Environmental exposure to fine particles in Gothenburg

- personal exposure and its variability, indoor and outdoor levels, and effects on biomarkers

Sandra Johannesson

Occupational and Environmental Medicine

Institute of Medicine

Sahlgrenska Academy at University of Gothenburg

UNIVERSITY OF GOTHENBURG

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"Hey what else can we do now, except roll down the window and let the wind blow back your hair" Bruce Springsteen, Thunder Road.

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ABSTRACT

Urban particulate air pollution has been associated with adverse health effects in epidemiological as well as experimental studies. The overall aim of this thesis was to characterize environmental exposure to fine particles ($PM_{2.5}$), black smoke (BS) and particulate trace elements among the general adult population in Gothenburg. Exposure was assessed during 24 hours by personal sampling on 30 subjects, along with parallel residential indoor and outdoor measurements and fixed-site urban background monitoring. Repeated samplings were performed for 20 individuals. In a subsequent study, short-term effects of exposure to urban air pollution on blood biomarkers were examined in healthy volunteers.

The mean personal exposure to $PM_{2.5}$ was 12 $\mu g/m^3$ (95% CI 9.6-14 $\mu g/m^3$). There was a strong correlation (r_s=0.71) between personal exposure and indoor levels of $PM_{2.5}$, and a moderate correlation between personal exposure and urban background levels (r_s=0.61). Personal exposure exceeded residential outdoor levels for $PM_{2.5}$ and for several of the trace elements also the urban background levels. Air mass origin affected urban background levels of $PM_{2.5}$, BS and several trace elements, and also personal exposure to some elements derived from combustion processes. Determinants of personal exposure to $PM_{2.5}$ were season, smoking and the urban background levels. The within-person variance component dominated the variability of personal exposure to $PM_{2.5}$, BS and trace elements for non-smokers. Large within-person variance components point to the importance of performing repeated sampling when assessing environmental exposures. Levels of biomarkers were not found to be increased after days with elevated levels of ambient air pollution compared with low levels in healthy adults. Since there is no evidence of a threshold level below which no health effects of PM occur, further reduction of exposure to particulate air pollution would result in significant health benefits within the population of Gothenburg.

Keywords: personal exposure, air pollution, fine particles, black smoke, trace elements, exposure variability, determinants, panel study, biomarkers

SAMMANFATTNING PÅ SVENSKA

Luftföroreningar har kopplats till många allvarliga hälsoeffekter, främst hjärtkärlsjukdomar och luftvägssjukdomar. Huvudsyftet med denna avhandling har varit att mäta exponering för fina partiklar ($PM_{2.5}$) bland allmänbefolkningen i Göteborg. Personlig exponering mättes på 30 vuxna individer under ett dygn, samtidigt utfördes mätning i och utanför bostaden och i urban bakgrund. Partiklarna analyserades med avseende på masskoncentration, svärtningsgrad (black smoke) och innehåll av ett antal olika grundämnen. För att undersöka påverkan av långdistanstransport beräknades ursprunget för den luftmassa som befann sig i Göteborg under dygnet som mätning pågick. Inom-individvariansen i exponeringen (dag-till-dag-variansen) och mellan-individvariansen undersöktes, och information från dagböcker användes i mixed-effects models för att identifiera vilka faktorer som påverkade den personliga exponeringen. I en senare studie undersöktes eventuell påverkan på ett antal biomarkörer i blod efter dygn med antingen höga eller låga halter av partikulära luftföroreningar i en grupp friska vuxna frivilliga försökspersoner boende i Göteborg.

Medelvärdet för personlig exponering för $PM_{2.5}$ var 12 µg/m^3 (95 % KI 9.6-14 µg/m^3). Den personliga exponeringen var starkt korrelerad till inom-hushalterna av $PM_{2.5}$ (r_s=0.71), korrelationer mellan personlig exponering och halter utanför bostaden samt i urban bakgrund var något lägre (r_s=0.67 resp. r_s=0.61). Personlig exponering för $PM_{2.5}$ och flera av grundämnena var högre än halterna utanför bostaden. Luftmassans ursprung påverkade uppmätta utomhushalter av $PM_{2.5}$, black smoke samt innehållet av olika grundämnen. Effekt på personlig exponering kunde ses för vissa grundämnen som härrör från olika förbränningsprocesser (S, V och Pb). Inom-individvariansen var större än mellan-individvariansen för personlig exponering för $PM_{2.5}$, black smoke och grundämnen för icke-rökare. Faktorer som påverkade personlig exponering för $PM_{2.5}$ var årstid, rökning samt halten i urban bakgrund. Vid upprepad blodprovtagning i en panel av friska försökspersoner kunde ingen signifikant ökning av någon av de undersökta biomarkörerna ses efter ett dygn med höga jämfört med låga halter av luftföroreningar. Resultatet kan dock inte generaliseras till hela befolkningen som även innefattar känsliga grupper som t ex äldre och personer med lung- eller hjärtkärlsjukdomar. Även om de nivåer av partiklar som uppmättes i Göteborg var generellt låga jämfört med många andra städer i Europa och övriga världen, har stora epidemiologiska studier visat att även låga nivåer av partiklar ger allvarliga hälsoeffekter. Därför skulle ytterligare reducering av luftföroreningshalterna i Göteborg leda till positiva effekter på människors hälsa.

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

I.   Johannesson S, Gustafson P, Molnár P, Barregard L, Sallsten G. Exposure to fine particles ($PM_{2.5}$ and PM_1) and black smoke in the general population: personal, indoor, and outdoor levels. *Journal of Exposure Science and Environmental Epidemiology 2007; 17(7): 613-624*

II.   Molnár P, Johannesson S, Boman J, Barregard L, Sallsten G. Personal exposure and indoor, residential outdoor and urban background levels of fine particulate trace elements in the general population. *Journal of Environmental Monitoring 2006; 8(5):543-551.*

III.   Johannesson S, Rappaport S M, Sallsten G. Variability of environmental exposure to fine particles, black smoke and trace elements among a Swedish population. *Journal of Exposure Science and Environmental Epidemiology 2011; 21(5): 506-514.*

IV.   Johannesson S, Andersson E M, Stockfelt L, Barregard L, Sallsten G. Urban air pollution and effects on biomarkers of systemic inflammation and coagulation: a panel study in healthy adults. *Submitted manuscript*

CONTENT

1 INTRODUCTION

1.1 Air pollution and health

Air pollution continues to pose a significant threat to human health worldwide. The World Health Organization (WHO) have estimated that approximately two million premature deaths each year can be attributed to indoor air pollution, mostly in developing countries. Urban outdoor air pollution is estimated to cause about 1.3 million premature deaths per year worldwide (WHO, 2011). Also in Sweden, the health impact of air pollution is significant. It has been estimated that about 5000 premature deaths per year in Sweden can be attributed to PM exposure (Forsberg, et al., 2005).

Air pollution epidemiology

Several severe incidents have drawn attention to the hazards of urban air pollution and are regarded as the starting point for air pollution epidemiology. One of these incidents occurred in London, in December 1952, when stagnant air conditions resulted in a rapid increase of air pollution from domestic coal-burning, power plants and factories. This extreme smog episode was followed by a rapid increase in the number of deaths. It has been estimated that some 4000 extra deaths occurred during the following weeks (Harrison and Yin, 2000; Schwartz, 1994). A similar extreme air pollution episode took place in 1948 in the small-town Donora in Pennsylvania, USA, which resulted in a death rate more than six times the norm for that area (Bascom R, 1996).

The Harvard six cities study linked long-term exposure to fine particulate air pollution (measured as ambient $PM_{2.5}$) to increased mortality (Dockery, et al., 1993). Mortality rates were found to be higher in cities with higher mean concentrations of ambient $PM_{2.5}$ than in cities with lower levels. Another large cohort study, the American Cancer Society (ACS) study, also found an association between mortality (all-cause) and long-term exposure to ambient fine particulates and sulfate (Pope, et al., 1995). Fine particulate air pollution was associated with cardiopulmonary mortality and lung cancer but not with mortality due to other causes. These studies have, since published, undergone extensive re-analyses which have supported the original conclusions (Laden, et al., 2006; Lepeule, et al., 2012), and several other studies have confirmed

the association between long-term exposure to particulate air pollution and mortality e.g. (Beelen, et al., 2008; Ostro, et al., 2010; Pope, et al., 2002).

Short-term elevations in PM air pollution have in numerous time-series studies been associated with adverse health effects. Increases in ambient PM_{10} and black smoke have been associated with cardiovascular diseases (Le Tertre, et al., 2002) and with cardiovascular and respiratory mortality (Analitis, et al., 2006) within the European multi-city APHEA2 project. In a large study from the US (11.5 million individuals ≥65 years), short-term exposure to $PM_{2.5}$ was linked to an increased risk of hospital admission for cardiovascular and respiratory diseases (Dominici, et al., 2006). According to a recently published meta-analysis, short-term exposure to one or more of the main air pollutants, including PM ($PM_{2.5}$ and PM_{10}) but not ozone (O_3), were associated with a near-term increase in myocardial infarction risk (Mustafic, et al., 2012). However, this was not found in a case-cross over study in Stockholm (Berglind, et al., 2010). Coarse particles ($PM_{10-2.5}$) were associated with an increase in daily mortality in Stockholm, also after adjustment for other pollutants (including $PM_{2.5}$), and a stronger effect for November through May indicated re-suspension of road dust as an important factor (Meister, et al., 2012). A large US study found an association between coarse particles and hospital admissions for cardiovascular or respiratory diseases, but the association was not significant after adjusting for $PM_{2.5}$ (Peng, et al., 2008).

