000	.034
	Age_Group(4)	-3.850	.116	1107.957	1	.000	.021
	Age_Group(5)	-3.368	.101	1114.658	1	.000	.034
	Age_Group(6)	-3.269	.107	940.788	1	.000	.038
	Age_Group(7)	-3.426	.124	759.928	1	.000	.033
	Age_Group(8)	-2.723	.104	679.945	1	.000	.066
	Age_Group(9)	-2.202	.092	570.824	1	.000	.111
	Age_Group(10)	-2.297	.133	299.501	1	.000	.101
	Age_Group(11)	-2.549	.358	50.751	1	.000	.078
	Sex(1)	.005	.050	.010	1	.919	1.005
	SES_1			118.083	4	.000	
	SES_1(1)	.122	.078	2.450	1	.118	1.129
	SES_1(2)	-.320	.093	11.873	1	.001	.726
	SES_1(3)	-.620	.096	41.798	1	.000	.538
	SES_1(4)	-.677	.079	73.191	1	.000	.508
	Season(1)	-.618	.052	141.639	1	.000	.539
	Road_Type			25.516	4	.000	
	Road_Type(1)	.617	.152	16.593	1	.000	1.854
	Road_Type(2)	.451	.124	13.192	1	.000	1.570
	Road_Type(3)	.233	.117	3.949	1	.047	1.263
	Road_Type(4)	.333	.117	8.137	1	.004	1.395
	Constant	-3.173	.123	664.492	1	.000	.042

a Variable(s) entered on step 1: Age_Group, Sex, SES_1, Season, Road_Type.

Table 4.42: Results of the binary logistic regression with ICD-J admissions as the dependent variable and where road type is the independent variable of interest

Briefly, this study involved examining three different factors relating to traffic in an effort to determine whether living in certain areas resulted in an increased odds of hospital admissions for cardiovascular and/or respiratory disease. The data were analysed for all admissions, respiratory admissions only and cardiovascular diseases only and logistic regression analysis was conducted to investigate how these three dependent variables were affected by a variety of other factors, including road type, road category and distance from city centre (full details on page 206). Overall the results of this section show that there is an increased odds of being admitted to hospital with cardiovascular or respiratory illnesses for people living in different areas. Whether or not this is as a result of increased exposure to ultra fine particles from vehicular traffic is not yet clear and the results of this work require some investigation and discussion before it can be stated with any conclusiveness that road type can in fact be used as a proxy for exposure to ultra fine particulate pollution from vehicular traffic.

Chapter Five
Discussion and Conclusion

Many international studies have reported an association between levels of particulate pollution and various health endpoints including mortality. Although various different measures of particulates have been examined, little to no evidence exists regarding the effects of ultrafine particles on human health endpoints. The first part of this study attempted to measure levels of ultrafine particles and relate the levels measured to the level of various biomarkers of effect measured in the blood of persons living in areas of differing ultrafine particle exposure levels.

5.1 An exposure comparison study designed to examine the effects of exposure to traffic pollution on health

Overall, although a clear difference was observed between the mean outdoor particle numbers measured in the exposed and unexposed areas, no significant differences were found in the levels of the majority of the blood biomarkers examined.

Analysing the data for the presence of a dose response relationship failed to show a statistically significant difference in the levels of biomarkers measured in the three exposure groups notwithstanding the fact that again the difference between the three groups in terms of their mean number of outdoor particles/cm^3 was statistically significant.

These results would appear to suggest that there was no affect of traffic pollution on the levels of these cardiovascular or respiratory risk factors which would in turn indicate that these healthy individuals are not in fact at risk for developing such illnesses in later life as a consequence of alteration in these biomarkers of effect. Other reasons for the lack of differences between the two groups in terms of their levels of biomarkers may be that the biomarkers chosen to be examined were not the most appropriate choice. This is unlikely however as there is a large body of evidence to suggest that all of the biomarkers chosen are likely to be in some way affected, either directly or indirectly, by exposure to particulate pollution. In fact one of the particular strengths of the study is the wide variety of biomarkers that were chosen for examination. Many studies evaluating the effect of particles on various biomarkers concentrated on only one or two rather than the variety examined in this study. For example, Schwartz examined three cardiovascular risk factors, platelet counts, plasma fibrinogen and white blood cells, all of which were measured in this study, and found significant associations with PM_{10}.

Personal exposure is another factor that may have affected the outcome of this study. The levels of ultra fine particulates that each individual is exposed to will differ according to their lifestyles and measuring the particle numbers using the P-trak may not have adequately reflected this. The idea of personal exposure monitoring is discussed later.

Despite the fact that many studies have been conducted to examine the effects of exposure to particulate pollution, the majority of them have been time-series studies examining the effects of acute exposure to particulate pollution, which means that only limited data exists regarding the effects of chronic exposure.

There are a number of reasons why the results of this study did not show a statistically significant difference between the exposed and unexposed groups or when investigating the presence of a dose-response relationship.

5.1.1 Recruitment process

The recruitment process is often the most difficult undertaking in any study such as this, convincing people to take part in a study in which they perceive no individual benefit can be an incredibly difficult and sometimes impossible task. This study differs from most in the sense that it was more concerned with the possible effects of chronic exposure to particulates on healthy people rather than susceptible populations, and so recruiting people using traditional methods such as through hospital admissions, pre-existing cohorts or GP visits was not appropriate. The only alternative was to identify areas of interest for the study and to try and recruit residents of the areas by sending letters to every residential address point on the streets. Without knowing anything about the persons to which these letters were being sent there was no way to tell how responsive they were likely to be and with so much junk mail nowadays a common problem with blind mailing of this sort is the chance that many potentially interested parties would simply throw the letter away without reading it, or that persons interested in taking part may forget to return their letter. This also made follow-up on the letters particularly difficult as it was only possible to follow up on letters for which replies were received.

Many people who had expressed an interest in taking part in the study asked when contacted what they would get out of taking part. Several eligible people refused to take part on hearing that they would not be financially reimbursed for doing so. Monetary remuneration was discussed as a possible approach to enticing people to take part in the study; however the financial constraints meant that this was not an option, although it may be a possible option when considering recruitment for similar future studies.

The difficulty in recruiting people to this study is evidenced by the fact that of more than 8,000 letters sent out, only about 450 were returned and many of these were from people asking not to be contacted in the future either because they were ineligible for participation or because they were not interested in taking part. And although many of the people who responded were interested and willing to take part, most were ineligible due to age, smoking status or health status.

Time constraints presented another problem for the recruitment stage of the study, some epidemiological studies, particularly cohort studies will have a recruitment stage lasting several years with dedicated staff concentrating solely on the recruitment, in this study however the recruitments stage was often conducted alongside the data collection part of the study making it difficult to concentrate entirely on the recruitment. Other factors affecting the number of participants included difficulty arranging times to visit participants as this had to be done to suit the participants and often this meant evenings.

For the majority of studies such as this the big question will always be 'how representative of the total population is the sample?' Examining the NHSAR database indicated that there were more than 60,000 people in Cardiff between the ages of 50 and 70 years and of these just over 31,000 was male in 2002. Roughly the same numbers of letters were sent to the exposed and unexposed areas and the response from the two areas was more or less the same, with 250 people responding from the exposed area and 189 from the unexposed. Not all of these responses however were positive, in fact the majority of the responses in both groups were either negative or the willing participant was ineligible to participate according to the criteria of the study. Given some of the comments made on the letters that were returned as well as examination of the areas from which the letters were returned it is thought that there is something about the group of people that chose to respond that is different, be it in terms of their education or their perceptions, that made them more likely to reply. For example, of the people who replied and were willing to take part in the study, a number stated that they felt that they suffered the supposed effects of pollution including wheeze and shortness of breath or asthma and felt sure that the volume of traffic in their area was the reason for this.

It was felt that for a study examining effects in a healthy population there was always going to be a problem with representativeness and there is little that can be done to overcome this other than to perhaps find another way to recruit people rather than blind mailing, which allows direct access to known persons, maybe by enlisting the help of different GP's in recruiting people off their register, and thus recruiting a group of people more representative of the total population. As it is, this population of this study are likely to represent a small subset of the total population and most probably consists of persons with a reasonable level of education and an interest in the possible public health implications of the results of such a study. It is still felt however that there is no real way to remove the type of 'self selection' bias observed in this study as it was felt that the people who responded and were willing to take part in this study were likely to be more concerned about their health than those who didn't and this is always likely to be the case when attempting to recruit people into a study such as this.

5.1.2 Using local traffic data to identify the areas of high traffic volume in Cardiff

When it came to selecting the streets from which to draw participants for each group in the study the method of choice was to use traffic data collected by Cardiff transport authority to identify streets with high and low traffic volumes. The study highlighted the lack of quality traffic data that could be used for research purposes that was available not only in Cardiff but in Wales and the UK as a whole. Traffic data existed for only a small number of the most trafficked streets in Cardiff and meant that for streets to be included in the unexposed group, local knowledge of the city was the only method by which the areas of low traffic volume could be identified.

High traffic volumes can be problematic for a number of reasons other the various respiratory and cardiovascular health effects thought to be associated with the higher pollution levels around busy roads. For instance high volumes of traffic result in traffic jams, road traffic accidents and increased stress levels in commuters resulting in accidents and incidents of road rage. It is strange therefore that for many cities in Wales, such poor data regarding the numbers and types of vehicles using the roads exists; as with so many problems associated with traffic, such data would be invaluable to researchers in their efforts to elucidate the frequency and severity of such effects. Clearly better traffic data is needed for many of the cities in Wales. Swansea City Council have already begun the process of building a comprehensive profile of Swansea traffic and in the future it is hoped that the same will be done in other major cities in the UK. Traffic pollution has become the main focus of particulates in cities in the UK and so the lack of accurate traffic data severely hinders the ability to conduct scientific research into the effects of traffic pollution on the health of the population.

5.1.3 Blood sample collection

Collecting the blood samples was an area that presented many concerns for the study. As mentioned previously the blood samples were unable to be collected from participants at the time of interview as was originally desired. This meant that participants had to be convinced to come into the outpatients department and provide their blood sample in the phlebotomy unit. This raised a number of issues the key one being that the outpatients department do not have the facility to make appointments and so it was up to the individual participants to come to the unit whenever they had the time or the inclination and the risk of participants forgetting or deciding against coming to give blood was therefore much higher than if they had been told when to be there. This fact is reflected in the fact that indeed quite a number of participants, particularly in the unexposed group, did not donate a blood sample despite a reminder letter and follow-up phone call. This will also have had the potential to affect the results of the blood tests as beyond requesting that persons donating blood were to have been in residence in their immediate area for at least three weeks before giving the blood sample and to have allowed a minimum of six weeks between any infectious illness such as a cold or flu before giving blood to allow the various inflammatory biomarkers to return to normal, there was no way to ensure that this had been the case when people gave the blood samples. Another factor that might possibly have affected the outcome of the blood tests includes acute exposures to particles or to other pollutants in the hours prior to providing a blood sample, for example a participant may have walked from their home to the hospital along busy streets and been exposed, for a short time, to levels of pollution that are higher than they would normally have been.

In the future if blood samples are to be collected in this manner it might be prudent to have someone meet with participants when they come to the hospital to provide their sample and administer a brief questionnaire in an effort to ascertain whether there may have been anything that might affect the outcome of the test results and which could then be used to accounted for during the analysis of the results. Ideally however in a study requiring blood samples for analysis it would be much better if a nurse or phlebotomist could visit the participants in their home and take the samples there, having first spoken to the participant to ensure that they fulfil the requirements.

5.1.4 Using the P-trak to measure the number of particles per cm^3 in the exposed and unexposed areas

Like so many studies before it, one of the main issues for this study was how to most accurately gauge the levels of particulate pollution to which the participants were exposed. Air pollution distribution is not homogenous across different areas and an individual's exposure levels are likely to vary very much with respect to their day to day routine, something that is very difficult to account for when estimating their individual levels of exposure. The primary option available, and one that is commonly used in exposure studies, was to use the measurements of particulates made by local air quality monitoring stations. The main issue with this was that the monitoring stations in Cardiff only measure levels of PM_{10} and as this study was primarily concerned with the effects of ultrafine particles measurements of PM_{10} were not suitable, particularly given a lack of evidence of how representative PM_{10} measures would be of the levels of ultrafine particles in the area. Personal exposure monitors would be the ideal monitoring method by which the levels of exposure for every individual in the study could be more accurately determined and accounted for during analysis, as in addition to providing information about chronic exposure levels; they can also be used to provide information about acute exposures, which may have occurred immediately prior to providing a blood sample that may affect the outcome of any tests conducted. Personal exposure monitors have been widely used in an occupational setting and becoming increasingly more utilised in environmental epidemiology, although as to our knowledge, no technology currently exists to carry out personal exposure measurements to ultrafine particles hence ruling out personal exposure monitors as a viable option for this study.

Some analysis has been carried out to investigate how well the measures from fixed monitoring stations correlate with personal exposure measures. Janssen took repeated personal measurements of PM_{10} and fine particles and compared them to measurements made at fixed monitoring stations. She found that for the total population the measurements were poorly correlated, but when people exposed to environmental tobacco smoke were excluded from the analysis the correlation coefficient was considerably higher, particularly for fine particles, suggesting that apart from confounding by environmental tobacco smoke, measurements from fixed monitoring stations adequately reflect the level of exposure to fine particles for an individual, suggesting that for epidemiological time series studies concerned with the association between daily variations in exposure levels and health effects, measurements from fixed monitoring stations provide adequate indication of exposure levels. If it is true that stationary monitoring stations provide satisfactory indications of personal exposure to fine particles then perhaps the same would be true for ultrafine particles; however the evidence base supporting the adequacy for fine particles is at best sparse and a lack of any data relating to ultrafine particles means that this cannot be assumed with any degree of certainty. There is also a question mark over whether the use of a central monitoring station can in anyway reflect the variation in particle levels within the city. While studies suggest that these stationary monitors can be used as an adequate measure of exposure, all they really say is that when particle levels go up and one site, they tend to go up and another but do not provide any information as to the actual difference in particle levels measured. This study found that the mean numbers of particles/cm^3 measured outdoors in Cardiff are almost double in the exposed area compared with the unexposed area, and examining the mean numbers of particles measured outdoors on each street a large degree of difference is seen from the lowest to highest measurements made, suggesting that while fixed monitoring stations may well provide an adequate measure of what is going on in terms of the levels of particles in a city (i.e. whether they are increased above a certain level), they most probably cannot adequately reflect the personal exposures of individuals in different parts of the city.