Human experimental studies

Controlled experimental exposure studies on humans and animals using concentrated ambient particles (CAPs) have provided a causal link between PM exposure and adverse health effects in the lung and cardiovascular system (Ghio and Huang, 2004). Experimental studies on humans have shown evidence of pulmonary inflammation after inhalation to both CAPs and dilute diesel exhaust. Exposure to dilute diesel exhaust has also demonstrated impairment of vascular functions in healthy adults. Moreover, clinical studies have shown that exposure to PM is associated with small, but significant increases in diastolic and systolic blood pressures (Mills, et al., 2009).

Health effects linked to urban PM exposure

There are numerous epidemiological studies, associating adverse health effects with long-term, as well as short-term exposure to urban particulate air pollution (Brook, et al., 2010; Pope and Dockery, 2006; Ruckerl, et al., 2011). However, long-term exposures have shown larger, more persistent cumulative health effects (Pope, 2007), possibly due to progression of

underlying diseases. Long-term effects are not the sum of all short-term effects. It has been concluded that the overall evidence confirms a causal relationship between exposure to $PM_{2.5}$ and cardiovascular mortality and morbidity (Brook, et al., 2010).

The range of health effects linked to long-term urban air pollution has broadened over the years; and there is now also epidemiological evidence for a reduced lung function in children (Gauderman, et al., 2004; Gotschi, et al., 2008; Nordling, et al., 2008; Schultz, et al., 2012). A study in three Swedish cities (Gothenburg, Uppsala and Umeå), found an association between levels of vehicle exhaust outside the home and an increase in the risk of onset of asthma in adults (Modig, et al., 2009). However, the role of traffic-related air pollution in adult-onset asthma is less conclusive than in childhood asthma (Jacquemin, et al., 2012). Evidence is increasing for an association between ambient fine PM and birth outcomes, including low birth weight and preterm birth according to a recent review (Shah, et al., 2011).

For the various health outcomes that have been linked to PM exposure, no threshold below which adverse health effects would not be anticipated has been indicated (WHO, 2006). Populations characteristics that may lead to increased susceptibility to PM-related health effects have been identified in epidemiological studies and include life stage, specifically children and elderly; individuals with preexisting cardiovascular and respiratory diseases, genetic polymorphisms and low socioeconomic status. More limited epidemiological evidence suggests an increased susceptibility also for individuals with diabetes, COPD and obesity (Sacks, et al., 2011).

1.2   Particulate matter

Ambient PM is derived from a variety of sources. Primary PM is emitted directly from its source, whereas secondary PM is generated through atmospheric chemical reactions of gases (Schlesinger, et al., 2006). Moreover, airborne PM then continues to undergo chemical and physical transformation in the atmosphere. Particulate matter has both natural and anthropogenic origin; some examples of natural sources of PM are the oceans (sea salt), volcanoes, the earth's crust (soil dust), wildfires and biological sources. Anthropogenic sources of PM are attributable to human activities such as combustion processes (e.g. burning of biomass and fossil fuels, traffic exhausts), industry, agriculture and various residential activities (Schlesinger, et al., 2006; WHO, 2006). Different sources along with continuous

transformation contribute to a diverse and complex composition of airborne PM.

Particles are usually described and categorized on the basis of their aerodynamic diameter (the size of a unit-density sphere with the same aerodynamic characteristics), usually referred to as simply the particle size. Particle size determines the particles' transport in air and their removal from the air, and also which region within the human respiratory tract the particles are likely to deposit. The size categories are based on the particles that pass a size-selective inlet with 50% cut off (e.g. a cyclone or an impactor). The thoracic fraction is the fraction of particles that enters the thorax. These particles have a diameter less than 10 µm and are referred to as PM_{10}. Fine particles ($PM_{2.5}$), are particles smaller than 2.5 µm, and can reach deep into the alveolar region. Particles between 2.5 and 10 µm of size are referred to as coarse particles ($PM_{10-2.5}$). Ultrafine particles have a diameter smaller than 0.1 µm. The respirable fraction (with a 50% cut off diameter of 4µm) reaches the alveolar region and is often the measure for dust at work places and is regulated by occupational exposure limits (OELs).

Which characteristics make PM in ambient air more harmful?

Although there is clear evidence for the association between ambient PM and adverse health effects, the relationship between specific physiochemical properties of PM and health effects remains largely unsolved (Schlesinger, et al., 2006). To gain further insight, knowledge derived from different disciplines is needed, such as atmospheric chemistry, exposure assessment, toxicology and epidemiology (Schlesinger, et al., 2006; WHO, 2006). The health hazards of particulate matter seem to be highly dependent on its nature; physiological properties (e.g. size, shape, surface area), the chemical composition (chemical species, solubility, etc.), toxicological and biological properties, and oxidative potential. Furthermore, it is the particles' ability to deposit in the human respiratory tract (the deposited dose) that determines a health response. The most important particle characteristics, with regard to deposition in the airways, were size and the particle's ability to grow by absorption of water vapor (Löndahl, 2009).

Epidemiological evidence for health effects of PM rely, in most of the studies, on ambient PM mass concentration as the measure of exposure. This may be regarded as an adequately real-life exposure, incorporating also other pollutants in the ambient air pollution mixture. However, in epidemiological studies it is difficult to disentangle various PM constituents due to possible correlations between the various pollutants as well as correlations between

pollutants and potential confounders such as weather conditions. However, a large time-series study in the US found that ambient levels of elemental carbon and organic carbon matter were associated with the largest risk of emergency hospital admissions for CVD and respiratory diseases across seven major chemical components of ambient $PM_{2.5}$ (Peng, et al., 2009).

Toxicological studies and human experimental studies have the advantage of using controlled exposure designs (concentration, dose, particle properties, etc.) in order to relate the exposure to a specific response. These studies have provided a causal link between PM exposure and adverse health effects in the lung and cardiovascular system (Brook, et al., 2010).

While toxicological studies indicate that particle characteristics determine the toxicity, definitive links between specific characteristics and health effects have not yet been identified. Toxicological and epidemiological studies do, however, indicate that PM generated from combustion processes (e.g. vehicle emissions, industry, energy production and biomass burning), play a significant role in causing the adverse health effects (WHO, 2007). These particles have a high content of elemental carbon as well as various carbonaceous substances and also some metals. The organic carbon content of PM consists of a wide range of compounds, among which polycyclic aromatic hydrocarbons (PAHs) or their nitro- or oxy-derivatives have been regarded as having a high toxicological potency (Bolling, et al., 2012; WHO, 2006).

Black smoke

Black smoke (BS) was monitored in Europe for urban air quality assessment decades ago, with start in the 1920s, as an indication of black particles from combustion of coal, biomass and oil. The first guideline value for protection of human health was set by the WHO for BS in 1979, as health effects of combustion related air pollutants had been identified (WHO, 2012). Black smoke was used in the European multi-city time-series study APHEA2, as one of the measures of exposure for assessing short-term effects of air pollutants with mortality (Katsouyanni, et al., 2001). Difficulties in standardizing BS monitoring combined with increased attention to health effects of total PM mass concentration (incorporating also the non-black particulate components), led to that BS no longer is addressed by air quality guidelines (WHO, 2012). Black carbon (BC) refers to the dark components of PM and is measured by optical light absorption techniques. Measurements of light reflectance from a PM sample (on a filter) are usually referred to as BS or absorbance (Abs), and light transmission trough the filter sample as BC. Elemental carbon (EC), on the other hand, is usually measured with thermal-

optical methods. All measures aim to capture the fraction of PM derived from various combustion sources (e.g. traffic, energy production, biomass burning etc.). Cohort studies have provided sufficient evidence of associations between BC and mortality (all-cause and cardiopulmonary) (WHO, 2012). Currently, there is no generally accepted standard method to measure BC or EC.

Most epidemiological studies base the risk estimate on the total PM mass concentration, thus assuming that all PM has the same potential to cause health effects regardless of its chemical composition or physical properties. Air quality guidelines are based on these risk estimates and, consequently, the current health based air quality guidelines are set for PM mass concentration as the measure of exposure (WHO, 2006).

Air quality regulations and guidelines

National regulations and environmental objectives

Sweden currently has 16 national environmental objectives, adopted by the Swedish Parliament. These objectives describe the quality of the environment that Sweden wishes to achieve by 2020. One of the environmental objectives is *clean air,* specified as: "The air must be clean enough to not represent a risk to human health or to animals, plants or cultural assets". Outdoor air quality in Sweden is regulated by air quality standards (Swedish Environmental Protection Agency). The standards for PM_{10} and $PM_{2.5}$ are included in the air quality regulation (Regulation 2010:477), and should contribute to the protection of human health and fulfillment of the EU-directive 2008/50/EG. The air quality standard for $PM_{2.5}$, 25 µg/m^3 as annual mean, is valid from 2010, and must not be exceeded after January 1st 2015. For PM_{10} there are two air quality standards; 40 µg/m^3 (annual mean), and 50 µg/m^3 (24-hour mean). The standards are valid for outdoor air, excluding outdoor work places and tunnels.

In 2009, the city of Gothenburg adopted a local environmental objective for clean air, which reads: "The air should be so clean that it is not harmful to human health and should not cause frequent annoyance". The local environmental objective for urban background levels of $PM_{2.5}$ in Gothenburg, 12 µg/m^3 as annual mean, is to be achieved by 2013. Moreover, the 24-hour

mean for PM_{10} should be <35 µg/m^3, exceeded at a maximum of 37 days at street level (Miljöförvaltningen, 2012).

Air quality guidelines from the World Health Organization

The first WHO guidelines were produced in 1987, updated in 1997 (WHO, 2000), and had a European scope. In the second edition of Air quality guidelines for Europe (WHO, 2000), experts came to the conclusion that no guideline values for PM could be recommended based on the current scientific database. Risk managers were instead referred to risk estimates from epidemiological studies on air pollution and health.

In early 2001, the Clean Air for Europe (CAFE) program was launched with the aim to establish a strategy on air pollution under the Sixth Community Environment Action Program within the European Union. The WHO project "Systematic review of health aspects of air quality in Europe" provided the CAFE program with a systematic and scientifically independent review of the health aspects of air quality in Europe. The systematic review recommended that guidelines for $PM_{2.5}$ would be further developed and that the guideline for PM_{10} would be revised. This resulted in a global update; Air Quality Guidelines for Europe, global update 2005 (WHO, 2006). For $PM_{2.5}$ the guidelines are 10 µg/m^3 as an annual mean and 25 µg/m^3 as a 24-hour mean. For PM_{10} the corresponding guidelines are 20 µg/m^3 and 50 µg/m^3, respectively. The guidelines for annual mean levels are based on the lowest levels at which total, cardiopulmonary, and lung cancer mortality have been shown to increase with more than 95% confidence in response to long-term exposure to $PM_{2.5}$. The numerical guideline value for PM_{10} is based on a $PM_{2.5}/PM_{10}$ ratio of 0.5. With regard to the ultrafine particles, no guideline concentrations could be provided. The WHO concluded that while there is considerable toxicological evidence for potential harmful effects of ultrafine particles, the epidemiological evidence is still insufficient to reach a conclusion on the exposure-response relationship.

1.3   Exposure assessment

Exposure assessment includes the design of the study, the collection of data as well as interpretation of the data (Nieuwenhuijsen, 2003). Quantitative exposure assessment aims to determine exposure levels for individual

subjects as well as a population. Exposure to particulate matter occurs both in occupational settings and in the general environment. Exposure levels may however differ substantially, work place exposures are often measured in milligrams per cubic meter whereas environmental exposures tend to be measured in micrograms per cubic meter. Also the duration of exposure differs, work place exposures normally last for up to eight hours per day, while environmental exposures may last for up to 24 hours a day. Lower air concentrations require more sensitive methods for sampling and chemical analysis. Lower exposure levels in combination with smaller health risk estimates also call for a refined exposure assessment in environmental epidemiology (Nieuwenhuijsen, 2003). Quantifying exposure levels usually involves monitoring, which can be done stationary (at fixed-sites) and by personal sampling.

Personal sampling involves, in air pollution monitoring, the attachment of an air pollution sampler to a person in order to measure the exposure of the individual. Personal exposure sampling has been widely used in occupational settings, and is also being more frequently used to monitor exposure in the general environment (Nieuwenhuijsen, 2003). Personal monitoring is generally more labor intensive and costly than stationary sampling, but can provide more informative and relevant information about the exposure. Personal monitoring can also provide insight into determinants of exposure (WHO, 2006).

1.3.1 Exposure variability

Environmental exposures to air pollutants are known to be highly variable in space and time (Clayton, et al., 1993; Egeghy, et al., 2005; Rappaport, 1991). Concentrations vary from day to day for a given subject and from subject to subject. As a consequence of exposure variability a range of exposure levels will be obtained during exposure monitoring, which complicates characterization of exposure levels. In a paper published as early as 1952 (Oldham and Roach, 1952), analysis of variance was applied on exposure data from the coal-mining industry. The authors found that significant variation occurred in dust concentrations from one worker's exposure to another's, and from one day to another for the same worker, and concluded that exposure variability (both in duration and intensity) must be taken into account in a proper evaluation of industrial dust exposure.

When exposures are highly variable in time and space, single measurements will not be sufficient to adequately assess exposure levels (Brunekreef, et al., 1987; Peretz, et al., 1997). With a study design that incorporates repeated measures of exposures, the total variance of exposure in a population can be partitioned into its variance components. The between-person variance component - a measure of the variation in average exposure levels between subjects, and the within-person variance component - a measure of the day-to-day variance in exposure levels for a given subject (Rappaport, 1991). If the population is divided into groups, there is also the between-group variance component. Between- and within-person variances have been estimated for exposures to various air pollutants in occupational settings e.g.(Hagstrom, et al., 2008; Heederik, et al., 1991; Kromhout, et al., 1993; Liljelind, et al., 2003; Mamuya, et al., 2006; Peretz, et al., 1997; Rappaport, et al., 1999; Spaan, et al., 2008; Symanski, et al., 2006). The same methods for analysis of variance have been applied for air pollution exposure in the general environment, however, the number of publications is still fewer than for work place exposures (Rappaport and Kupper, 2008). Exposure variability should be acknowledged since it will be of significance for exposure assessment strategies, and it will have an impact on epidemiological studies (Nieuwenhuijsen, 2003).

Attenuation of exposure-response relationships

Exposure measurements are often used in occupational and environmental epidemiology to estimate relationships between exposure concentrations and health effects in humans (exposure-response relationships). If exposure levels are not accurately characterized, the estimated exposure-response relationship (the estimated regression coefficient) tends to be underestimated. This underestimation (or suppression) of the risk estimate is referred to as attenuation (Brunekreef, et al., 1987; Nieuwenhuijsen, 1997; Rappaport and Kupper, 2008).

In an individual-based study, where both exposure and health outcome are measured in each subject (in log-scale), the ratio of the observed regression coefficient to the true regression coefficient is related to the ratio of the variance components (within/between) and the number of repeated samples per subject (Brunekreef, et al., 1987; Heederik, et al., 1991). Attenuation increases with increasing variance components ratio and decreases with increasing number of samples per subject. Estimated variance components ratios can be used as an indicator of the least biasing measure of exposure for estimation of an exposure-response relationship (Rappaport and Kupper, 2008).

Exposure variability may in some way be troublesome, due to its ability to complicate the quantitative assessment of exposure levels, which in turn has an effect on the estimation of dose-response relationships. However, the sources of variability also contain valuable information, which can be used to develop appropriate control measures to reduce exposure levels (Burdorf, 2005; Rappaport and Kupper, 2008). Furthermore, considerable within-person variability in personal exposure concentrations is valuable when performing time-series analyses.

1.3.2 Determinants of exposure

Determinants of exposure are factors that are associated with elevated or reduced exposure levels (Burstyn and Teschke, 1999). A determinant of exposure is a variable with a constant and repeatable effect on the exposure levels. With the use of a linear mixed-effects model, exposure determinants (fixed-effects) can be estimated, along with estimates of the variance components (Rappaport and Kupper, 2008). Given that factors which may influence exposure levels are recorded during exposure monitoring, a subsequent data analysis could identify which are important sources of exposure (Burstyn and Teschke, 1999). These methods have been applied in studies for assessing exposure determinants in various occupational settings (Burstyn, et al., 2000; Hagstrom, et al., 2012; Lillienberg, et al., 2008; Peretz, et al., 2002; Preller, et al., 1995; Rappaport, et al., 1999).

Determinants of personal exposure to $PM_{2.5}$ in elderly subjects with coronary heart disease in Helsinki and Amsterdam was investigated by incorporating data from questionnaires, time-activity diaries, housing characteristics as well as outdoor levels (Lanki, et al., 2007). A similar approach was applied in an exposure study of subjects with COPD living in Boston (Rojas-Bracho, et al., 2004). Another study investigating factors that could predict personal exposure to $PM_{2.5}$ and BS was performed among students living in Copenhagen (Sorensen, et al., 2005). In studies of environmental exposure to PM, factors that may influence the relationship between personal exposure and ambient levels have been assessed in several studies, e.g. (Adgate, et al., 2007; Brown, et al., 2009; Ebelt, et al., 2000; Janssen, et al., 1998; Liu, et al., 2003; Sarnat, et al., 2006).

The variance in the measured exposure levels can be partly explained by the fixed determinants of exposure, thereby reducing the between- and/or within-

persons variance (Burdorf, 2005; Burstyn and Teschke, 1999; Rappaport and Kupper, 2008). Comprehensive evaluations of the influence of exposure determinants on the between-worker and within-worker variances, respectively, have been performed in occupational settings e.g. (Burstyn, et al., 2000; Peretz, et al., 2002; Rappaport, et al., 1999). (Egeghy, et al., 2005) and colleagues evaluated effects of exposure determinants on the between- and within-person variances for environmental exposures to lead, phenanthrene and chloripyfos (a pesticide), by incorporating data from questionnaires and time-activity diaries.

1.4 Particulate air pollution and biomarkers

An increased risk for cardiovascular events following both short- and long-term exposure to ambient PM has been shown in epidemiological studies. The biological mechanisms leading to cardiovascular effects are, however, not fully understood. One of the suggested pathways is that inhaled particles induce pulmonary oxidative stress and inflammation leading to systemic inflammation and increased blood viscosity, and the progression of atherosclerosis, resulting in increased risk of cardiovascular events (Brook, et al., 2010).

Biological effects after exposure to air pollution can be investigated by analyzing biomarkers of inflammation and coagulation in blood. Exposure can be carried out under controlled conditions, e.g. in chamber studies, or by utilizing the day-to-day variation in ambient levels of air pollution that occurs within, for example, a city (often referred to as panel studies).