The P-trak was seen as the best compromise – a method of measuring the levels of ultrafine particles to which an individual was exposed that was more accurate than those measurements provided by a fixed monitoring station because the measurements could be made in the area of residence of the participants but less accurate than measurements from personal exposure monitors, because the measurements from the P-trak still constitute environmental rather than personal exposure measurements resulting in less well defined exposure estimates. The P-trak has not however been used in a similar capacity in the past making it an entirely unknown quantity and this was something that would need to be accounted for. The P-trak is most commonly used in indoor settings to determine the quality of indoor air and determine the source of any potential particle problems and so using it within the setting of this exposure comparison study presented it's own set of problems, some of which could be identified at the outset but most of which presented themselves during the course of the study.

On examining the measurements taken using the P-trak some interesting points were noted. One point, which has already been mentioned, is that the P-trak is a very sensitive machine, and rapidly picks up on changes in particle number in the environment. There is a small display screen on the P-trak which shows the number of particles/cm^3 that are being recorded every second by the machine and from watching how these numbers change through the course of any given monitoring period there is a lot of information that can be gleaned from monitoring these changes in number and recording when they occur and any changes in the area that may be attributed to these changes. It seemed, from watching how different passing vehicles affected the particle counts, that each vehicle creates its own individual particle cloud with the numbers depending on various factors such as vehicle type and speed. Buses and goods vehicles seemed to increase the particle numbers to levels higher than cars and smaller vans, thus creating the characteristic peaks in particle numbers seen on the graphs of particle counts, particularly in the exposed areas. A variety of other factors were noted to affect the numbers of particles throughout the course of the study including; environmental tobacco smoke, and various household activities including cooking, vacuuming. One concern which was raised during the course of the study was whether or not the fact that the P-trak only measured up to 500,000 particles/cm^3 would affect the outcome in any way. For the most part this did not prove to be a problem as levels of particles measured were below this number. On a small number of occasions however the levels of particles recorded did reach and most likely exceed 500,000 particles/cm^3 but there was no way to tell by how much the numbers exceeded 500,000 and this may represent a problem, particularly when it comes to identifying possible hot spots of high particulate pollution. For this study however it was not deemed to be a serious problem as the number of times the levels exceeded the 500,000 particle cut-off were few.

Although only ten minute samples were taken outdoors at each interview, for most streets in the study more than one person was participating and therefore for most streets is a cumulative particle number count in the region of hours rather than minutes. Extra measurements, not related to the individual participants were also taken throughout the course of the study. Measurements were made at times convenient to the participants and times varied between 8am and 9pm, as well as varying in the time of year the measurements were made. This meant that for each street the average measurement was calculated from a large group of individual measurements and to some degree reflected any variation in particle numbers by time of day or year. With the benefit of hindsight however it would have been better that a much more comprehensive profile of particle counts for each street was obtained especially as with such a sensitive machine, there is a chance that the average measurements for some of the streets could be thrown out as a result of a small number of excessively high measurements, thus giving an inaccurate particle exposure estimation, and result in over or indeed under estimation of the health effects of exposure to particulate pollution. Another factor which may have affected the ultrafine particle data is the possibility of variability in the levels of ultrafine particles throughout the day. It was noticed during the course of the data collection that passing vehicles affected the levels of ultrafines and it stands to reason that the more vehicles there are when measuring the higher the levels of ultrafines that will be recorded, suggesting that peak hours in the morning and evening are likely to be the times for which the levels of ultra fine particles will be at their highest. For this study however the measurements were made at varying times through out the day and it was felt that this possible variation is adequately accounted for. Unaccounted variation in ultra fine particle number in different micro-environments may also explain the lack of significant associations.

Little evidence is available to enable comparisons of the particle numbers recorded in this study with particle numbers recorded in similar work, but a study by Hughes et al[197] found that in Pasadena, California, ultra fine particle numbers measured were in the range of $1.3\text{-}8.9\text{x}10^4$ particles/cm^3, while in Erfurt, Germany, Wichmann et al[198] found that the daily average number of ultra fine particles was $1.8\text{x}10^4$ particles/cm^3. In this study the outdoor average number of particles/cm^3 ranged from $1.197\text{x}10^4$ to $3.57\text{x}10^4$; numbers which are similar to those found in previous studies.

The possibility that the numbers of particles will differ in different microenvironments is very real, and one for which little data currently exists. As mentioned previously, when taking the indoor measurements, the P-Trak was placed in a room towards the front of the house, or a room which faced onto the road of interest. This was most often a sitting room or a dining room and not a kitchen. The number of particles generated in a kitchen is quite high due to the type of activities going on (e.g. cooking) and to have placed the P-trak in the kitchen may have resulted in an inflated indoor exposure estimate. A study in Abeerdeen University[199] found that the highest exposure to particle numbers occurred in traffic, during cooking and when people were smoking. Cooking with gas in particular raised the number of ultrafines as did using oil or butter during cooking and cooking fatty foods. The conclusion of this work was that there is a clear difference in the numbers of ultrafines in some microenvironments and that these levels can sometimes be higher than those measured outdoors. A study by Levy[200] also found that particle counts differed significantly among some indoor environments with higher levels again being found close to traffic and other combustion sources such as cookers. This suggests that when considering particle number as an exposure variable perhaps personal exposure monitoring methods are more appropriate and that if unable to employ such methods then the use of detailed activity diaries outlining the daily routine of each individual being studied, along with more detailed particle number data in an effort to calculate and more accurate level of exposure for each individual should be considered.

One of the primary advantages of using a piece of kit such as the P-trak is it's portability, being a hand held machine, it is easily transported between locations and is user friendly in terms of using the machine. It does not require very much by way of maintenance and is easy to set up. Recently the department has acquired three new condensation particle counters that would in many ways have been better for use in this study than the P-trak. The main advantage over the P-trak is that the new models can take measurements for up to 24 hours as compared with the measurements of only 4-6 hours that can be taken by the P-trak and with three of them simultaneous measurements could be made indoors and outdoors for each participant. Simultaneous measurements could also be made at outdoor locations in the exposed and unexposed areas. The new models are not without their problems however, chief of which is the inability of the machine to log and store particle measurement data for later download. This means that unlike the P-trak, the new condensation particle counters require that a computer be connected at all times so that the information is downloaded directly to the software where it is stored for future analysis. The new condensation particle counters also generate quite a bit more noise than the P-trak and one thing that came out of this study was that people really do not want anything that is going to infringe on their daily lives and noisy machines were something they were not prepared to have in their homes for prolonged periods. Given the difficulty with recruiting people to the study, it may not have been possible to use the newer condensation particle counters except in a small number of homes for comparison purposes.

A statistically significant difference was found between the mean number of particles/cm^3 measured outdoors in the exposed area and in the unexposed area. Results for both the ANOVA and the Mann-whitney U tests were highly statistically significant with p values of 0.0005. This difference in the two areas is highly likely be the result of the differences in traffic volumes but equally there may have been a confounding effect by one or more unknown factors. It was thought that the levels of indoor particles between the two areas should not differ significantly although a question was raised as to whether ultrafine particles from outside were likely to penetrate indoors thus increasing the levels of indoor particles in the exposed areas. For some individual measurements the levels of indoor particles were indeed higher than the outdoor levels but the cumulative mean of the indoor levels was not significantly different for the two groups, as was shown by the results of the ANOVA and mann-whitney tests. This might suggest that the numbers of particles penetrating the houses was similar in both areas although the number of particles penetrating will depend on the concentration gradient and it is therefore possible that the levels of particles measured outdoors in the exposed area were not high enough to result in increased penetration of particles from outdoors to push up the levels of indoor particles. It is more likely that it is indicative of the fact that there are various factors indoors which affect the numbers of particles and these are likely to be similar for both areas. Further support for this second theory comes from the fact that various household activities such as cooking, dusting, vacuuming and even ironing were noticed to result in higher indoor particle counts. The results of the additional analysis, comparing the levels of indoor particles to the levels of outdoor particles measured in each area can be seen to further support the theory that particles from traffic are the most likely reason for the differences in outdoor levels in the two areas. Results of ANOVA and mann-whitney U tests show that there was a statistically significant difference in the levels of outdoor particles compared with indoor particles in the exposed areas, whereas in the unexposed areas the difference was not statistically significant (see appendix 4 for full results). With levels of indoor and outdoor particles in the unexposed areas virtually the same it would appear to suggest that barring some unknown and unaccounted for confounder, the elevated levels of ultrafine particles in the exposed areas are the result of higher traffic volumes.

5.1.5 Examining the correlation between measured particle numbers and traffic volume

Some traffic data was made available by Cardiff City Council at the outset of the study and so this could be used in conjunction with the particle number data collected in an effort to further consolidate the theory that the elevated levels in the exposed areas were due to traffic.

Much of the epidemiological evidence supporting the theory of health effects associated with exposure to particles is provided by time series studies that examine the effects of short-term variations in particle numbers and primarily examines the effect of such particle size fractions as PM_{10} or $PM_{2.5}$. There is very little epidemiological evidence to support the associations between traffic generated particles and various health endpoints other than from a small number of studies conducted by Brunekreef and others in the Netherlands who have examined the levels of particulates at varying distances from the roadside and found that the levels decline in a curvilinear pattern up to 300 metres from the roadside, which if true would suggest that persons living within 300 metres of a road with high traffic volumes would be exposed to higher levels of particulate pollution. In this study an attempt was made to correlate actual mean particle data with traffic data for a small number of streets and although not significant a small, positive correlation was found (r=0.137). From the scatter plot there appeared to be a number of outliers which may be affecting the results and reducing the correlations. Two streets in particular, Cowbridge Road West and North Road, had higher traffic counts than perhaps would have been expected given their particle counts. Both of these streets however are main routes into and out of the city centre and as such are heavily trafficked throughout the day. Also both streets link to major junctions at one end; North road to the Gabalfa interchange and Cowbridge Road West to the Coryton interchange, it is likely therefore that the traffic counts are going to have been affected by where the on the road the count was taken as both roads are long roads with links to several other roads along their length which may mean that the traffic counts overestimate the number of vehicles per day. It is more likely however that it is the particle number data that if inaccurate, with only a small amount of data for each street, it is likely that the mean outdoor particle count obtained for these streets is not an accurate reflection of the true particle count.

Another street, Ninian road seemed to show the opposite on the scatter plot, with a mean outdoor particle count that seemed to be rather high by comparison to the volume of traffic recorded on the street. As Ninian road is situated almost parallel to the main Cardiff city by pass and is less than 500 metres as the crow flies from the route, it was felt that perhaps the mean particle number obtained for the street was possibly being affected by it's proximity to this heavily utilised by pass resulting in higher particle numbers than would have been expected when taking into consideration the traffic volume and for this reason it was felt that there was a logical justification for removing Ninian road data from the analysis to see how it affected the correlation coefficient. Although removing the data from the analysis did in fact increase the correlation coefficient there is still the possibility that the particle number measurements made during the study were not enough to provide a representative mean or that an unexplained or unusual event was responsible for the particle numbers that were higher than the normal level and thus resulted in an over-estimation of the mean particle number. This is particularly plausible given the sensitivity of the p-trak particle counter to sudden changes in the number of particles/cm^3.

The low correlations and the statistically insignificant results obtained in the correlation analysis suggest that while it is possible that there is an association between particle number and traffic volume, it is very likely that errors in both the traffic count and particle counts affect the observed correlation. Traffic volume does affect the level of particles, with transport thought to contribute approximately 24% of to the total UK burden of PM_{10}, whether or not traffic is the reason for the differences in the levels observed in the different areas in this study however may be a question for further study.

5.1.6 Investigating the level of biomarkers of effect in the blood samples of participants

Coagulation factors have been the subject of much investigation over the past few years, particularly fibrinogen concentration, which has been put forward as a risk factor for non-fatal myocardial infarction, stroke and cardiovascular death.

In this study no significant difference was found between the exposed and unexposed groups for any of the coagulation factors measured apart from prothrombin time. There may be a number of reasons why there were seven patients with prothrombin times of 14 seconds or above. For example if any of them had been taking aspirin or other blood thinning drugs for any reason, this would result in an increased prothrombin time. Alternatively, in a random sample of fifty patients it is likely that a few will have some degree of liver impairment, possibly caused by alcohol consumption, and this may explain the elevated prothrombin times observed in the exposed group. Overall however, although as a group, the difference between prothrombin time in the exposed participants was statistically significant compared with the unexposed group (p=0.004) the individual results do not present with any clinically significantly high values and it is fair to say that the difference found between the groups may be to do with the smaller numbers of people in the unexposed group rather than the presence of a random group of people within the exposed group with some other underlying problem causing elevated prothrombin times. The only significant result on investigation of the presence of a dose-response relationship was again for prothrombin, overall there was a significant difference between the three exposure groups (p=0.01) and further investigation revealed that there was a statistically significant difference between groups one and three (p= 0.004). An almost significant difference (p=0.084) was observed between exposure groups two and three and no significant difference was observed between groups one and two (p=0.387). While on the face of it the results may seem to indicate that there is no difference between prothrombin time between either the exposed and unexposed groups or between the three different exposure groups, the presence of some statistically significant results may be the first step in showing that exposure to traffic generated particulate pollution does have an effect on prothrombin time and may be more of a reflection of how small numbers can mask an effect suggesting that with larger numbers and further investigation the true effect could be elucidated.

With respect of fibrinogen, the weight of evidence is in support of the theory that exposure to particulate pollution is associated with increased fibrinogen concentrations but there is some evidence to suggest this is not always the case and the results of this study support the latter, with no significant difference found in fibrinogen concentrations between the exposed and unexposed groups. Neither was any significant dose-response relationship observed but the fibrinogen concentrations in the third group was marginally higher than the in other two but this difference was not significant.