There are some specific biomarkers that have been associated with cardiovascular diseases. The acute-phase proteins C-reactive protein (CRP) and fibrinogen are consistently found to be predictors of cardiovascular disease, and associations have been found for serum amyloid A (SAA) as well (Danesh, et al., 2005; Kaptoge, et al., 2010; Pearson, et al., 2003). Fibrinogen is also a major determinant of blood viscosity. There are several biomarkers which are considered to be involved in the inflammatory process, e.g. the coagulation factor VIII in plasma (Liao, et al., 2005), p-selectin, and the cell adhesion molecules, soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (VCAM-1) (Elangbam, et al., 1997; Haverslag, et al., 2008). The pneumoproteins Clara

cell protein 16 (CC16) and surfactant protein D (SP-D) in the epithelial lining fluid of the lung are believed to protect the respiratory tract against inflammation and oxidative stress. Increased serum-levels have been found in a number of pulmonary diseases and after acute exposure to air pollution, and may reflect increased permeability of the air-blood barrier (Hermans and Bernard, 1999).

The first panel study on ambient air pollution and blood biomarkers was conducted in Germany, where plasma viscosity was found to be increased during an episode of elevated air pollution compared with before and after the episode (Peters, et al., 1997). A large number of epidemiological studies have followed, however, study designs, investigated populations, and blood measures vary between studies. According to a recent review, the epidemiological evidence for an association between air pollution and blood biomarkers suggests that positive associations are indicated for blood markers of inflammation, whereas results for fibrinogen and adhesion markers are still inconsistent (Ruckerl, et al., 2011). Another recent review of epidemiological studies on the effect of PM air pollution on CRP solely, found results in studies of healthy adults to be inconsistent, but there were suggestive evidence that higher PM levels tended to induce stronger inflammatory responses (Li, et al., 2012).

2 AIMS OF THE THESIS

Personal exposure to fine particles had not previously been measured among the general population of Gothenburg, nor in any other Swedish city, when this project was started. One main objective of this thesis was to address this gap in knowledge.

The specific aims of this thesis were to:

- Characterize the personal exposure to fine particles ($PM_{2.5}$ and PM_1), black smoke (BS) and particulate trace elements in the general adult population of Gothenburg (**Paper I and II**).

- Assess the relationship between the personal exposure and the simultaneously measured residential indoor and outdoor concentrations and urban background levels of PM, BS and trace elements (**Paper I and II**).

- Investigate the influence of different air mass origin on the measured levels of $PM_{2.5}$, BS and trace elements (**Paper I and II**).

- Estimate the between- and within-person variance components for the personal exposure to $PM_{2.5}$, BS and the trace elements (**Paper III**).

- Identify determinants of the personal exposure to $PM_{2.5}$, BS and trace elements (**Paper III**).

- Examine if exposure to the varying levels of urban air pollution in Gothenburg would be associated with a short-term effect on biomarkers of inflammation and coagulation in healthy adults (**Paper IV**).

3 MATERIALS AND METHODS

3.1 Paper I, II and III

3.1.1 Study group

Paper **I, II** and **III** are based on the results of a study conducted in Gothenburg during 2002-2003. The study group consisted of 30 adults living in Gothenburg. Twenty of the 30 subjects were randomly selected from the population register, and ten were volunteers recruited among the employees at the Department of Occupational and Environmental Medicine in Gothenburg. Inclusion criteria were to be between 20 and 50 years of age at the time of recruitment and live in Gothenburg. In total, the study group consisted of eight men and 22 women between 23 and 51 years of age. At the time of monitoring, 24 of the study subjects were gainfully employed, three were students, one was on maternity leave, one was unemployed and one had a sabbatical year. There were three smokers among the 20 randomly selected subjects, and none among the ten staff volunteers.

All study subjects completed a short questionnaire about age, occupation, type of home, lifestyle factors, workplace exposure to dust and/or fumes etc. The subjects also completed a time-activity diary for the 24-hour monitoring period, including smoking (number of cigarettes), exposure to environmental tobacco smoke (ETS), time at home, time at work, time indoors elsewhere, time outdoors, time spent in car or on a bus. Information about the study group and data from the questionnaires and diaries is presented in Table 1. All three smokers participated in the repeated sampling. Exposure to ETS was reported by two non-smoking subjects in each sampling session (different subjects in 1st and 2nd sampling). According to the time-activity diaries, over 90% of the sampling time was spent indoors.

Table 1. Data on the study group in Paper I, II and III

	1st sampling	2nd sampling (repeats)
Study subjects (N)	30	20
Women	22	16
Men	8	4
Median age, years (range)	37 (23-51)	36 (23-50)
Smokers (N)	3	3
Cigarettes per day, median (range)	7 (4-13)[*]	12 (6-15)[*]
Non-smokers exposed to ETS	2	2
Time-activity data from diaries		
Time spent, median (%)		
Indoors, total	94	95
Indoors at home	58	58
Indoors at work	30	33
Outdoors	4	4
In cars or buses	3	2

* For the smokers

3.1.2 Monitoring

Fine particles were measured for 24 hours using both personal and stationary monitoring equipment. Personal sampling of $PM_{2.5}$ was carried out simultaneously with measurements of $PM_{2.5}$ and PM_1 indoors in living rooms and outside the home (residential outdoor), on a balcony, porch, etc. In addition, parallel urban background $PM_{2.5}$ levels were measured. The urban background monitor was placed on the roof of the Department of Occupational and Environmental Medicine, located at Medicinaregatan 16, somewhat south of the city center and not near any major highway.

Personal monitoring was performed in two different ways. The 20 randomly selected subjects carried personal monitoring equipment for $PM_{2.5}$, while the ten staff members carried two pieces of personal monitoring equipment at the same time. During the first sampling, one $PM_{2.5}$ together with one PM_1 sampler were carried, while during the second sampling duplicate $PM_{2.5}$ samplers were used. The personal monitoring equipment was carried by the subject during awake time. During the night, it was placed in the living room. Repeated measurements (personal and parallel urban background levels) were performed for ten of the 20 randomly selected subjects. For the ten staff volunteers, repeated measurements were performed also indoors and outside

the home. The repeated samplings were collected from about two to eight weeks after the first sampling (median: 26 days between repeats, range: 14-54 days). All measurements were performed during the spring and fall seasons of both 2002 and 2003. Samples were collected on weekdays only. In total, 29 of the personal samples were collected during spring and 21 during fall. Apart from a few exceptions, monitoring was carried out for only one subject per day. Altogether, 50 sampling sessions (on 47 different days) resulted in a total of 270 filters.

One subject among the randomly selected participants reported being highly exposed to dust and paint at work during the day of sampling, and this subjects' personal sample was excluded from the data set in the further statistical analyses.

3.1.3 Particle sampling equipment

For personal, indoor and residential outdoor sampling, the BGI personal sampling pump (BGI 400S) was used together with the GK2.05 (KTL) cyclone for $PM_{2.5}$ sampling and the Triplex cyclone SCC1.062 for PM_1 sampling (BGI Inc., Waltham, MA, USA). A flow rate of 4 L/min was used for $PM_{2.5}$ sampling, while 3.5 L/min was used for PM_1. The flow rate was adjusted prior to monitoring and controlled at the end of the sampling period using a DryCal DC-Lite flowmeter (BIOS International Corporation, Butler, NJ, USA). The average flow rate was then used to calculate the total volume of air drawn through the filter. For personal monitoring, the pump was placed in a small shoulder bag and the cyclone was attached to the shoulder strap near the subject's breathing zone, see Figure 1 (a). For duplicate sampling, the two pumps were placed in the pockets of a vest, Figure 1 (b).

Figure 1. (a) Personal sampling equipment for $PM_{2.5}$ (b) Duplicate personal sampling equipment ($PM_{2.5}$ and PM_1)

For the stationary indoor and outdoor sampling, the pumps were placed in a box (to reduce the noise from the pumps and for rain shelter, respectively), and the two cyclones (one for $PM_{2.5}$ and one for PM_1) were placed about 1.5 m above the floor on a tripod. Urban background monitoring was carried out using the PQ100 Basel $PM_{2.5}$ sampler (EPA WINS) (BGI Inc., Waltham, MA, USA), which has an impactor cutoff system. The flow rate of the EPA WINS is 16.7 L/min.

Teflon filters (2 µm pore size) were used for all samplings (Pall Teflo, Pall Corporation, Ann Arbor, MI, USA), 37 mm filters for personal and stationary indoor and outdoor sampling, and 47 mm filters for the urban background sampler.

3.1.4 Analyses

Paper I

Mass concentration

The filters were conditioned for 24 hours prior to weighing in a climate chamber controlled for temperature and humidity (temperature: 23 ±0.5°C, relative humidity (RH): 50±5 %). The weighing followed a, where three field blanks followed each batch of filters. The weighing procedure was a modified version of the standard operating procedure used in the ULTRA study (Pekkanen et al., 2000). Filter mass before and after particle sampling was determined using a CAHN C-30 microbalance. Prior to weighing, the filters were deionized on both sides using an alpha radiation source (Po-210) in order to remove static charge. Each filter was weighed twice, and if the two results differed by more than 2 µg a new pair of weighing results was required. The procedure was repeated until this requirement was met. The average field blank mass increase (or decrease) was subtracted from each sampled filter mass in the batch. Filters were placed in plastic filter cassettes that were checked for potential leakage, and stored at room temperature prior to sampling.

The limit of detection (LOD) was evaluated by weighing batches of blank filters according to ISO/CD 15767 (International Organization for Standardization, 1998) and resulted in a lowest detectable sample mass of 18

μg. With flow rates of 4.0 and 3.5 L/min for 24 hour sampling of $PM_{2.5}$ and PM_1, respectively, the corresponding mass concentrations were 3.2 μg/m^3 and 3.6 μg/m^3. The coefficient of variation for duplicate personal $PM_{2.5}$ samples was 15 %. Among a total of 142 sampled $PM_{2.5}$ filters, 7 were below LOD. None of these filters were from personal sampling (4 indoors and 3 outdoors). For PM_1, 17 out of a total of 89 filters were below LOD (2 personal, 7 indoors and 8 outdoors). None of the $PM_{2.5}$ filters from the urban background station (EPA WINS) were below LOD since the sampled volume was substantially larger.