The results of the Mann Whitney U test showed no significant difference between the levels of C reactive protein either between the exposed and unexposed groups (p=0.823) or between the groups when investigating the presence of a dose-response relationship using the Kruskal-Wallis test (p=0.601). As mentioned previously there was a concern with the C reactive protein in the sense that all the results provided were given in whole numbers and it was unknown whether this might have possibly masked any effect. Discussing this with the laboratory personnel revealed that C reactive protein results are reported in whole numbers both from the instrument used to measure the levels and from the laboratory computer presenting the results. The instrument examines the results down to two decimal places before rounding meaning that results of 0.54 and below would be rounded down while results of 0.56 and above would be rounded up. According to laboratory personnel results of 0.55 are rather more tricky as it is not clear whether they go up or down although it is thought that the instrument goes down further decimal places before rounding in these cases. It was considered unlikely that the rounding of the results would affect the outcomes due to the sensitivity of the methods and so the lack of a significant difference between the groups may very well be a real result. However there is also the possibility that the rounding of the results is not a problem for individual persons receiving the result of a CRP test as provided the concentration falls within the normal range there is little more an individual needs to know. In terms of conducting statistical analysis on a population however, small differences in measures may well be significant and not having this information means that there may be a difference in the groups that cannot be identified.

White blood cells, neutrophils and platelets are all related to inflammation and have all been examined, usually in conjunction with fibrinogen concentrations, for an association with exposure to particles. In this study, no statistically significant difference was found between the exposed and unexposed groups for any of the three biomarkers or indeed between the three groups on investigating the presence of a dose-response relationship. Considering the results for Fibrinogen concentrations, C Reactive protein concentrations, and the various cell counts and finding no statistically significant difference between the exposed and unexposed groups and also not finding any indication of a dose-response relationship for any of the aforementioned biomarkers of effect it appears that the results of this study do not support the findings in the various laboratory studies, which have that exposure to elevated levels of particles lead to increases in concentrations of various inflammatory biomarkers.

Exposure to elevated levels of particles has been shown to cause oxidative cell damage, of which CRP is also a marker. Two other markers of oxidative damage that were examined in this study were TBARS and protein carbonyl concentrations. Once more, no statistically significant difference was observed between the levels of either factor in the exposed and unexposed groups, although for protein carbonyl concentration the difference between the unexposed and exposed groups was almost statistically significant ($p=0.056$). With respect to protein carbonyl concentrations, when investigating the presence of a dose-response relationship the difference between the three exposure groups did not reach statistical significance ($p=0.191$), from the box plot (fig. 4.46) however it seemed that exposure group one differed from the other two, with a median value that appeared much larger than in the other two groups. Despite this obvious difference however, none of the differences between exposure groups reached statistical significance although the difference between groups one and three was close ($p=0.072$).

No studies appear to have used either TBARS concentrations or protein carbonyl concentration as markers of oxidative damage and so no results are available for comparisons, however in light of the lack of significant results for other biomarkers, particularly CRP, these results support the overall findings that no significant difference exists between the levels of the various biomarkers of effect in persons exposed to elevated levels of ultrafine particles.

5.1.7 Conclusion

With so little evidence on the effects of ultrafine particles, either in a healthy population or in susceptible populations, this study was rather adventurous in its aims. There was a statistically significant difference between the exposed and unexposed groups in terms of the mean number of particles/cm^3 measured and taken in conjunction with the lack of a significant difference between the levels of biomarkers of effect in the exposed and unexposed blood samples, this would appear to suggest that there is no effect of chronic exposure to ultrafine particles from traffic on the healthy population. Although this is distinctly possible, the lack of ability to collect more comprehensive particle number data or to recruit more people to the study to provide blood samples means that that there is a distinct possibility that there is a difference in blood biomarkers of effect and that has been missed due to the small numbers in the study. The overall conclusion for this study is therefore that it cannot be taken as any more than a pilot investigation into the methodology required to suitably measure the levels of ultrafine particles in cities so as to adequately reflect personal exposure levels and that although the results seem to show that there was no effect of elevated levels of particles on the various biomarkers of effect, the lack of numbers, particularly in the unexposed areas, and the inability to adequately control for various confounders such as acute exposures to ultrafine particles means that the results found may reflect the effects of factors other than ultrafine particle levels. Future work may show that the results of this study are in fact a true reflection of the situation but until the results are substantiated by further results it cannot be said with any great certainty that the levels of these biomarkers of effect are not affected by the levels of ultrafine particles.

5.2 Does proximity to different road types increase ones likelihood of being admitted to hospital with cardiovascular or respiratory complaints?

As observed during this study, the levels of ultrafine particles are not homogenous across the city and can vary largely depending on location. It was felt that the primary reason for the differences in particle levels is to do with traffic volume, supported by the fact that the mean numbers of ultrafine particles/cm^3 measured in the exposed area was almost twice as high as that of the unexposed area.

A study was conducted in an effort to elucidate whether proximity to certain road types increased individuals' odds of being admitted to hospital with for a range of cardiovascular and respiratory illnesses. As roads with higher traffic volumes were thought to be likely to have higher levels of particulates it was thought that road type may be used as a proxy for exposure to ultrafine particulate pollution in future studies.

5.2.1 Grouping the roads into categories

The main theory behind the choice of road categories was that certain road types, such as National A roads are likely to have much higher volumes of traffic than would residential cul de sacs. Other roads for which it was thought there would be high traffic volumes included National B roads and any roads with a bus route as these represent the primary routes of access in and out of the city centre and are therefore likely to be the most heavily utilised. Consequently the first stage of creating the different road categories involved grouping roads according to type. The second point that was raised at this stage was regarding how distance from the city centre might affect traffic volumes on each of these road types. There is a distinct possibility that the traffic volumes increase towards the city centre as numerous roads converge, forcing more traffic onto a smaller number of roads, thus increasing the volume of traffic closer to the city centre. For this reason the decision was taken to split the roads not only by type but by distance from the city centre as well. Cardiff has historically been an industrial city and as with most coastal industrial cities, the main city centre has built up and radiated out from the port. The port in Cardiff is no longer as active and much of the area around it has become pedestrianised in an effort to turn it into a modern social area. For this reason Cardiff central station was identified as the point best suited to be the point from which the distances would be calculated as it is serviced by major routes from all sides of the city and close to the port and thus much of the city and suburbs radiate out from the station. It is also the main station for both buses and trains into and out of the city, thus making the roads leading to it some of the busiest and most congested in the city.

5.2.2 The use of PEDW and NHSAR to populate the study

Once the database was complete with respect to the streets and their postcodes, distance from city centre and road categories, it was passed on to Health Solutions Wales (HSW) along with a list of ICD codes of interest and staff there used the information to create two separate databases containing information on both persons admitted to hospital with a diagnosis corresponding to one of the ICD codes of interest as well as information on the base population of Cardiff. HSW staff used the postcodes provided for each street to search the PEDW database and extracted all persons admitted with a diagnosis corresponding to one of the ICD codes of interest, along with information about each person including id, age, sex, diagnosis, socioeconomic status and date of admission. Similarly they extracted details on every person living within these postcodes from the NHSAR database again with information such as age, sex, and socioeconomic status. In both cases HSW staff added into the databases the road category in which each individual case resided, thus providing two separate data files which were then merged and used to conduct logistic regression analysis. Merging the two data files presented some problems as the information fields in each of the files were not identical, on top of which one file was an excel file and the second was an access database making a straight merge impossible. The first thing to be done was to identify the information fields of interest in the two data files and to create an SPSS file in preparation for importing the data. Since the PEDW data contained more data fields and the majority of the fields in the NHSAR file were the same as those in the

PEDW file, the PEDW file was used to identify the fields of interest. Once the fields of interest were identified they were reordered so that the same fields were in the same place in each file. The relevant data from each file was then copied and pasted into SPSS for analysis creating a database with over 320,000 cases. Merging the data files was a difficult and slow process, made more so by the fact that the original files were in two different formats with differing fields making a straight merge or import into SPSS impossible and this could be something that needs to be addressed in the future.

The merits of using such centralised databases as PEDW and NHSAR as the primary information sources for studies such as this are obvious. The main benefit of using such centralised data is that all studies are conducted on data taken from the same information source and thus findings from different studies are far more comparable in terms of the results and so contribute towards building a profile of the Welsh population and identifying the various risk factors for sub-groups within the population. Not only are the results comparable for Welsh studies, but as the PEDW database was set up in line with the English system, results are also comparable within the UK.

The NHSAR data base consists of information about every single person in Wales who is registered with a GP and so is one of the most straight forward methods to finding a base population for a study. There are some issues however; the primary one being that if a person does not register with a GP then there will be no record of them in the database which could lead to underestimation of the population and subsequently over-estimation of the effect sizes. Students present a particular problem to the accuracy of NHSAR as they very often leave Wales on completion of their studies and do not remember to deregister with their GP resulting in overestimation of the population size and subsequent underestimation of the effects. The results of the 2001 census indicated that there were a population of 305340 people in Cardiff and the number of people registered with a GP in July 2002 in Cardiff was more than 316000 and only seven duplicate entries. It is important therefore that this system is examined closely to make sure it is as accurate as possible at any given time. There are possible methods of dealing with inaccuracies such as these. For example a way to make sure that every person in Wales is registered with a GP perhaps a system whereby every child born must be registered with a GP before leaving the hospital. When dealing with replicates in the NHSAR database however the situation may be a little more complex. In theory it should be a simple process of searching by an individuals NHS number, a unique identification number given to every person who registers with a GP. In practice however identifying persons by their NHS number alone is often not enough as numbers there are inconsistencies such as some people registered more than once resulting in different NHS numbers for the same person,

5.2.3 Variables in the regressions analysis

One of the primary strengths for this part of the study was the fact that there were a number of variables, known to possibly effect hospital admissions for the diseases of interest. An age effect has been recognised for a number of years, with the older members of the population more at risk for developing circulatory and/or respiratory problems, although given the rapid rise in obesity coupled with a lack of physical exercise, particularly in the younger population, it would not be surprising if age became less of a factor as younger members of the population become increasingly more at risk of developing cardiovascular problems as a result of their lifestyle. Very young children, particularly babies under a year old are also more susceptible to some cardiovascular and respiratory illnesses. Although not as great as in the older age groups, it can be seen from the bar graph that there is indeed and increase in cardiovascular and respiratory admissions for children under the age 12 months and to a lesser extent, children between the ages of two to five years.

Reviewing the bar graph it was noticed that although there appeared to be a month effect, there also appeared to be a seasonal effect, with lower numbers of admissions between May and October compared with the period November to April. The decision was therefore taken to include a variable for season rather than for individual month. Two seasons would be examined, the cold season (Nov-Apr) and the warm season (May-Oct) coded 1 for the cold season and 2 for the warm season. This decision was supported by the literature where many of the studies which include weather or temperature variables in their analysis use season as the variable of choice.

Including a variable for season in the regression analysis presents a unique problem. The nature of the database is such that there are approximately 300,000 cases with no season of admissions as they were not admitted to hospital during the course of the year. For this reason SPSS cannot work out the rates necessary to carry out the regression analysis when these cases are included in the analysis and so simply ignores them, leading to spurious results. There was no question that some variable for weather or temperature was to be included in the analysis as in much of the literature temperature, weather and seasonal effects have been observed and thus to not include it in some way in our analysis when the data was readily available could not be justified, especially in light of the fact that no temperature or weather data was available for the first part of the study and therefore could not be included. For many studies examining the effects of exposure to particulate pollution on hospital admissions the rates were not being considered and therefore the number of admissions was not being compared to a total population including those not admitted but rather against a subpopulation consisting only of those admitted.

For this study, where the rates of admissions were of interest, there were two ways in which this problem could have been handled, the first was to randomly assign each person not admitted a value between one and twelve to represent a month. The problem with this method is that it can misrepresent the proportions and can result in inaccurate results. The second and most accurate way to deal with it would have been to create a new database into which the cases not admitted were copied twelve times and each one given a value for all months, thus keeping the proportions correct.

5.2.4 All Hospital Admissions with a primary diagnosis corresponding to any ICD Code of interest.

As expected the dependent variables age-group, sex, season and socioeconomic status were all statistically significantly associated with hospital admissions. The variable road category was also statistically significantly associated with hospital admissions (p=0.0005).

Unsurprisingly the seasonal effect was a protective one (OR=0.869, p=0.0005) with a decrease in odds for the warm season. Respiratory disease episodes for illnesses such as influenza and pneumonia are known to increase during the winter months and so this would explain the decrease in odds for the warm season. The evidence in support of the seasonal effect being the result of the differences between respiratory illnesses can be found in the regression analysis using admitted/not admitted for a cardiovascular illness where the results for season are not significant whereas when examining respiratory admissions the seasonal effect if again significantly associated with a decrease in odds for the warms season (OR=0.539; p=0.0005) and the protective effect for respiratory illnesses alone is larger than for combined respiratory and cardiovascular illnesses. Ideally this would be further investigated by admissions for specific illnesses rather than group of illnesses however with limited admissions data the numbers of admissions for each individual illness would be too small to provide meaningful results.

Gender also showed a protective effect in favour of females for all admissions and for cardiovascular admissions. Again this was not a particularly surprising result and men have in the past been shown to be at increased risk for diseases such as COPD and heart disease as a result of several factors including occupation and lifestyle factors. Respiratory diseases however did not show any gender effect with an odds ratio of 1.005 (p=0.922) when considering road category as an independent variable. The odds ratio for sex was little changed in either size or significance when distance category or road type were considered as independent variables in the analysis. This may be due to the fact that the respiratory diseases admissions might be dominated by infectious diseases such as influenza where there it is likely that both men and women are equally at risk. The protective effect of gender in favour of females was greater for cardiovascular disease admissions than for all admissions combined suggesting that the protective effect for females for all admissions is influenced by the real protective effect for cardiovascular admissions.

Age group was expected to be significantly associated with admissions and in all cases it was highly significantly associated (p=0.0005). For all cardiovascular and respiratory admissions combined there was a significant protective effect for all ages between 2 years and 70 years (p=0.0005) as compared with babies under 12 months, for persons aged 71 upwards the odds increased through the age groups. This result was not unexpected as very young babies are prone to all kinds of infections, including such respiratory illnesses as pneumonia and bronchitis, as are the older members of the population. When investigating the associations with respiratory diseases only the effect of age-group was protective for all age groups compared with the 0-1year group, but this protective effect is greater in the younger populations and if the reference group is changed from 0-1years to 21-30 years the odds ratios for the older age groups increase, suggesting that there is something happening within the age-group 0-1 which increases the odds of being admitted with a respiratory disease above all other age groups.

For cardiovascular diseases the odds ratios appear to increase drastically for the older population, with an odds ratio of 242.606 for all persons over 90 years, though when one again considers that the group to which all others are being compared is 0-1years it then becomes apparent why such large odds ratios were obtained. Cardiovascular diseases are not common illnesses in the younger populations, particularly in children whereas they are one of the biggest reasons for requiring hospital treatment in the older population and so the hugely increased odds ratio is most probably reflecting the lack of incidences of cardiovascular illnesses in the younger population. There is a large increase in odds between age groups 31-40 years and 41-50 years and if the reference group is altered to use 41-50 years as the reference, by dividing the odds ratio for this group into all other odds ratios it was found that in fact the odds ratios, although still high, were much lower than when using the age group 0-1 years as the reference group. These elevated odds ratios reflect the fact that cardiovascular diseases such as COPD are much more prevalent in the older population in Cardiff.

Socioeconomic status showed a statistically significant protective effect for persons in the median, next affluent and most affluent groups for all admissions and for respiratory admissions. For all admissions, with road category and again with road type as the independent variables of interest there was a statistically significant increase in odds ratio from the most deprived to next deprived group but with distance as the variable of interest although there was an increase in odds it was significant. For respiratory admissions there was again an increase in odds ratio between the most deprived and next deprived group but it was not significant in any of the analyses. This may imply that in terms of being admitted to hospital with a respiratory diagnosis there is little difference between the two deprived groups. For cardiovascular diseases there was a significant protective effect for the median to most affluent groups when road category and when distance group were included as the variables of interest in the analysis. When road type was included however the odds ratios reduced in each group but they were no significant for any group other than most affluent. In all three analyses there was a statistically significant increase in odds for the next deprived group over the most deprived group.

Road category was significantly associated with hospital admissions for all admissions, cardiovascular admissions and respiratory admissions. Somewhat surprisingly all but one category had increased odds of admissions and most were statistically significant when compared with the reference category which was A&B roads within 0-2km of the city centre, the group which was thought most likely to have higher volumes of traffic.

Distance was significantly associated with hospital admissions for all three dependent variables. The distance group 2-4km showed reduced odds ratios for all admissions and for respiratory admissions, but this protective effect was only significant for respiratory admissions (OR=0.843; p=0.030). For cardiovascular admissions there was a statistically significant increase in odds in the 2-4km group. All other groups showed increases in odds ratios compared with the reference group (0-2km from the city centre).

For road type the variable was significantly associated with hospital admissions for all three dependent variables. There was a marked increase in odds for people living on B roads and Bus routes compared with people living on A roads. For those living in cul de sacs the effect was protective though not significant, nor was the marginal increase in odds for people in all other properties significant. Similar results were observed for cardiovascular diseases although this time the increase in odds for people living on B roads was not statistically significant. For respiratory diseases all road types resulted in significantly increased odds of hospital admissions compared with the reference group (A roads). There were no preconceptions for what results might be found when road type was included as the dependent variable, apart from the fact that it was felt that certain road types would be more heavily trafficked than others (e.g. A roads would be more heavily trafficked than cul de sacs). In relation to cardiovascular diseases the only road type to show a significant association with admissions was bus route with a statistically significant increase in odds of 22% ($p=0.003$) over persons living on A roads. In relation to respiratory diseases however, all road types showed a significantly increased odds of admission over A roads, the largest of which was for B roads ($OR=1.854$; $p=0.0005$).

It is more likely that the results for each of the exposure variable may be affected by one of the other factors in the analysis such as socio economic status. For example part of what determines a persons socioeconomic status is whether they have a car or not, in the most deprived group most people will not have a car and are therefore likely to live on or close to a bus route or other form of public transportation. Crosstabulations of road category and socioeconomic status reveal that apart from in the group 6-12km, many more people from the most deprived social category do in fact live on a bus route and this may be being reflected in the results of the logistic regression by the fact that for all admissions the largest increases in odds ratios were seen for between 0 and 6km of the city centre. Similarly for cardiovascular admissions and respiratory admissions separately the largest increases in odds ratios were observed for people living on bus routes. Similarly for the categories cul de sacs and all other properties the majority of people living in these areas are classed as being the most deprived group and most of these live within 0-4km of the city centre, further out an increasing number of the most affluent persons are living in the categories cul de sacs and all other properties.

Other reasons why the results were not what were expected may be because the exposure variables were not accurately classified. The distance category is the most accurate of the three exposure variables as it was calculated using GIS software and the results using this variable are probably therefore also the most accurate. The majority of persons living in the distance group 2-4km are classed in the two lower social groups, most deprived and next deprived (n=82660) and in the distance group 6-12 km the majority of persons are classed in the two uppermost social groups, most affluent and next affluent (n=109186). For all admissions there was a slightly protective effect for persons living 2-4km away compared with those living 0-2km and for persons living 6-12km there was a slightly increased odds although neither was significant.

There was always the chance that the exposure variables chosen for this study would not accurately reflect the levels of exposure in the population but until the data had been collated and interrogated there was no real way of knowing how, if at all the variables chosen would be affected by the other variables in the analysis. If the effects of socioeconomic status are truly reflected to a degree in the exposure variables chosen for this study then a new exposure variable should be considered which more accurately represents the level of exposure to particulate pollution.

5.2.5 Conclusions

The results for this study were not what would be expected if the road categories defined were indeed a good proxy for traffic volume. Based on the categories defined at the outset it there is no consistency between the results obtained, although statistically significant results were obtained, therefore there may be a confounding factor affecting the results. If it were the case that the road categories were a good proxy for traffic volume and hence particulate pollution from vehicular sources, one would have expected that similar categories of road would show similarly increased odds ratios for both respiratory and cardiovascular admissions, but this is not the case and thus leads to the conclusion that there may be something else at play and that the road categories are acting as proxy measures for this.

In relation to road type, it seems that there is something about bus routes in particular which result in increased odds of admission for both cardiovascular and respiratory diseases for persons living on bus routes compared with those living on A roads. It is possible that a disproportionate number of people in the most socially deprived groups live on bus routes and this might explain to some degree the significantly increased odds observed. It is unlikely that this is the only factor affecting the outcomes however, and it is possible that bus routes have higher volumes of traffic than might be anticipated when compared with A roads and as a result the results observed may be a true reflection of effects of living on the different road types. Another possible reason is that the emissions from buses differ in some way from those emitted by cars, although no real evidence currently exists, it appears that emissions from buses contain higher levels of ultrafines and this is something that was noticed when collecting the particulate data for the first part of this study, a passing bus tended to increase the levels of ultrafines recorded by the P-trak. This may point towards the fact that buses or other heavy goods vehicles using these routes may present more of a risk to the population than do the smaller cars. If this is the case then it raises questions about whether the current drive to reduce the number of cars on the road by encouraging the use of the public transport system is indeed the appropriate way forward. It is more likely however that if buses do currently present a larger risk to the population, then steps need to be taken to reduce emissions from buses and to continue with trying to reduce the number of vehicles on the roads.

Every attempt was made in this study to take account of factors which may have an effect on the outcome however it is possible and probably that there are other factors affecting admissions that were not identified at the outset. For example, point sources of ultrafine particles, such as a factory, may have resulted in an increased number of ultrafine particles or indeed some other pollutant thus exposing the surrounding population to an entirely different mix of pollutants resulting in the increased odds ratios observed.

5.3 Recommendations for future work

Ultrafine particle and the effects they have on health have, in the last few years, become the focus of many research studies. As yet however there are no monitoring sites in Wales that are capable of measuring ultrafine particle concentrations and this represents a large gap in useful research data. To recap briefly, the main problems presented in the first part of this study included lack of traffic data, less than comprehensive particle number data and a lack of any centrally monitored ultrafine particle data by which to compare the levels measured during this study as well as a lack of numbers of participants thus reducing the power of the statistical results.

Better methods of estimating individual exposure levels are also needed, the p-trak is a useful piece of kit but there is better and more suitable equipment available for measuring ultrafine particles. Therefore effort needs to be concentrated in the collection of raw data so that adequate and accurate data is available to any researcher wishing to examine the health effects of exposure to traffic pollution.

In relation to the second part of this thesis it would appear that the categories of road defined at the outset did not prove to be the proxy for exposure to particulate pollution from vehicular sources that was hoped and therefore the main conclusion must be that a better measure of traffic flow must be defined for it to be possible to use traffic volume to estimate the effects of exposure to particulate pollution from vehicular sources.

Overall the recommendations for future work can be seen to be similar for both sections of this thesis – which is that much of the effort needs to be concentrated on gathering comprehensive data on both traffic volume and ultrafine particle levels in the city.

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Appendix One

Assay Procedure for Measuring Human IL-6

Assay Procedure Summary

1. Add 50 μl ELISA Diluent to each well.
2. Add 100 μl standard or sample to each well.
   Incubate 2 hours at room temperature.
3. Aspirate and wash 5 times.
4. Add 100 μl prepared Working Detector to each well.
   Incubate 1 hour at room temperature.
5. Aspirate and wash/soak 7 times.
6. Add 100 μl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.
7. Add 50 μl Stop Solution to each well.
   Read at 450 nm within 30 minutes.
   λ correction 570 nm.

Assay Procedure for Measuring Human TNF-α

Assay Procedure Summary

1. Add 50 μl ELISA Diluent to each well.
2. Add 100 μl standard or sample to each well.
   Incubate 2 hours at room temperature.
3. Aspirate and wash 5 times
4. Add 100 μl prepared Working Detector to each well.
   Incubate 1 hour at room temperature.
5. Aspirate and wash/soak 7 times.
6. Add 100 μl TMB One-Step Substrate Reagent to each well.
   Incubate 30 minutes at room temperature.
7. Add 50 μl Stop Solution to each well.
   Read at 450 nm within 30 minutes.
   λ correction 570 nm.

TBA FLUORIMETRIC ASSAY FOR LIPID PEROXIDATION

Ref: Wojciech Wasowicz, Jean Neveand Anne Peretz.
CLIN. CHEM. 39/12, 2522-2526 (1993).

ASSAY PROCEDURE

1. Prepare the standard by adding 328μl (2mmol) Tetramethoxypropane to100ml absolute ethanol (20mmol /l).
   This is then diluted again by adding 500μl of this dilution to 1 litre distilled water (1/20000 dilution =10μmol/l).
   This standard is 1nmol/100μl
2. Prepare TBA reagent by dissolving 417mg TBA in 100ml 50% glacial acetic acid. This needs to be stirred.
3. Add 1ml HPLC grade water to glass pyrex test tubes in triplicate for standards and tests.
4. 100μl of serum is added to triplicate test tubes.
5. A calibration curve is prepared in triplicate by adding 0, 0.05(5μl standard),0.1(10μl standard), 0.2(20μl standard), and 0.3(30μl standard to 1ml of water.
6. 1ml TBA reagent is added to each tube, mixed and then put in the heating block at 95-100°C for 1 hour.
7. The tubes are cooled on ice.
8. 25μl of 1M HCl is added to each tube.followed by 3.5 ml n-butanol.
9. The tubes are rotated for 5 minutes on a mixer and then centrifuged at 1500g for 10 minutes.
10. The fluorescence of the butanol layer is measured at 525nm excitation 547 emission
11. The values of the serum are calculated from the standard curve.

Protein Carbonyl Measurement

Ref Buss and Winterbourn. Methods in Molecular Biology vol 186
Oxidative Stress Biomarkers

Reagants and Buffers

2,4-Dinitrophenylhydrazine Sigma
10 mM DNP in 6 M guanidine hydrochloride, 0.5 M potassium phosphate buffer, pH 2.5.

Mouse (Monoclonal) anti-DNP Sigma

Rat anti-mouse IgE HRP conjugated Serotec

20 mg tablet o-phenylene diamine Sigma

Methods

Reduced Protein

Serum albumin as purchased already contains carbonyls. Therefore fully reduced bovine (BSA) or human serum albumin (HSA) needs to be prepared for use in the standard curve

1. A 0.5 g/100 mL albumin solution in PBS is reacted with 0.1 g solid sodium borohydride for 30 min. Since this reaction produces hydrogen (notable through intense foaming of the protein), it should be carried out in a fume hood. Sodium borohydride breaks down over time, so the amount used may need to be increased after prolonged storage

2. The solution is then neutralized with HCl by adding a 2 M solution until pH 7.0 is reached. After overnight dialysis against PBS, the protein conc is checked and adjusted to 4 mg/mL.

Oxidized Protein

Oxidized BSA containing additional carbonyls is prepared for us standard curve by reacting with HOCl

1. The HOCl concentration in a stock solution of household bleach is determined spectrophotometrically by diluting an aliquot in 0.01 M NaOH and measuring the absorbance at 290 nm
2. BSA (50 mg/mL in PBS) is reacted with 5 mM HOCl for 24 h at 37°C. The reaction is complete after this time and the BSA typically contains about 9 nmol carbonyls per mg protein
3. The protein concentration is adjusted to 40 mg/mL for calibration and 4 mg/mL for use in the ELISA

Colorimetric Assay for Calibration of Standards

The carbonyl content of the oxidized and reduced BSA is determined using a modification of the standard colorimetric method

1. 10 mg protein in 250 ul PBS is reacted with 1 mL 10 mM DNP in 2 M HCl for 45 min, precipitated with 1 mL 28% TCA and washed three times with ethanol/ethyl acetate (1:1). A blank for each sample consisting of protein with 2 M HCl containing no DNP is carried through the procedure
2. Pellets should be broken up mechanically and by sonicating during the washing steps, then dissolved at 37°C in 1 mL of 6 M guanidine hydrochloride, 20mM potassium phosphate, pH 2.5, and the absorbance at 375 nm measured
3. The protein concentration of the final extract is determined.
The results can then be adjusted for the protein loss (about 10%) that occurs with this method (6). The absorbance of the subtracted.
4. Carbonyl content is determined as nmol/mg protein using ε_{375} 22000 M-1 cm -1 after subtracting the value for reduced albumin.
5. The fully reduced BSA consistently gives an A_{375} of about 0.13 per 10mg (equivalent of 0.6 nmoles carbonyls/mg), which is unaffected by further treatment with sodium borohydride. It is assumed to be nonspecific and not due to carbonyls.