Black smoke

The filters were analyzed for black smoke using a M43D EEL smokestain reflectometer (Diffusion Systems Ltd., London, UK), following a procedure also similar to the ULTRA study (Pekkanen et al., 2000; Götschi et al., 2002). Each filter was measured for reflectance five times on different locations according to the five-point method (in the center and in each of the four quadrants) and the average reflectance derived from the five measurements was used in the calculations. The absorption coefficient (a) was used to express the reflectance according to ISO9835 (International Organization for Standardization, 1993):

$a = (A/2V)*\ln(R_0/R_s),$

where *A* is the loaded filter area (m^2), *V* is the sampled air volume (m^3), R_0 is the average reflectance of field blank filters, and R_s is the average reflectance of the sampled filter. The absorption coefficient (a) is expressed in 10^{-5} m^{-1}. After every 25 filters, three filters were selected and measured a second time to ensure that the two results differed by a maximum of 3%.

Paper II

Elemental composition of the particle mass

The particle mass on the filters was analyzed for its elemental composition using an energy-dispersive X-ray fluorescence (EDXRF) spectrometer (Öblad, et al., 1982). The EDXRF spectra were processed and quantified using the Quantitative X-ray Analysis System (QXAS) and the Analysis of X-ray spectra by Iterative Least-square fitting (AXIL) (Bernasconi, et al., 2000; van Espen and Jansen, 1993). Samples were analyzed over a period of

1,000 seconds, a tube voltage of 55 kV, a tube current of 25 mA, and a molybdenum secondary target. For some filters with low mass concentrations, a more narrow and fine-tuned spectrum fit was obtained to improve the data recovery of the lighter elements (up to vanadium). The mean analytical precision was 5%, as calculated from repeated analysis (N=5) of two randomly selected filters, one having a low and the other, a high mass loading. In total, 65 field blanks were analyzed and concentrations were below the LOD for all elements except Fe and Zn, but their concentrations were low and did not change the results.

3.1.5   Air mass trajectories

The effect of long-range transport on measured air pollutants levels was investigated in **Paper I** (PM and BS) and **Paper II** (trace elements) by computing 96 hour air mass back trajectories using the NOAA ARL HYSPLIT Model (Draxler and Rolph, 2003). For each 24-hour sampling, five air mass back trajectories were computed; at startup time and 6, 12, 18, and 24 hours thereafter. Four different major air mass paths were identified as routes of the trajectories: a *Nordic* trajectory passing the Nordic countries and reaching Gothenburg from the north, a *Marine* trajectory originating from the North Atlantic, a *UK* trajectory, with the air mass passing the UK on its way to Gothenburg, and a *Continental* trajectory coming from the Central European continent. The classification was then made according to the criterion that all five trajectories during a sampling period must have a major path belonging to the same class. Trajectories not meeting this criterion (i.e. trajectories that shifted classes during the sampling day) were classified as undetermined.

3.1.6   Paper III

The variability in the personal exposure to $PM_{2.5}$, BS and trace elements was estimated, based on the 49 samples from **Paper I** and **II** (29 individual subjects and 20 repeats, work place exposed subject excluded). Only trace elements with more than 50% of the personal samples above the LOD were analyzed statistically, these were: Cl, K, Ca, Ti, Fe, Ni, Cu, Zn, Br, and Pb. However, Br was excluded from further analysis in the study because of the very low (though detectable) levels were too close to the LOD to provide usable data (Molnár et al., 2005).

A mixed-effects model was applied to each of the exposure variables ($PM_{2.5}$, BS, and the trace elements) to estimate the within-person and between-person variance components, and covariates were added for the purpose of identifying determinants of exposure (see Section 3.3). Exposure to ETS among the non-smokers was scarce, only reported for three sampling events (0.5 hour, 1 hour and 2 hours, respectively), and could therefore not be evaluated in the models.

3.2 Paper IV

3.2.1 Study group

The study group consisted of 16 subjects, eight men and eight women, all non-smokers and living in Gothenburg. Median age was 35 years (range 26-55 years) at the time of recruitment in 2007. All the study participants were volunteers employed at the Sahlgrenska University Hospital or at the University of Gothenburg. All the volunteers were healthy and did not suffer from any severe chronic disease (e.g. coronary heart diseases, COPD, diabetes or asthma), and none of the subjects was obese (body mass index (BMI): 21 to 27 kg/m^2). The study was approved by the Regional Ethical Review Board at the University of Gothenburg. All study participants gave a written informed consent before entering the study. The study participants lived between 1.7 and 7.6 km from the urban background monitoring station (median: 3.6 km).

3.2.2 Air pollution monitoring and criteria

Ambient levels of air pollutants were collected as 1-hour mean concentrations from the monitoring station run by the Environmental Department of the Municipality of Gothenburg. PM_{10} was measured by a tapered element oscillating microbalance (TEOM) instrument, and NO_2 and NO_x by the chemiluminescence technique. The monitors were located at roof top level (27 m above the ground) in downtown Gothenburg. Criteria for high and low pollution days were established before the start-up of the study, and the aim was to get as large a contrast as possible in air pollution levels (Table 2). Air pollution levels were classified as a high-pollution day based on 24-hour mean concentrations of PM_{10}, and as a low-pollution day based on

levels of PM_{10} and NO_2. Lag 0 represents the average concentration of the air pollutant from 8:00 am on the prior day until 8:00 on the sampling day. Lag 1 refers to the 24-hour average concentration 24 to 48 hours before blood sampling (see also Figure 1 in **Paper IV**).

Table 2. Criteria for 24-hour average air pollution concentrations for low- and high-pollution day, respectively ($\mu g/m^3$).

	Lag 0		Lag 1	
	PM_{10}	NO_2	PM_{10}	NO_2
Low-pollution day	<15	<35	<25	
High-pollution day	>30			

Data was also re-analyzed with air pollution levels classified according to ambient NO_2 and NO_x levels instead (as markers of traffic exhausts), regardless of the PM_{10} levels. This implied that two sampling days were shifted.

3.2.3   Blood sampling procedure

Sampling started with the first subject at 8:00 in the morning, and the other subjects were called about 10-15 min apart. Subjects were scheduled for about the same time on each sampling session, to account for circadian variations. The subjects answered a short questionnaire just before blood sampling (see **Paper IV**). Altogether, three tubes of blood were drawn, two for serum and one for plasma. Subjects with an ongoing infection (e.g. cold), and those who had been out of town during the past two days were not allowed to participate in that session.

A blood sample was drawn from subjects the next morning on the following day (follow-up sampling, Figure 1, **Paper IV**). During the follow-up sampling, the procedure was exactly the same as during the previous morning, and the subjects were scheduled for the same time. Blood samplings were performed from Tuesday to Friday, not on Mondays or during the weekend. On some occasions subjects were not able to participate on the follow-up day, and this was allowed according to the study design.

The first blood sampling was performed in September 2007 and the last in mid-June, 2009.

3.2.4 Biochemical analyses

The blood samples were analyzed for ten different biomarkers. Serum CRP was analyzed using immunoturbidimetry, fibrinogen in plasma was analyzed based on the coagulation time at high thrombin concentration, and factor VIII in plasma using one-stage clotting method. Commercial ELISA kits were used to analyze SAA, sICAM-1, sVCAM-1, p-selectin and the pneumoproteins CC16 and SP-D in serum, and PAI-1 in plasma. CRP, fibrinogen, factor VIII and PAI-1 were analyzed at Sahlgrenska University Hospital, Department of Clinical Chemistry. SAA, sICAM-1, sVCAM-1 and p-selectin were analyzed at The Wallenberg Laboratory at University of Gothenburg, and CC16 and SP-D were analyzed at Occupational and Environmental Medicine, Sahlgrenska Academy. For further information about methods and reagents, see **Paper IV**. Some of the samples (N=30) did not contain enough plasma to analyze also for PAI-1, and the total number of PAI-1 samples is therefore less than for the other biomarkers (Table Y).

3.3 Statistical analysis

Correlations between concentrations in different locations (personal, indoor, residential outdoor and urban background) of PM and BS (**Paper I**) and trace elements (**Paper II**) were assessed using the Spearman rank correlation coefficient (r_s). In **Paper IV**, r_s was used to estimate associations between the different biomarkers (separately for each subject, and presented as the median over the 16 subjects). Correlations between the possible covariates were assessed using r_s prior to inclusion in the mixed-effects model (**Paper IV**). *Differences* between pairs of personal, indoor, residential outdoor and ambient levels were tested using the Wilcoxon signed rank test (**Paper I and II**). Wilcoxon signed rank test was also used to test correlations between pairs of biomarkers for statistical significance (**Paper IV**). For unpaired observations, the Wilcoxon rank sum test was used.

The between- and within-person variance components (σ^2_{bY} and σ^2_{wY}) were estimated in **Paper I, III** and **IV**, using a one way random-effects model (model (1)):

$$Y_{ij} = \mu_Y + b_i + e_{ij} \tag{1}$$

where μ_Y represents the true fixed mean exposure level for the population, b_i represents the random effect for the ith subject, and e_{ij} represents the random effect of the exposure level Y_{ij} on the jth day for subject *i*. The random effects b_i and e_{ij} are assumed to be mutually independent, and normally distributed with means of zero and variances σ^2_{bY} and σ^2_{wY}, respectively.