NAME

C-REACTIVE PROTEIN

INTENDED USE

The C-Reactive Protein (CRP) assay is used for the quantitative analysis of C-reactive protein in human serum and plasma

SUMMARY AND EXPLANATION OF TEST

C-reactive protein (CRP) was discovered in 1930, and named for its reaction with the C polysaccharide of the pneumococcal cell wall. CRP is a cyclic pentamer with an approximate molecular mass of 118 kDa. Each subunit is composed of 206 amino acids. CRP is synthesized in the liver and released into the circulatory system in response to proinflammatory stimuli, the strongest of which is interleukin-6 (IL-6). It is this positive response to inflammation from which CRP derives its diagnostic utility and categorizes it with "acute phase reactants." CRP has been called the archetype of acute phase proteins due to its rapid (24 to 28 hours) and marked response to a wide variety of inflammatory conditions and diseases. These stimuli include bacterial and fungal components, as well as damaged cell membranes from injured tissue arising from trauma, arthritis, vasculitides, and a spectrum of autoimmune processes. A reactant common to these stimuli is phosphocholine, which shows calcium-dependent binding to CRP and is the major ligand. CRP shares some of the immunological functions of IgG. It activates the classical complement pathway, binds to Fc receptors, and acts as an opsonin. These CRP activities make it a critical component of the innate immunological system that provides early defense against infectious agents. As stated by Volanakis, the main biological function of CRP appears to be host defense against bacterial pathogens and clearance of apoptotic and necrotic cells.[1] CRP is clinically useful due to its 1,000-fold rise during an inflammatory response, which provides a nonspecific indication that an inflammatory process is present. Its concentration falls rapidly when the condition resolves, due to a short half-life (19 hours). The biology and chemistry of CRP have been reviewed by Bienvenu et al.,[2] Du Clos,[3] and Volanakis.[1]

PRINCIPLES OF PROCEDURE

CRP is an in vitro diagnostic assay for the quantitative determination of CRP in human serum and plasma.

When an antigen-antibody reaction occurs between CRP in a sample and polyclonal anti-C-reactive protein antibody which has been adsorbed to latex particles, agglutination results. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration.

The increase in absorbance at 572 nm is proportional to the CRP concentration.

REAGENTS

Reagent Kit

C-Reactive Protein, List No. 8G65, is supplied as a liquid, ready-to-use, two-reagent kit which contains:

- Reagent 1 (R1) 3 x 38 mL
- Reagent 2 (R2) 3 x 43 mL

Estimated tests per kit are 620. Calculation based on minimum reagent fill volume per kit.

Reactive Ingredients

Ingredient	Concentration
R1 Ethylenediaminetetraacetic Acid Disodium Salt Dihydrate	1.86%
Sodium Azide	0.09%
R2 Latex particle adsorbed anti human CRP	0.15%
Sodium Azide	0.09%

REAGENT HANDLING AND STORAGE

Reagent Handling

Mix reagent cartridges by gentle inversion prior to placing on the instrument.

Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles.

CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration which could impact results.

Reagent Storage

The unopened reagents are stable until the expiration date when stored at 2 to 8°C. R1 should be clear. R2 should appear milky.

Reagent stability is 54 days if the reagent is uncapped and onboard.

WARNINGS AND PRECAUTIONS

Precautions for Users

1. For in vitro diagnostic use
2. Do not use components beyond the expiration date
3. Do not mix materials from different kit lot numbers
4. Reagent 1 (R1) and Reagent 2 (R2) contain sodium azide and are classified per applicable European Community (EC) Directives as Harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases

R22	Harmful if swallowed
R32	Contact with acids liberates very toxic gas
S35	This material and its container must be disposed of in a safe way
S36	Wear suitable protective clothing
S46	If swallowed seek medical advice immediately and show this container or label

NOTE: Refer to *Section 8* of the instrument-specific operations manual for proper handling and disposal of reagents containing sodium azide

SPECIMEN COLLECTION AND HANDLING

Suitable Specimens

Serum and plasma are acceptable specimens

Serum: Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. Separate serum from red blood cells as soon after collection as possible. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results

Plasma: Use plasma (acceptable anticoagulants: lithium heparin, sodium heparin, and EDTA) collected by standard venipuncture techniques into glass or plastic tubes without gel barriers. Separate plasma from red blood cells as soon after collection as possible. Ensure centrifugation is adequate to remove platelets

For total sample volume requirements, refer to the instrument-specific ASSAY PARAMETERS section of this package insert and *Section 5* of the instrument specific operations manual

CAUTION: This product requires the handling of human specimens. It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens[4] Biosafety Level 2[5] or other appropriate biosafety practices[6] should be used for materials that contain or are suspected of containing infectious agents

Specimen Storage

Serum and plasma:

Temperature	Maximum Storage	Bibliographic Reference
20 to 25°C	15 days	8
2 to 8°C	2 months	8, 9
20°C	3 years	8
70°C	indefinitely	10

Guder[8] suggests storage of frozen specimens at 20°C for no longer than the time intervals cited above. However, limitations of laboratory equipment make it necessary in practice for clinical laboratories to establish a range around 20°C for specimen storage. This temperature range may be established from either the freezer manufacturer's specifications or the laboratory standard operating procedure(s) for specimen storage

NOTE: Stored specimens must be adequately mixed and centrifuged to remove precipitates prior to testing

PROCEDURE

Materials Provided

C-Reactive Protein Reagent Kit, List No. 8G65

Materials Required but not Provided

- AEROSET System or ARCHITECT c8000 System
- C-Reactive Protein Calibrator, List No. 8G68
  CAL 1-6, 1 x 2 mL
- Control Material
- Saline (0.85% to 0.90% sodium chloride) for specimens that require dilution

Assay Procedure

For a detailed description of how to run an assay, refer to *Section 5* of the instrument-specific operations manual

PROCEDURE (Continued)

Specimen Dilution Procedures

The AEROSET System and the ARCHITECT c8000 System have Automatic Dilution features; refer to *Section 2* of the instrument-specific operations manual for additional information.

Serum and plasma: Specimens with CRP values exceeding 30.00 mg/dL are flagged and may be diluted using the Automated Dilution Protocol or the Manual Dilution Procedure.

Automated Dilution Protocol

If using the Automated Dilution Protocol, the system performs a 1:10 or 1:25 dilution of the specimen and automatically corrects the concentration by multiplying the result by the appropriate dilution factor.

Manual Dilution Procedure

Manual dilutions should be performed as follows:

- Use saline (0.85% to 0.90% sodium chloride) to dilute the sample.
- The operator must enter the dilution factor in the patient or control order screen. The system uses this dilution factor to automatically correct the concentration by multiplying the result by the entered factor.
- If the operator does not enter the dilution factor, the result must be multiplied by the appropriate dilution factor before reporting the result.

NOTE: If a diluted sample result is flagged indicating it is less than the linear low limit, do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to *Section 5* of the instrument-specific operations manual.

The EXT and LH result error codes (AEROSET) and patient result flag ">" (ARCHITECT c8000) may indicate antigen excess. Dilute specimens and rerun. Specimens with CRP values greater than 30.00 mg/dL (300.0 mg/L) up to 149.70 mg/dL (1,497.0 mg/L) were tested and the results were flagged appropriately.

CALIBRATION

Calibration is stable for approximately 30 days (720 hours) and calibration is required with each change in reagent lot number. Verify calibration with at least two levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary.

For a detailed description of how to calibrate an assay, refer to *Section 6* of the instrument-specific operations manual.

For information on calibrator traceability, refer to the C-Reactive Protein Calibrator package insert.

QUALITY CONTROL

The following process is the recommendation of Abbott Laboratories for quality control during the CRP procedure. As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions.

- Two levels of controls (normal and abnormal) are to be run every 24 hours or each day of use.
- If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.
- If quality control results do not fall within an acceptable range defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory.
- If quality control results fall outside acceptance criteria, recalibration may be necessary.
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

RESULTS

Refer to the instrument-specific operations manual for information on results calculations.

- **AEROSET System Operations Manual**—*Appendix A*
- **ARCHITECT System Operations Manual**—*Appendix C*

Representative performance data are given in the EXPECTED VALUES and SPECIFIC PERFORMANCE CHARACTERISTICS sections. Results obtained in individual laboratories may vary.

LIMITATIONS OF THE PROCEDURE

Refer to the SPECIMEN COLLECTION AND HANDLING and SPECIFIC PERFORMANCE CHARACTERISTICS sections of this package insert.

EXPECTED VALUES

Reference Range

Serum

	Range (mg/dL)	Range (mg/L)
Adult[11]	0.01 to 0.82	0.1 to 8.2

To convert results from mg/dL to mg/L, multiply mg/dL by 10.

A study was conducted using 120 serum samples from adult volunteers. Data were analyzed as described by NCCLS document C28-A2.[12] From this study, results in the 95th percentile were less than 0.58 mg/dL with samples ranging up to 0.70 mg/dL.

CRP is a nonspecific indicator for a wide range of disease processes. It is recommended that each laboratory determine its own appropriate upper limit for the reference interval, as reference intervals are affected by many factors that may differ for each population studied.

Appendix Two

CONSENT FORM

A Research Study of the effects upon health of exposure to air pollution from motor vehicles.

Participant identification number of this research project...

Contact Telephone Number: ....

I confirm that I have read and understood the information sheet dated September 2002 for the above study and have had the opportunity to ask questions.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

I agree to take part in the above study.

Name **Date:..**

Signature...

Researcher **Date:..**

Signature...

Coleg Meddygaeth Prifsgol Cymru
University of Wales College of Medicine

The Resident
«House_number» «street»
«area»
«town»
«postcode»

«ID»

Dear Resident

A research study of the effects upon health of exposure to air pollution from motor vehicles

The University of Wales College of Medicine is investigating the ways that air pollution might affect breathing and heart problems. We are asking healthy men between the ages of 50 and 70, who do not smoke, if they would be willing to take part in this study. If you are male between these ages with no history of chest disease, heart disease, diabetes or arthritis we would be grateful if you would consider taking part. A researcher will then describe the study to you in more detail and answer any questions you may have.

Please answer the questions below and return this sheet in the **FREEPOST** envelope enclosed, you do **NOT** need to use a stamp.

	YES	**NO**
I am willing to have a member of the research team contact me to discuss my taking part in the study		
I am male and between the ages of 50 and 70		
Are you a smoker		
Do you suffer from chest or heart disease, diabetes or arthritis		
My name is	--------------------------	
My date of birth is	--------------------------	
My telephone number is	--------------------------	

THANK YOU FOR YOUR HELP

Professor Ian Matthews

INFORMATION SHEET

A Research Study of the effects upon health of exposure to air pollution from motor vehicles

We invite you to take part in a research study which is investigating the links between air pollution caused by motor vehicles and health. Before you decide we would like to explain why the research is being done and what it will involve. Please take time to read the following information carefully and ask us if you would like more information.

There is growing evidence to suggest that very small and invisible particles from motor vehicle exhausts may cause minor but long term inflammation of the lung. In turn this may, over a period of years, lead to breathing problems and possibly heart symptoms.

The purpose of this research is to investigate whether in healthy people:

i) the levels of certain pollutants from vehicle exhausts is higher in their urine than would be expected.
ii) the levels of certain pollutants from vehicle exhausts is higher in their hair than would be expected.
iii) measurements of their blood indicates that they have signs of minor inflammation of the lung.

We would like healthy men between the ages of 50 and 70 who do not smoke, to take part in this study. We are writing to households close to roads and households distant from roads and aim to recruit a total of one hundred and fifty men.

If you decide to take part then a researcher from the University of Wales College of Medicine will answer any questions which you may have and you will be asked to sign a consent form and you will be given a copy of this to keep. If you decide to take part you are still free to withdraw at any time and without giving a reason.

If you do take part this will involve:

- answering a short questionnaire relating to cardio-respiratory health
- donating a venous blood sample on two occasions which are six months apart
- donating a urine sample on two occasions which are six months apart
- donating a few fibres of head hair on one occasion

We will arrange for a clinician to take the blood sample in the University Hospital of Wales, Heath Park, at a date and time convenient to you and reimburse you for your travel expenses. Any information which you provide is kept strictly confidential.

This research is needed to decide if the current legal standard for air quality is adequate to protect health and the results of the research may show that air pollution should be reduced below current levels.

The research is organised by the Housing and Neighbourhood and Health (HANAH) research team at the University of Wales College of Medicine. HANAH is funded by the Medical Research Council, the National Asthma Campaign and the Welsh Office of Research and Development. We intend to publish the result of this study in approximately eighteen months time in a medical research journal and at that time we will also inform all participants of the results. If you would like any further information then please telephone Professor Ian Matthews on 029 20 742324.

September 2002

<u>Traffic Questionnaire</u>

Road:
ID Number:

Do you think Traffic is a problem?

Yes No

Explain

When is traffic at its worst?

AM PM Both All Day

What type of traffic is mostly in the area?

Cars Buses Heavy Vehicles All

Any other Comments Regarding traffic in your immediate area?

AWDURDOD IECHYD
BRO TAF
HEALTH AUTHORITY

3rd October 2002 DEBP/JS/JJL

Professor Ian Matthews
Professor Environmental Epidemiology
Dept Epidemiology, Statistics and Public Health
University of Wales College of Medicine
Heath Park
Cardiff

Dear Professor Matthews

02/4740 - A Research Study of the effects upon health of exposure to air pollution from motor vehicles

The Bro Taf Local Research Ethics Committee (Panel D) reviewed the above application for ethical approval at its meeting on the 3rd October 2002.

I am pleased to be able to inform you that the Panel agreed that full ethical approval should be granted to this study.

I confirm that Panel D reviewed the following documents at its meeting on the 3rd October 2002:

LREC application form	-	***signed, undated***
Invitation to participants	-	***no version, undated***
Information sheet	-	***no version, undated***
Health questionnaire	-	***no version, undated***
Consent form	-	***no version, undated***

I enclose for your information a copy of the Bro Taf Membership list on which the Members of Panel D, who were present at the meeting on the 3rd October 2002, are indicated. I confirm that the Bro Taf Local Research Ethics Committee complies with the ICH Guidelines for Good Clinical Practice as they relate to an Independent Ethics Committee. A copy of the Committee's Constitution and Terms of Reference is available on request.

You will no doubt realise that whilst the Local Research Ethics Committee has given approval for your project on ethical grounds, it is still necessary for you to obtain approval, if you have not already done so, from the relevant Clinical Director and/or Chief Executives of Trusts (or U.W.C.M.) in which the work will be carried out.

NHS CYMRU WALES
HEADQUARTERS:
Churchill House
17 Churchill Way, Cardiff, CF10 2TW
PRIF SWYDDFA:
Tŷ Churchill
Ffordd Churchill, Caerdydd, CF10 2TW
Temple of Peace and Health
Cathays Park, Cardiff, CF10 3NW
Teml Heddwch ac Iechyd:
Parc Cathays, Caerdydd, CF10 3NW

The committee attach certain standard conditions to all ethical approval. These are that if staff conducting research should change, any new staff should read the research programme submitted to the committee for ethical approval and this letter (and any subsequent letter I may write concerning this application for ethical approval); that if the procedures used in the research programme should change or the programme itself should be changed you should consider whether it is necessary to submit a further application for any modified or additional procedures to be approved and if the employment or departmental affiliation of the staff should change you should notify me of that fact. Any material changes to the structure or operation of the trial (including the recruitment of subjects) must be submitted to, and approved by, the Committee before being adopted. The Committee also ask that if any serious adverse events occur or if you should encounter any unexpected ethical issues, you will inform them of what these are. Full ethical approval needs to be resought if any study does not begin within two years of the date of this letter.

Yours sincerely

Ms. C. Latus,
Acting Chairman, Panel D
Local Research Ethics Committee

Appendix Three

Appendix Three

The following is a list of graphs in which the levels of particles measured indoors are compared to those measured outdoors for each individual in the study.