Natural logarithmic transformation of exposure data was performed in **Paper I** (PM and BS), and in **Paper III** (PM$_{2.5}$, BS, and trace elements) since data were right-skewed. Then $Y_{ij} = \ln(X_{ij})$ and the mean μ_Y represents the mean logged exposure level for the population, and the natural-scale mean exposure level can be estimated as $\mu_X = \exp(\mu_Y+0.5\sigma^2_Y)$, which was done in **Paper III**. In **Paper IV**, log-transformation was needed for some of the biomarkers (data were skewed to the right). However, for some of the biomarkers untransformed data were used (fibrinogen, factor VIII, p-selectin, sICAM-1, and CC16). Then $Y_{ij} = X_{ij}$ and the mean μ_Y in model (1) represents the mean natural-scale biomarker level of the population.

Papers III and IV involve applications of a mixed-effects model (model (2)), including additional fixed effects for U covariates $C_1, C_2, \ldots, C_U$ in order to identify and estimate significant determinants of exposure along with estimates of the variances between and within persons:

$$Y_{ij} = \mu_Y + \sum_{u=1}^{U} \partial_u C_{uij} + b_i + e_{ij} \tag{2}$$

$Y_{ij} = X_{ij}$ for untransformed data, and $Y_{ij} = \ln(X_{ij})$ for log-transformed. The ∂_u:s are regression coefficients representing the U covariates. In **Paper III**, a compound symmetry covariance structure was used.

Before applying the mixed-effects model to each biomarker in **Paper IV**, a likelihood test was used to assess whether common variances could be used for men and women. For each biomarker, the model (2) (containing gender as the only extra variable) was estimated with three different variance structures: common between- and within-person variances for men and women, distinct between-person but common within-person variance for men

and women, and distinct between- and within-person variances for men and women, respectively (Rappaport and Kupper, 2008). The difference in -2loglikelihood follows a chi square distribution and p-values below 0.05 were considered significant. Common between- and within-person variances could be used for men and women for CRP, fibrinogen, PAI-1, p-selectin, sICAM-1, sVCAM-1 and SP-D, whereas distinct between- and within-person variances had to be used for men and women, respectively, for factor VIII, SAA and CC16.

Regression coefficients for the U covariates were estimated using Proc Mixed. Backwards stepwise regression was used to eliminate non-significant variables.

As a measure of exposure variability, *fold-ranges* ($R_{0.95}$) containing the middle 95% of the exposure concentrations were calculated in **Paper III**. $R_{0.95}$ is defined as the ratio of the 97.5[th] to the 2.5[th] percentiles of the exposure concentrations. For a log-normal distribution, the between-person fold-range, including 95% of the individual mean exposure levels, was calculated as $_bR_{0.95} = e^{3.92\sigma_{bY}}$. The within-person fold-range representing 95% of the daily measurements experienced by a given person, was calculated as $_wR_{0.95} = e^{3.92\sigma_{wY}}$ (Rappaport, 1991; Rappaport and Kupper, 2008).

Potential attenuation was assessed in **Paper I** and **Paper III** using the equation $B = (\beta_o/\beta_t) = (1+\lambda/n)^{-1}$, where B represents the ratio of the observed linear regression coefficient (β_o) to the true linear regression coefficient (β_t), bias is defined as (1-B), λ is the ratio of the estimated variance components ($\lambda=\sigma^2_{wY}/\sigma^2_{bY}$), and *n* is the number of repeated samples per individual (Brunekreef, et al., 1987; Heederik, et al., 1991).

In **Paper III,** the estimated variance components from the models with and without significant fixed effects were compared to determine the impact of the fixed effects on the between- and within-person variances. The comparisons were made with the same numbers of measurements, which differed between the investigated compounds depending on whether or not urban background levels were included in the final models (49 observations without and 43 observations with urban background included).

In **all four papers**, statistically significant refers to significance level 5% in two-tailed tests (p<0.05). For values below the LOD, the LOD divided by the square root of 2 was used in the calculations (Hornung and Reed, 1990). In **Paper I**, Proc Nested of SAS was used to estimate the variance components, and in **Paper III and IV** Proc Mixed of SAS using the restricted maximum

likelihood (REML) method. All statistical analyses have been performed with the SAS System for Windows, version 9.1 (**Paper I-III**) and version 9.2 (**Paper IV**).

4 RESULTS

4.1 Paper I

Personal exposure, indoor and outdoor levels of PM and BS are presented in Table 3 below (Table 2, in Paper I).

Table 3. Particle mass concentrations and black smoke (first sampling only)

$PM_{2.5}/BS_{2.5}$	N	Particle mass ($\mu g/m^3$)			Black smoke (10^{-5} m^{-1})		
		Median	Mean	Range	Median	Mean	Range
Personal	29	8.4^1	11.0	3.9–40	0.49	0.65	0.13–2.0
excl. smokers	26	8.3^2	9.5	3.9–21	0.41^3	0.62	0.13–2.0
Residential indoor	30	8.6	9.7	2.2–29	0.45	0.56	0.003–2.3
excl. smokers	27	8.5	9.2	2.2–25	0.40	0.52	0.003–2.3
Residential outdoor	29	6.4	7.8	2.1–28	0.45^4	0.68	0.17–1.9
excl. smokers	26	6.9	8.2	2.2–28	0.45^5	0.71	0.17–1.9
Urban background	28	5.6	8.8	3.0–31	0.46	0.63	0.25–1.6
all measurements	42	6.3	10.1	3.0–43	0.55	0.68	0.23–1.8
PM_1/BS_1							
Personal	10	5.4	6.1	2.5–11	0.56	0.55	0.22–0.8
Residential indoor	30	6.2	7.7	2.6–31	0.46	0.54	0.007–2.1
excl. smokers	27	5.9	6.9	2.6–20	0.44	0.49	0.007–2.1
Residential outdoor	29	5.2	5.9	2.4–17	0.46^6	0.66	0.17–1.9
excl. smokers	26	5.5	6.2	2.4–17	0.46^7	0.68	0.17–1.9

[1]Significantly higher than residential indoor (p=0.046), residential outdoor (p=0.003), and urban background (p=0.03) $PM_{2.5}$
[2]Significantly higher than residential outdoor $PM_{2.5}$ (p=0.02) for non-smokers
[3]Significantly higher than residential indoor $BS_{2.5}$ (p=0.04) for non-smokers
[4]Significantly higher than residential indoor $BS_{2.5}$ (p=0.008)
[5]Significantly higher than residential indoor $BS_{2.5}$ (p=0.0002) for non-smokers
[6]Significantly higher than residential indoor BS_1 (p=0.04)
[7]Significantly higher than residential indoor BS_1 (p=0.003) for non-smokers

Particle mass concentrations

The median personal exposure to $PM_{2.5}$ was 8.4 µg/m^3 (95% confidence interval (CI) 6.5–12.0 µg/m^3) for the 29 study subjects (workplace exposed subject excluded). Excluding the three smokers resulted in a median personal exposure of 8.3 µg/m^3 (range: 3.9-21 µg/m^3), which was significantly higher than the parallel residential outdoor levels (matched pairs test).

Personal exposure to $PM_{2.5}$ was strongly correlated with indoor $PM_{2.5}$ levels (r_s=0.71 p<0.0001) for non-smokers, and the correlation was slightly lower for personal *versus* outdoor $PM_{2.5}$ levels (r_s=0.67 and r_s=0.61, residential outdoor and urban background, respectively). Residential outdoor $PM_{2.5}$ was highly correlated with the simultaneously measured urban background levels (r_s=0.90, p<0.0001).

Median ratio $PM_1/PM_{2.5}$ was between 0.71-0.83 for the parallel personal, indoor and residential outdoor samplings. Median personal exposure to PM_1 (N=10) was 5.4 µg/m^3, and correlated well with indoor levels (r_s=0.76, p=0.01), whereas the correlation with residential outdoor concentrations was non-significant (r_s=0.60, p=0.07). No statistically significant differences were found between levels of PM_1 in the different microenvironments.

Black smoke

The median personal exposure to $BS_{2.5}$ was 0.49 10^{-5} m^{-1} (Table 3). Residential outdoor levels were significantly higher than indoors for both $BS_{2.5}$ and BS_1. Also for $BS_{2.5}$, the correlation between personal exposure and indoor levels was strong (r_s=0.77, p<0.0001). Personal exposure was also correlated with residential and urban background levels (r_s=0.60 and r_s=0.65, respectively). Like $PM_{2.5}$, there was a strong correlation between the residential outdoor measurements at the subjects' homes and the urban background station for $BS_{2.5}$ (r_s = 0.77, p<0.0001). The ratio between BS_1 and $BS_{2.5}$ was very high, 0.98 for parallel personal, indoor and residential outdoor samples.

Correlations between particle mass and black smoke

There were relatively weak, but statistically significant, correlations between particle mass concentrations and black smoke for $PM_{2.5}$ vs. $BS_{2.5}$ indoors and outdoors (r_s=0.38-0.48) and for PM_1 vs. BS_1 (indoors: r_s=0.45) for non-smokers. For residential outdoor samples the correlation was somewhat stronger (r_s=0.63). For personal exposure there were no significant correlations.

Repeated measurements and variability of PM and BS

Correlations between the two repeated measurements for each individual were poor for measurements in all microenvironments. For personal exposure, the within-person variance component dominated in the non-smokers for both $PM_{2.5}$ and $BS_{2.5}$ (84% and 95%, respectively). However, if the three smokers were included, the within-person variance component was reduced to 50% for $PM_{2.5}$ and 80% for $BS_{2.5}$. For indoor and residential outdoor $PM_{2.5}$, the within-home and between-home variance components were of similar size. Analysis of log-transformed data using a mixed-effects model showed that smoking was a significant factor (p=0.003) for predicting personal exposure to $PM_{2.5}$. Determinants of personal exposure were further investigated in Paper III.