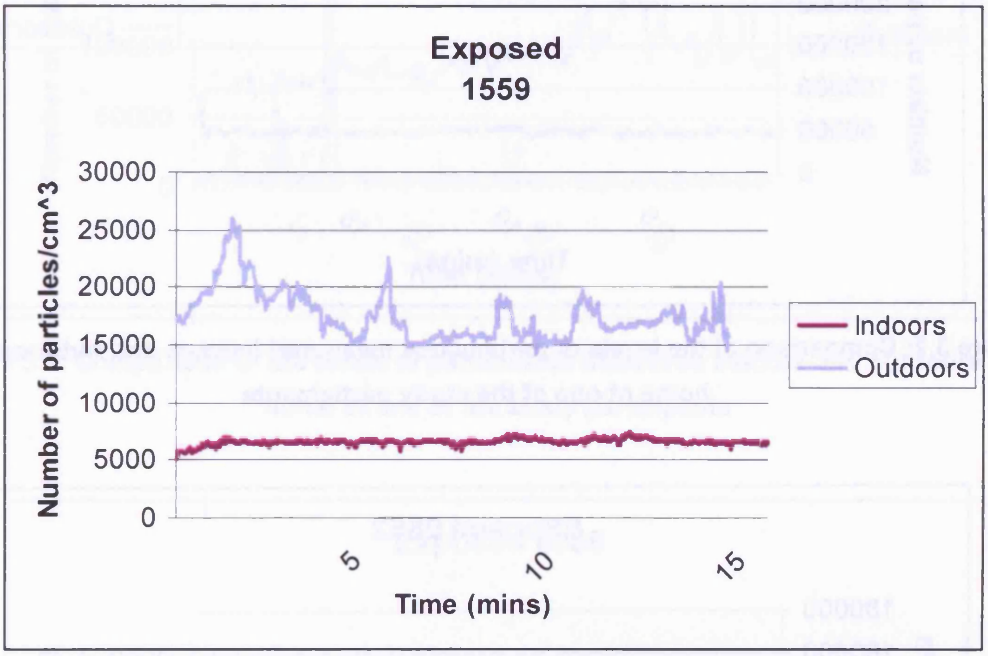

Figure 3.1: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

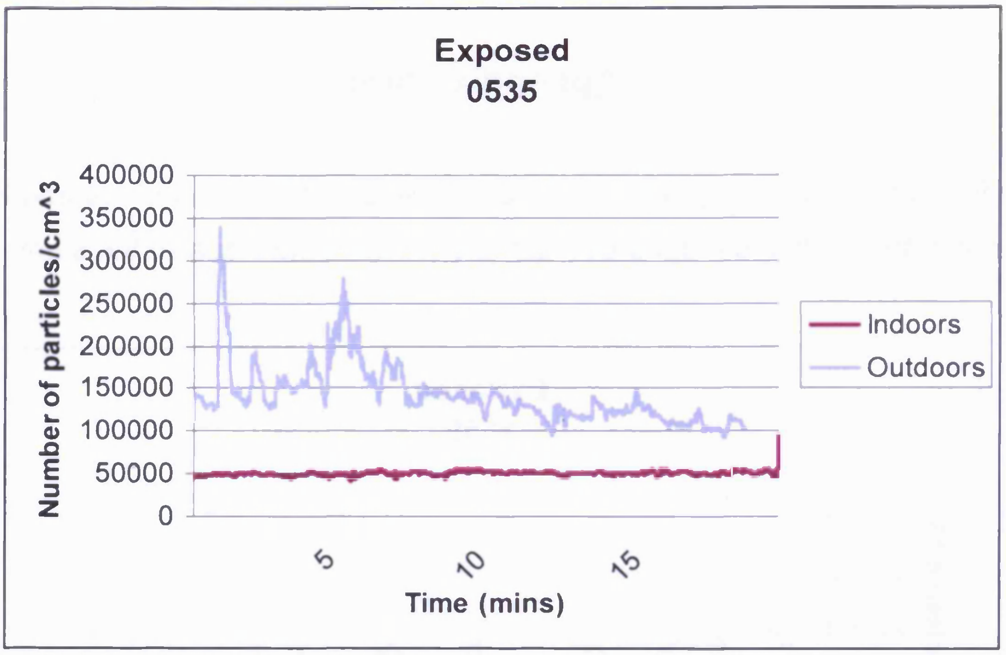

Figure 3.2: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

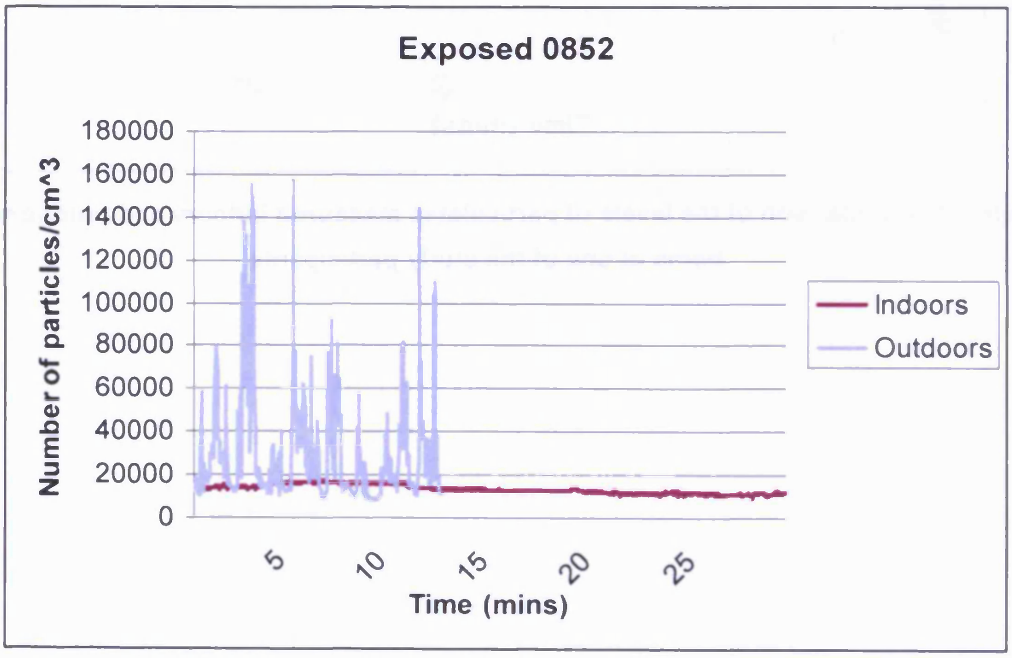

Figure 3.3: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

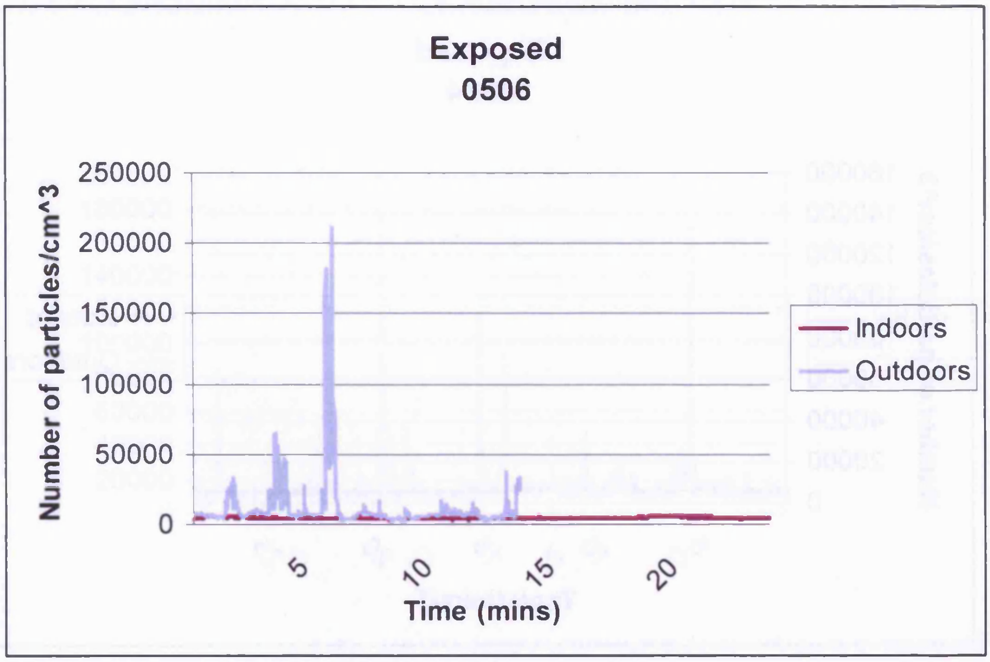

Figure 3.4: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

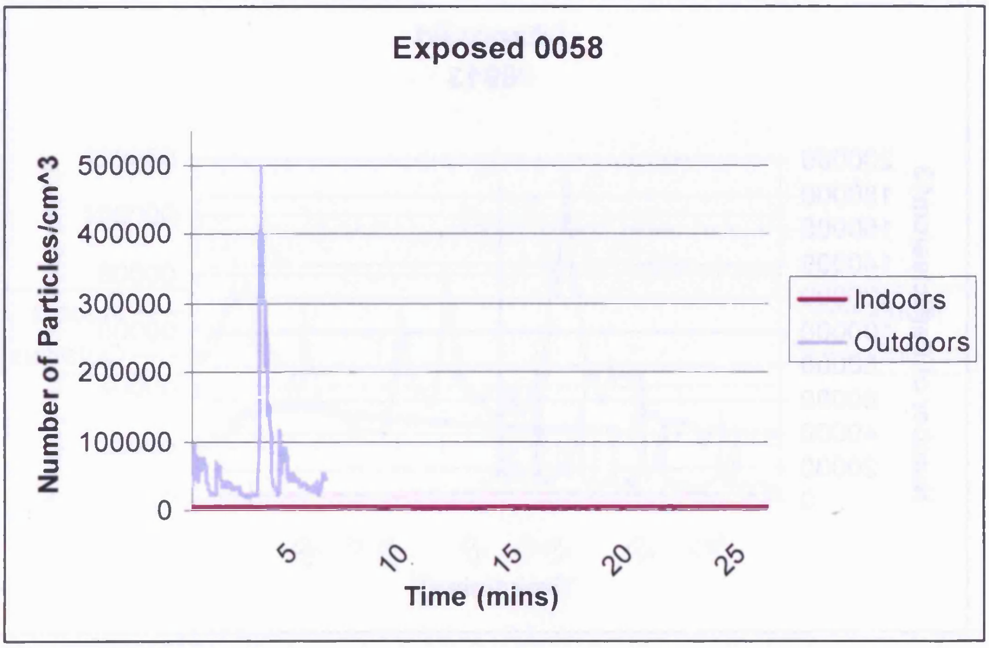

Figure 3.5: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

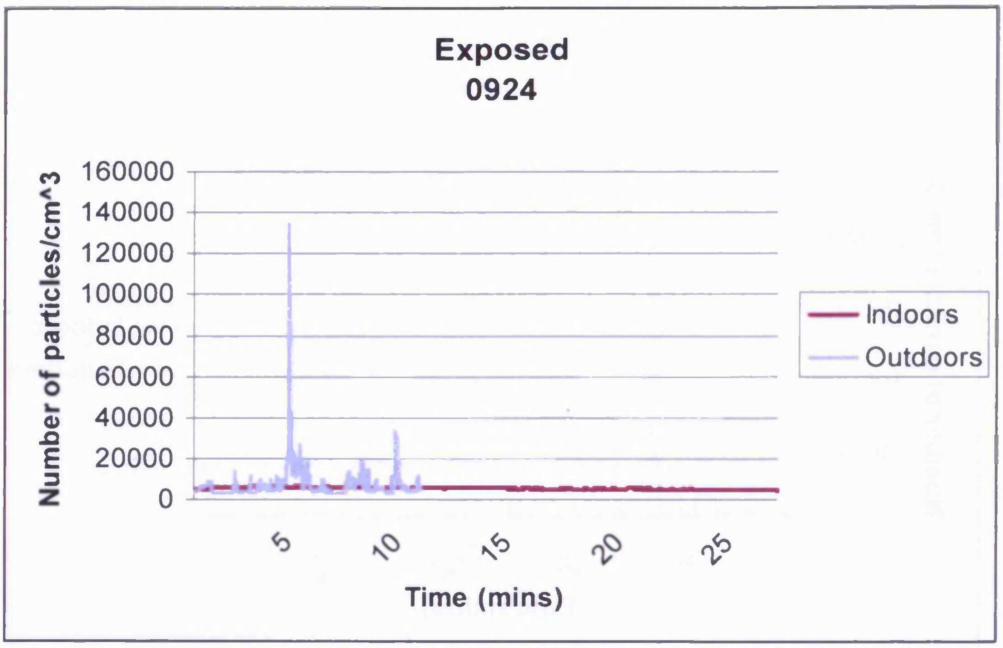

Figure 3.6: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

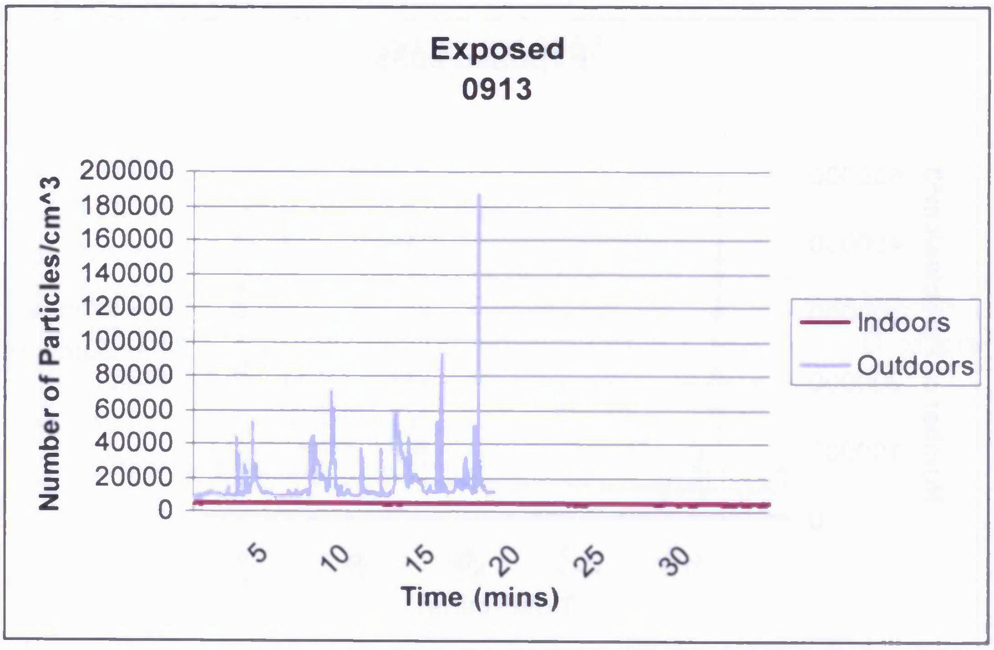

Figure 3.7: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

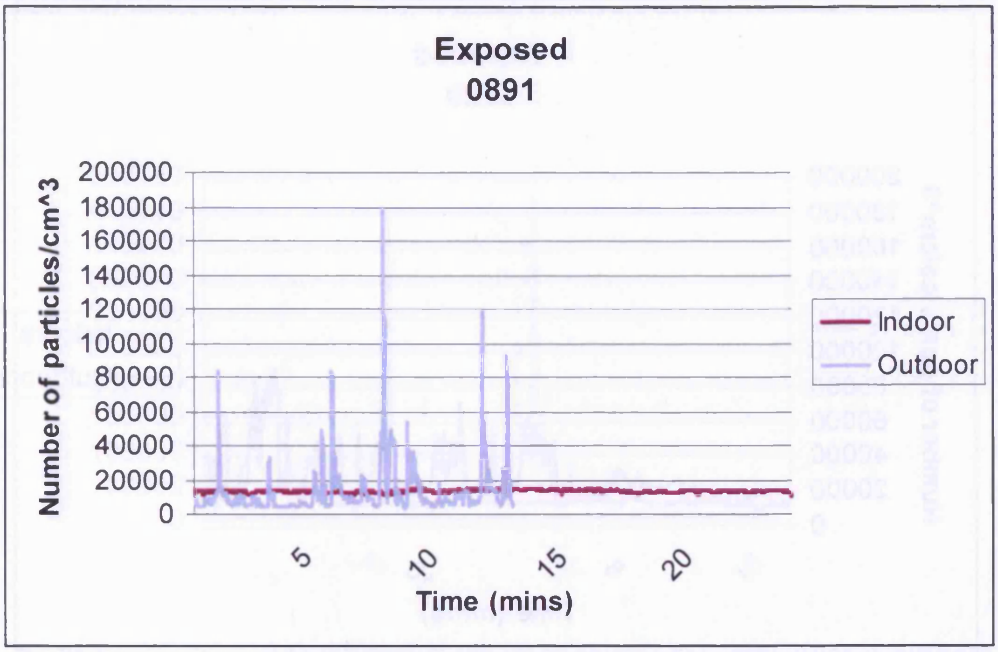

Figure 3.8: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

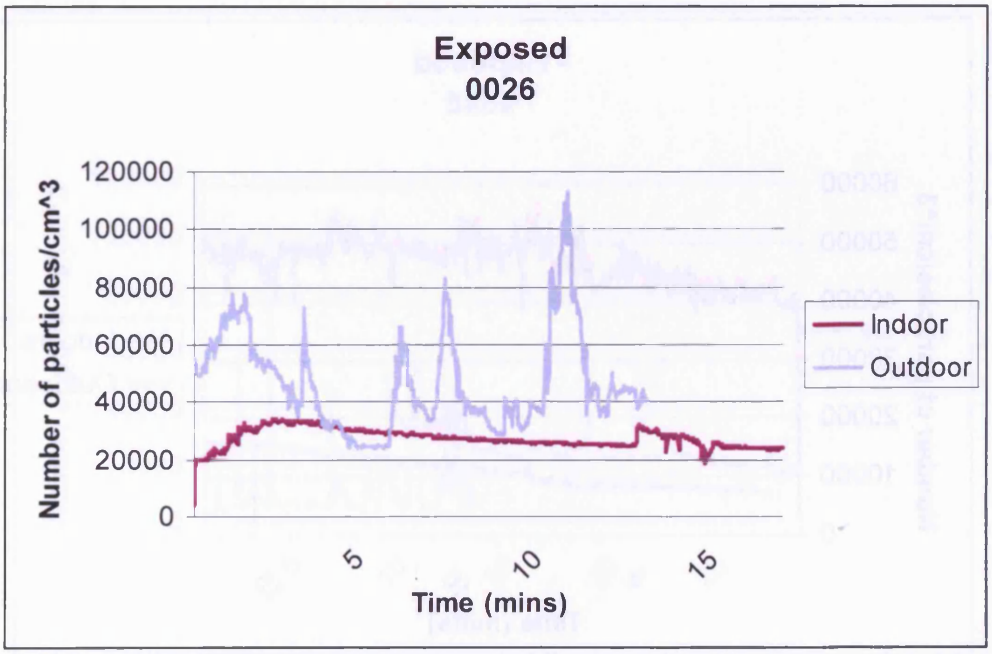

Figure 3.9: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

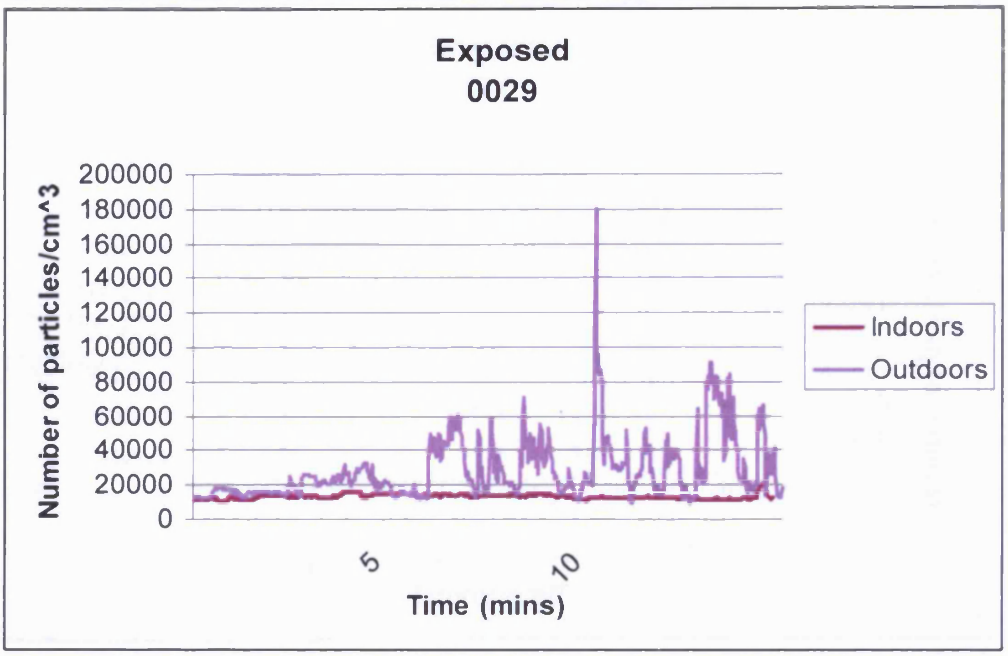

Figure 3.10: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

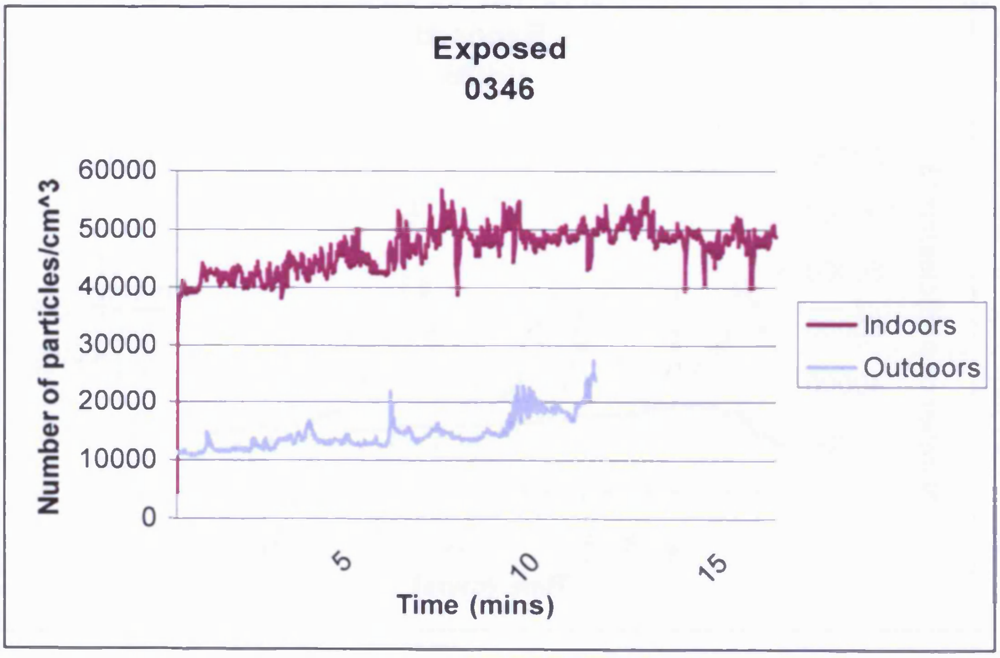

Figure 3.11: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

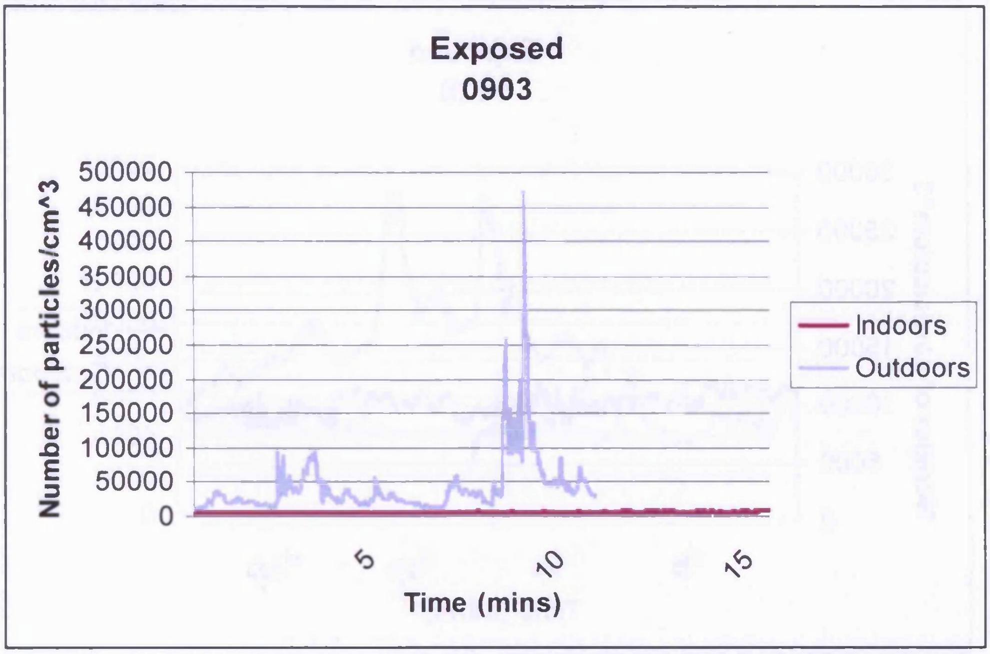

Figure 3.12: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

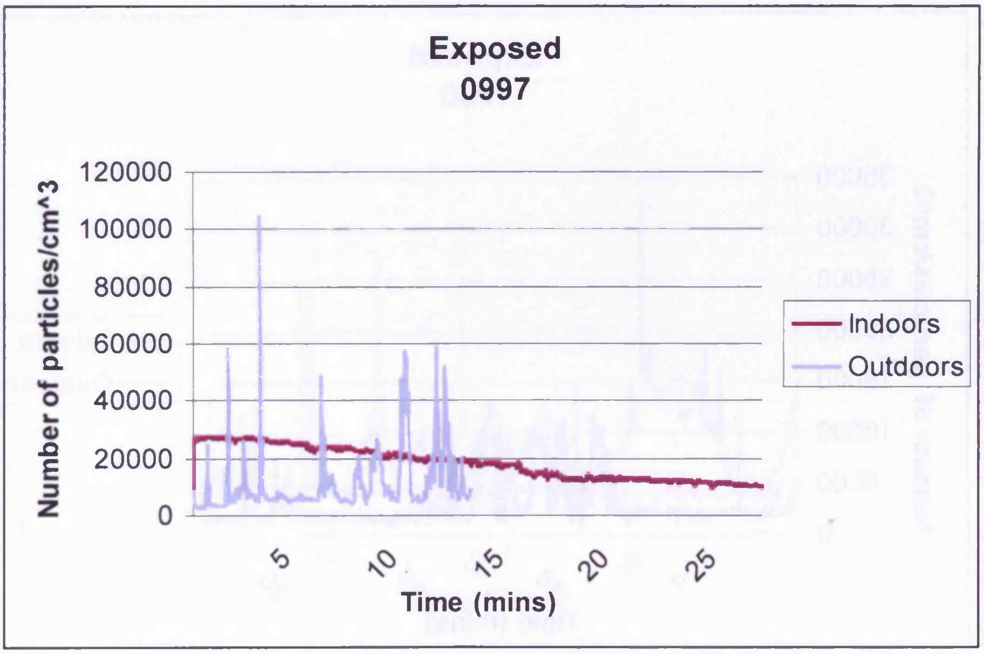

Figure 3.13: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

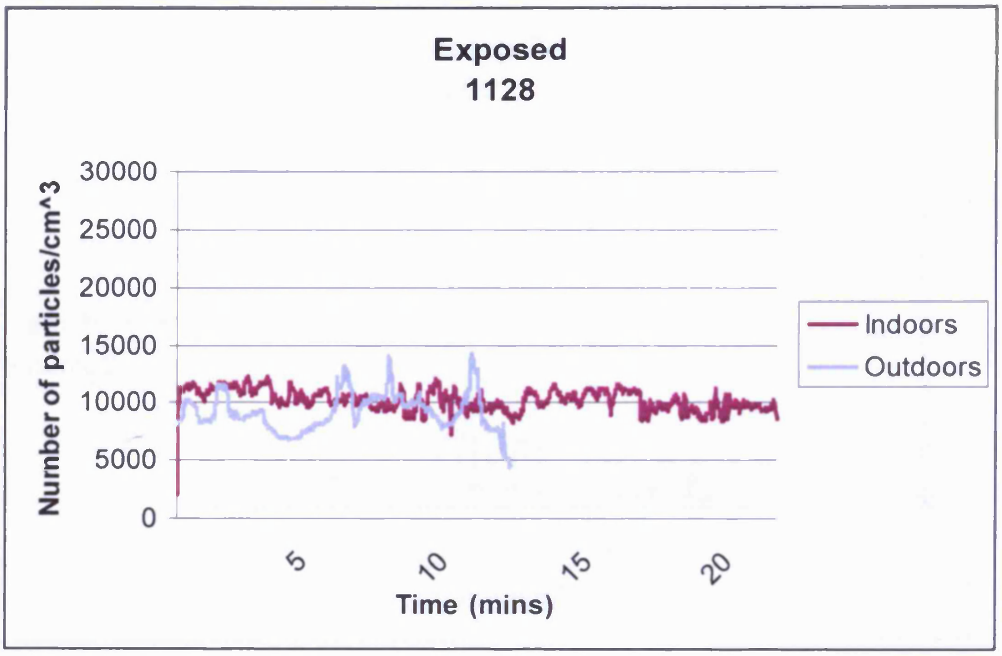

Figure 3.14: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

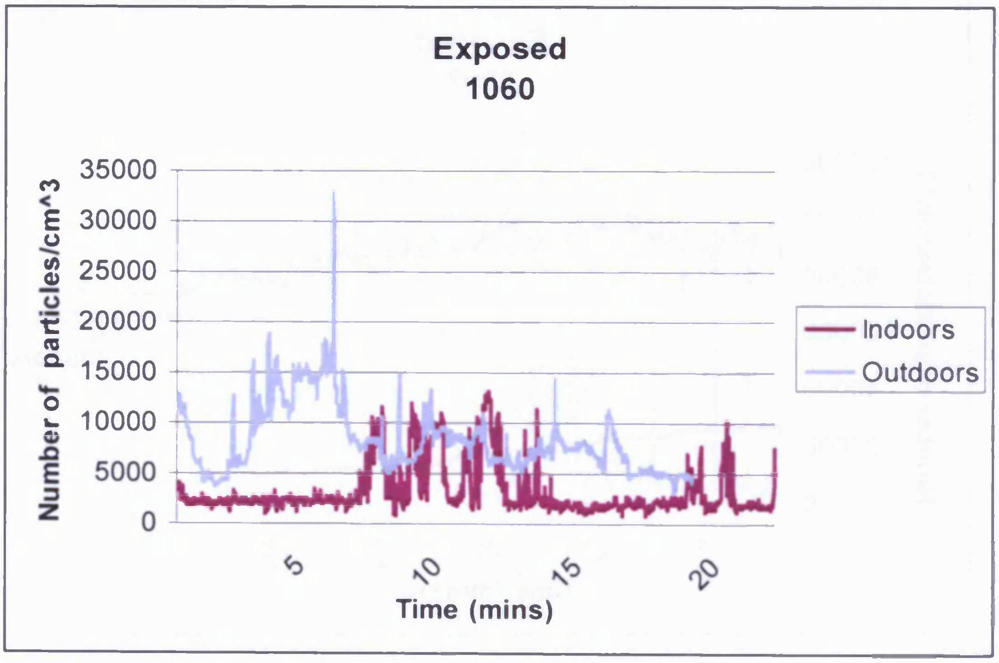

Figure 3.15: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

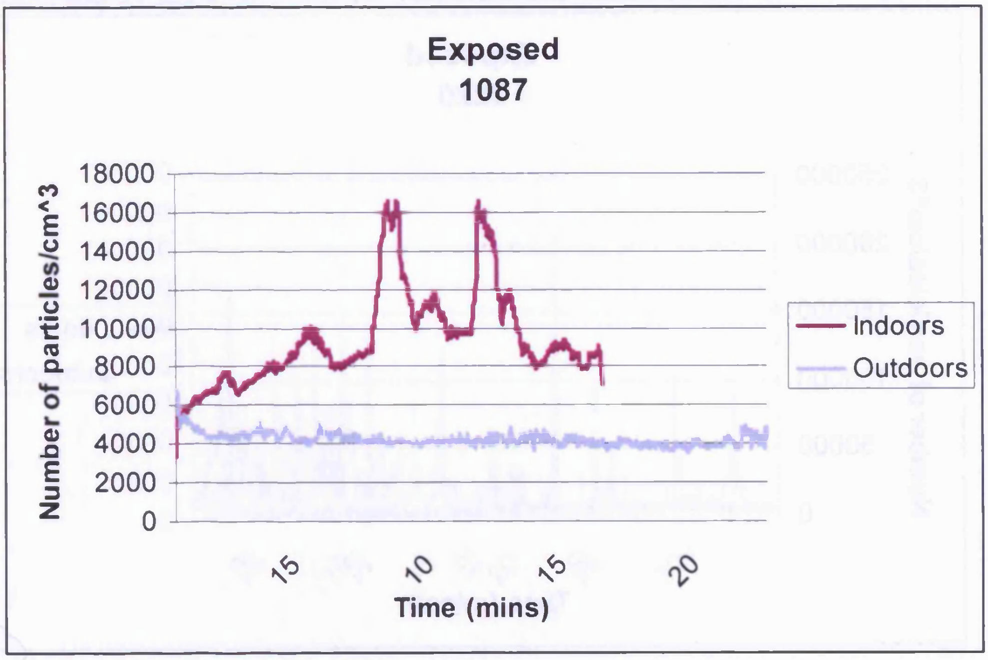

Figure 3.16: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

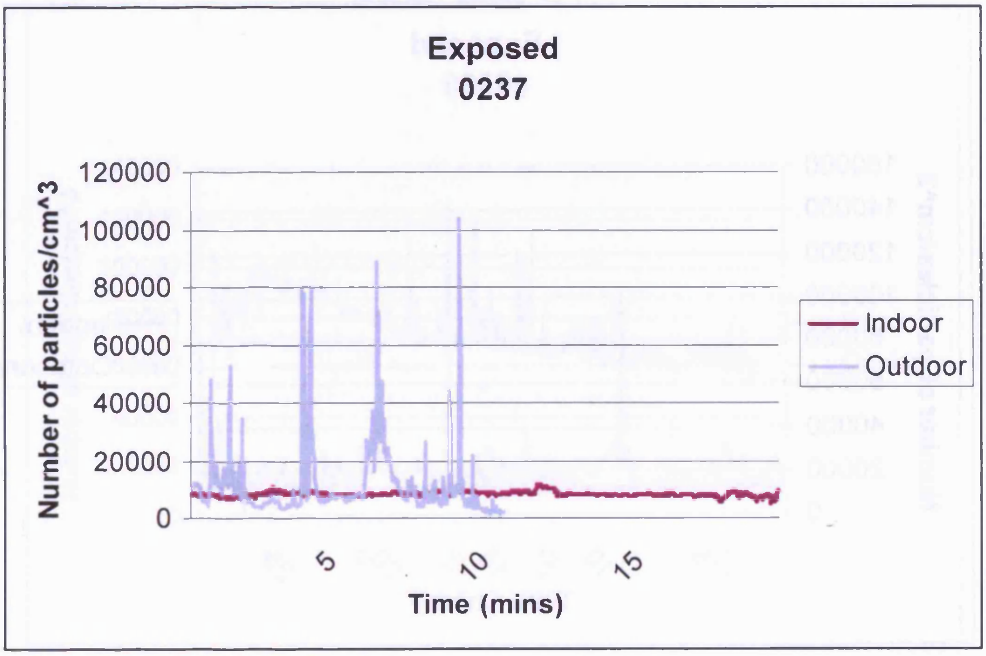

Figure 3.17: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

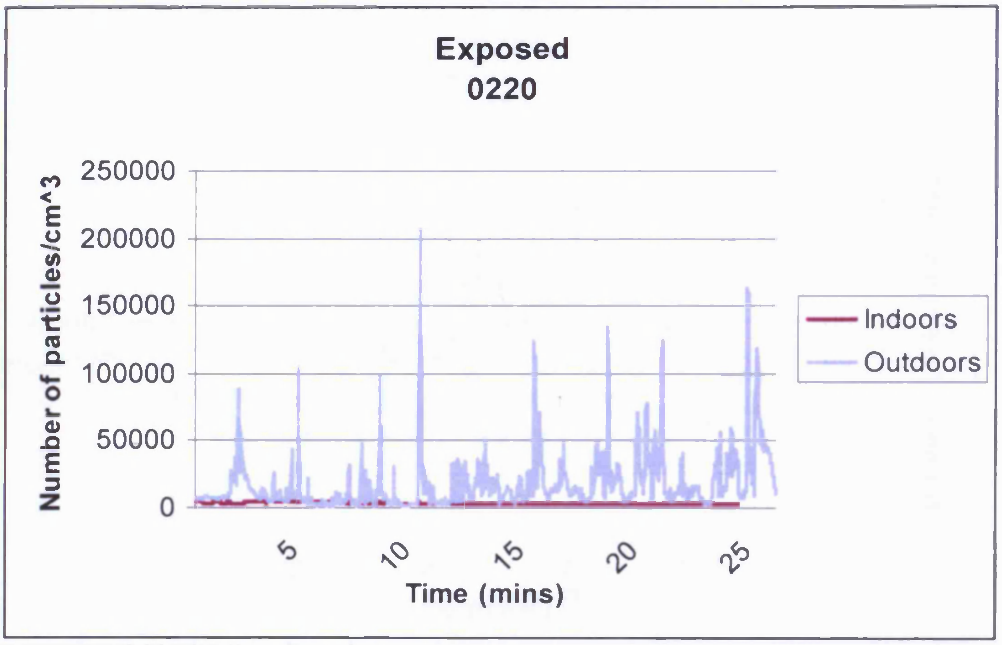

Figure 3.18: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

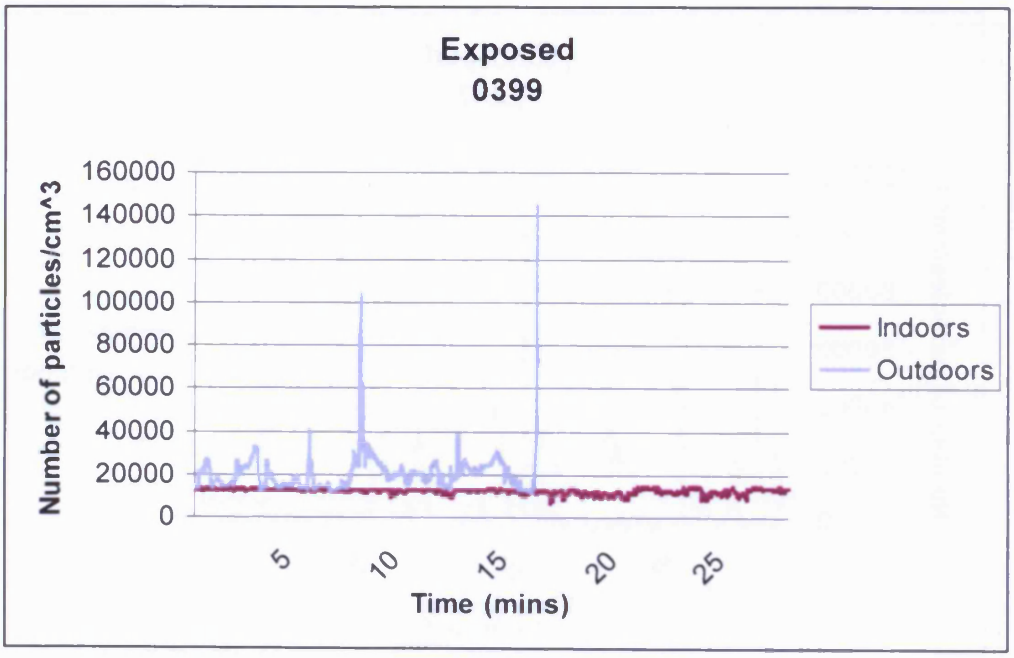

Figure 3.19: Comparison of the levels of particulates measured indoors and outdoors in the home of one of the study participants

Appendix Four

Appendix Five

Appendix Six