Influence of air mass origin

Measured outdoor levels were affected by the origin of the air masses reaching Gothenburg; the highest median levels of both $PM_{2.5}$ and $BS_{2.5}$ were measured on days when air masses originated from Central Europe (Continental trajectory). Higher residential outdoor and urban background levels of $PM_{2.5}$ were seen for Continental compared with Nordic and Marine air mass trajectories, and air masses originating from the UK gave higher residential outdoor $PM_{2.5}$ compared to Nordic air masses. Continental air masses resulted in higher urban background and residential outdoor levels of $BS_{2.5}$ than Marine. For the personal and indoor measurements, no significant differences were seen for PM or BS.

4.2 Paper II

Personal exposure was significantly higher than residential outdoor and urban background concentrations for the elements Cl, K, Ca, Ti, Fe and Cu (both with and without smokers included). Personal exposure was also higher than indoor levels for Cl, Ca, Ti, Fe and Br (smokers included), but after excluding the three smokers the difference was significant only for Ca and Fe. For Pb, a trace element with mainly outdoor sources, residential outdoor concentrations were higher than indoors and personal samples. The residential outdoor concentrations were also higher than urban background levels for most of the elements.

Personal exposure to trace elements in $PM_{2.5}$ samples is presented in Table 4.

Table 4. Concentrations of trace elements in personal $PM_{2.5}$ samples (ng/m³).

	Personal samples (N=29)			
Element	Median	Mean	# > LOD	Range
S	<470[a]	-	12	270-1400
Cl	170	270	21	61-920
K	96	140	29	39-690
Ca	80	110	29	27-670
Ti	9.5	11	25	3.7-27
V	4.0	4.7	15	2.7-9.4
Fe	69	68	29	23-150
Ni	2.6	4.2	20	0.89-46
Cu	6.6	10	28	1.1-81
Zn	16	21	29	6.6-70
Br	1.3	2.0	23	0.91-14
Pb	2.6	2.9	21	0.92-8.3

[a] Median value below LOD

The correlations between personal exposure and the indoor, residential outdoor and urban background levels were relatively strong for Zn, Br and Pb (r_s=0.47-0.81), while for Ca and Cu the correlations were low or non-significant. Residential outdoor levels were well correlated with urban background levels for S, V, Br and Pb (r_s>0.7), whereas the associations were moderate for Cl, Fe, Cu and Zn ($0.5 < r_s > 0.7$). The indoor to outdoor ratio of S and Pb were calculated as an indication of infiltration of PM of outdoor origin, since these elements have very limited indoor sources. The median ratio was about 0.7 for both S and Pb.

PM mass concentration and trace elements
For personal exposure, there were significant correlations between PM mass and concentrations of Ca, Fe and Br ($PM_{2.5}$ and PM_1) and K and Zn ($PM_{2.5}$ only). Moderate to strong correlations were found for K, Zn, Br and Pb (residential outdoor and urban background $PM_{2.5}$), and for Ca and Fe (residential outdoor only). Significant correlations for residential outdoor PM_1 samples and Fe, Zn, Br and Pb were found.

Influence of air mass origin
Higher personal exposure of Pb was found for Central European (continental) compared to Marine and Nordic air mass trajectories. Continental and UK air mass trajectories resulted in higher personal exposure to V compared to Nordic. Also, for personal exposure, UK air masses showed higher levels of S compared to Marine air. At the urban background station, significantly higher levels of S, Br and Pb were observed for air masses of Continental

origin compared to Marine and Nordic. Furthermore, UK air masses were higher in S, V and Ni than Nordic air. For the crustal elements Ca, Ti, Mn, Fe, Cu and Zn, no differences between the different air mass trajectories were found.

4.3 Paper III

Estimated variance components, fold-ranges, mean exposures and variance components ratios as well as the number of repeated measures per individual needed to restrict bias in a hypothetical exposure-response relationship to a fixed (20%) level are presented in Table 5.

Table 5. Parameters estimated under model (1) for $PM_{2.5}$, $BS_{2.5}$, and trace elements based on 49 personal samples from 29 subjects (20 repeats) [$PM_{2.5}$ ($\mu g/m^3$), $BS_{2.5}$ (10^{-5} m^{-1}), trace elements (ng/m^3)]. (Table 3, Paper III)

	N	σ^2_{bY}	σ^2_{wY}	$_bR_{0.95}$	$_wR_{0.95}$	μ_Y	μ_X	λ	n
$PM_{2.5}$	49	0.163	0.162	4.9	4.8	2.3	12	1.0	4
BS	49	0.090	0.377	3.2	11	-0.56	0.72	4.2	17
Cl	49	0.045	0.435	2.3	13	5.2	230	9.8	39
K	49	0.153	0.246	4.6	7.0	4.7	140	1.6	6
Ca	49	0.117	0.201	3.8	5.8	4.4	92	1.7	7
Ti	49	0.071	0.151	2.8	4.6	2.2	10	2.1	8
Fe	49	0.096	0.230	3.4	6.4	4.1	71	2.4	9
Ni	48	0.160	0.868	4.8	39	0.98	4.5	5.4	22
Cu	49	0	0.742	1.0	29	1.7	7.9	-[*]	-[*]
Zn	49	0.202	0.171	5.8	5.0	2.8	20	0.8	3
Pb	49	0	0.665	1.0	24	0.87	3.3	-[*]	-[*]

N = number of personal samples
σ^2_{bY} = between-person variance component (log scale)
σ^2_{wY} = within-person variance component (log scale)
$_bR_{0.95}$ = between-person fold-range (natural scale)
$_wR_{0.95}$ = within-person fold-range (natural scale)
μ_Y = mean (log scale)
μ_X = estimated mean ($\mu_X = \exp(\mu_Y + \sigma^2_Y/2)$) (natural scale)
$\lambda = \sigma^2_{wY}/\sigma^2_{bY}$
n = number of samples needed to reduce bias to 20%
[*] could not be estimated

The estimated within-person variance components dominated the total variability for all the substances except for $PM_{2.5}$ and Zn (where estimates of σ^2_{bY} and σ^2_{wY} were about equal), see Table 5. Expressed as fold ranges, daily exposure levels for a given subject varied from about 5-fold ($PM_{2.5}$, Ti and

Zn) to 39-fold (Ni), while average exposure levels varied from about 1-fold (Cu and Pb) to 6-fold (Zn), (Table 5). Excluding the three smoking subjects, reduced $_bR_{0.95}$ for $PM_{2.5}$ from 4.9 to 1.8; and $_bR_{0.95}$ for K from 4.6 to 1.0, but did not demonstrably affect estimates of $_bR_{0.95}$ for the other substances. Removing smokers did not noticeably affect estimates of $_wR_{0.95}$. The estimated natural-scale mean exposure level of $PM_{2.5}$ (i.e. the mean of the Xij:s) was estimated (using the estimated variances) as $\mu_X = 12$ μg/m^3 (95% CI: 9.6-14 μg/m^3).

The ratio of the within- and between-person variance components (λ) ranged from about 1 (for $PM_{2.5}$ and Zn) to 9.8 (for Cl) (Table 5). Listed in Table 5 is also the number of repeated measurements (n) per subject that would be needed to restrict bias to 20% in the hypothetical exposure-response relationship. For $PM_{2.5}$, BS and the trace elements, between 3 and 39 repeats per subject would be needed. For $PM_{2.5}$, n was 4, however, if the three smokers were excluded from the data set, reduction in the estimate of σ^2_{bY} would lead to a much larger λ (λ=9.5), yielding a corresponding estimate of n of 38 measurements per person.

Exposure determinants
For personal exposure to $PM_{2.5}$, significant determinants were season, smoking and urban background levels. For season, monitoring during fall was found to reduce the personal exposure compared with spring. The urban background levels were also significant determinants for the personal exposure to BS and the trace elements Cl, Zn and Pb, and smoking was a determinant for the personal exposure to BS, K and Ti. Time spent outdoors and in traffic (hours) was only found to significantly affect the personal exposure to Fe, however, these times were quite small for most participants (median 1.3 hours; range 0.25 to 6.0 hours). For personal exposure to the trace elements Ca, Cu and Ni, none of the variables included in the model were significant.

Table 6. Variance components estimated under models (1) and (2) for personal exposures to PM$_{2.5}$, BS and trace elements (N=43 or 49) and the reduction (%) of the between-person or within-person variance component. (Table 5 in Paper III).

	N	Variance	Model (1)	Model (2)	Reduction (%)
PM$_{2.5}$	43	σ^2_{bY}	0.241	0.067	69
	43	σ^2_{wY}	0.133	0.076	43
BS	43	σ^2_{bY}	0.013	0.076	-*
	43	σ^2_{wY}	0.468	0.253	46
Cl	43	σ^2_{bY}	0.029	0.014	52
	43	σ^2_{wY}	0.478	0.245	49
K	49	σ^2_{bY}	0.153	0.014	91
	49	σ^2_{wY}	0.246	0.232	6
Ti	49	σ^2_{bY}	0.071	0.050	30
	49	σ^2_{wY}	0.151	0.151	0
Fe	49	σ^2_{bY}	0.096	0.060	38
	49	σ^2_{wY}	0.226	0.201	11
Zn	43	σ^2_{bY}	0.347	0.278	20
	43	σ^2_{wY}	0.108	0.078	28
Pb	43	σ^2_{bY}	0	0.077	-*
	43	σ^2_{wY}	0.645	0.133	79

* could not be estimated

The variance components from the final model (2) including fixed effects compared with those from model (1) for PM$_{2.5}$, BS and the trace elements are presented in Table 6. For PM$_{2.5}$, inclusion of the fixed effects season, smoking and urban background levels, lowered the between-person variance component by 69%, and the within-person variance component by 43%. A considerable reduction (91%) of the between-person variance component was seen for K, with smoking as a single determinant in the model. For the total variance (σ^2_Y), addition of determinants reduced it by about half for PM$_{2.5}$, Cl and Pb.

For the purpose of investigating the impact of the urban background levels on the variance components for PM$_{2.5}$, model (2) was run without this determinant (with only season and smoking, but the same number of measurements (N=43)). This resulted in a similar reduction of the between-person variance component as previously but no reduction of the within-person variance component (σ^2_{wY}). It therefore appears that urban background levels of PM$_{2.5}$ mainly affected the within-person variance component.

4.4 Paper IV

Twelve sampling sessions were performed (six sessions after high air pollution and six after low pollution). In 7 of the 12 sessions, there was a subsequent follow-up sampling the next morning. The 16 study subjects each participated in between 5 and 11 of the 12 sampling sessions (median: 8 sessions). All study subjects participated in both high- and low-level sampling sessions. For air pollution levels preceding the 12 sampling sessions see Table 1, **Paper IV**. The ratio between high- and low-level days was about five for PM_{10} and two for NO_2 and NO_x (Table 1, **Paper IV**).

No significant increase in blood levels of any biomarkers was found the mornings after days with high levels of PM_{10} compared to days with low levels. On the contrary, a significant negative association was seen for CRP, SAA and SP-D (see Table 3, **Paper IV**). Negative associations were found between temperature and levels of sICAM-1 and sVCAM-1 (lower temperature was associated with increased levels of these biomarkers), whereas a positive association was found for SP-D. No significant fixed effects were found for fibrinogen, PAI-1 and p-selectin. When the exposure instead was classified by the urban background NO_x concentrations, p-selectin was found to decrease slightly after high-pollution days, whereas the negative associations previously found for CRP, SAA and SP-D were no longer significant. The results were essentially unchanged for all biomarkers when the analysis was repeated without temperature in the model.

For the follow-up samplings, a significant increase in sVCAM-1 levels (p=0.005) after high-pollution days compared with low pollution days (high PM_{10} versus low PM_{10}) was found. A significant negative association was again found for CRP, and also for p-selectin and fibrinogen. However, the number of follow-up samples was fewer (69 samples from 7 sampling days).

The between-person variance component dominated the total variability for most of the biomarkers (Table 2, **Paper IV**). Significant correlations were found between CRP and fibrinogen, CRP and SAA, fibrinogen and SAA, p-selectin and sICAM-1 and between CC16 and SP-D.

5 DISCUSSION

This study was the first to characterize environmental personal exposure to fine particles ($PM_{2.5}$) in a Swedish city. Furthermore, it was the first study to simultaneously measure personal exposure to PM_1.

5.1 Personal exposure concentrations

The mean personal exposure to $PM_{2.5}$ was about 10 µg/m^3 (range 4-21 µg/m^3) for the non-smoking subgroup (**Paper I**). This average exposure was below the 24-hour mean air quality guideline of 25 µg/m^3, but equivalent to the annual mean guideline (WHO, 2006). Personal exposure to $PM_{2.5}$ was found to exceed indoor and outdoor concentrations (residential outdoor as well as urban background) for the entire study group of 29 subjects (matched paired samples). With smokers excluded, only the difference between personal and the residential outdoor concentrations was significant (Chapter 4.1, Table 3).

Personal exposure to $PM_{2.5}$ found in this study was comparable to the levels found in the EXPOLIS-study in Helsinki (Koistinen, et al., 2001), and also to levels measured in Seattle ((Liu, et al., 2003)) and in Boston (Brown, et al., 2008). Levels were comparable to (Helsinki) or slightly lower than (Amsterdam) levels found within the ULTRA-study (Janssen, et al., 2005; Lanki, et al., 2007), and also slightly lower than those measured among students in Copenhagen (Sorensen, et al., 2005). Lower personal exposure was found in our study group compared with levels found in Basel within the EXPOLIS-study (Oglesby, et al., 2000), Oxford (Lai, et al., 2004), New York City (Kinney, et al., 2002), Minneapolis (Adgate, et al., 2002), Vancouver (Ebelt, et al., 2000), Toronto (Pellizzari, et al., 1999), Baltimore (Sarnat, et al., 2000), Ohio (Sarnat, et al., 2006), Boston (Rojas-Bracho, et al., 2000) and within the RIOPA-study (Houston, Los Angeles County and Elisabeth) (Meng, et al., 2005). Much higher personal exposure levels were measured for adult, non-smoking, office workers in Beijing, China, with average personal $PM_{2.5}$ exposure of about 120 µg/m^3 measured on workdays (Du, et al., 2010). Even higher exposures can be found in developing countries where

particles generated from wood burning for household heating and cooking give rise to very high indoor concentrations (Naeher, et al., 2007).

Personal exposure to BS was lower than in Copenhagen (Sorensen, et al., 2005), and in Helsinki and Amsterdam (Janssen, et al., 2005; Lanki, et al., 2007). Indoor and outdoor levels measured in Gothenburg were well below corresponding levels in cities such as Athens and Prague (Gotschi, et al., 2002). For elemental constituents in $PM_{2.5}$, the personal exposure presented in **Paper II** was generally similar to levels measured in Helsinki (Janssen, et al., 2005) except for Cl, which was higher in Gothenburg. Personal exposure concentrations among non-smokers in Minneapolis (Adgate, et al., 2007) were also comparable to our results, apart from Ti and K (higher in Gothenburg) and Ca (higher in Minneapolis). Much higher personal exposure to Pb and S was measured in Basel (Oglesby, et al., 2000) and in Oxford (Lai, et al., 2004), and outdoor levels of trace elements in these cities far exceeded the levels measured in Gothenburg. One reason for the lower levels of $PM_{2.5}$ and BS in Gothenburg could be that the area is less densely populated than Central Europe, UK and the US, which have substantially larger cities with higher traffic densities. Also, coal burning, which generates substantial emissions of BS, S as well as PM, is more common in Eastern Europe and in the UK than in Sweden, and even more common in China.

Comparison of results from different studies should always be made with caution. The study populations in the studies mentioned above were quite different. The European ULTRA-study (Janssen, et al., 2005; Lanki, et al., 2007), involved elderly subjects with coronary artery disease, and also some of the measurement campaigns performed in US or Canadian cities were designed to characterize exposure among senior adults (Brown, et al., 2008; Sarnat, et al., 2000) or susceptible subjects with chronic diseases like COPD or CHD (Ebelt, et al., 2000; Liu, et al., 2003; Rojas-Bracho, et al., 2000). The EXPOLIS-study focused, like us, on an adult urban population of working-age (Koistinen, et al., 2001; Oglesby, et al., 2000) as did Pellizzari, et al., (1999), Lai, et al., (2004)and Adgate, et al., (2002), whereas Sorensen, et al., (2005) and Kinney, et al., (2002) recruited their study subjects among students. Time-activity patterns (e.g. time spent outdoors, at home, commuting in cars or buses, etc.) are likely to differ between these different subpopulations and will influence the personal exposure to air pollutants (for example, the elderly subjects within the ULTRA study spent nearly 90% of the sampling time indoors at home (Lanki, et al., 2007)). Other factors that will affect exposure levels are smoking habits and exposure to ETS, personal activities, season and weather conditions, ventilation and air conditioning,

etc. All in all, there are many factors which should be considered when comparing personal exposure levels obtained in different studies.

5.2 Personal exposure in relation to ambient concentrations

Performing personal exposure measurements is far more labor intensive and time consuming than performing continuous monitoring at a fixed site. One may ask the question - is it really necessary to carry out personal sampling?

Associations between personal exposure and ambient levels can be assessed using cross-sectional correlations (within a study population) as well as longitudinal correlations (within subjects). In the present study, the cross-sectional correlation between personal $PM_{2.5}$ and urban background (ambient) $PM_{2.5}$ levels was found to be moderate (r_s=0.61) (calculated for non-smokers, first sampling only, **Paper I**). The correlation was weakened when the smokers were included (r_s=0.55). From other studies assessing cross-sectional associations between personal and ambient $PM_{2.5}$, somewhat varying results have been reported. No significant correlation was found within the EXPOLIS-project in Basel (with or without smokers included) (Oglesby, et al., 2000). In Helsinki, the correlation between personal and ambient $PM_{2.5}$ was poor for workdays (r=0.34), but stronger for leisure time (r=0.69) for non-smokers, and correlations dropped when smokers and ETS-exposed subjects were included (Kousa, et al., 2002). In Beijing, China, a strong correlation was found, but personal exposure was significantly lower than the ambient levels (Du, et al., 2010).

Personal exposure to $PM_{2.5}$ among the subjects in Gothenburg was found to be significantly higher than residential outdoor but not urban background levels, for the non-smokers. With smokers included, personal exposure exceeded also the indoor and urban background concentrations (**Paper I**). Personal exposure exceeding outdoor concentrations is consistent with several other studies (e.g. (Koistinen, et al., 2001; Lai, et al., 2004; Meng, et al., 2005; Nerriere, et al., 2005; Pellizzari, et al., 1999; Sorensen, et al., 2005), and has been attributed to personal activities, smoking, ETS-exposure and other indoor generated particles (from cooking, cleaning, laundry etc.).

When personal versus ambient PM correlations were assessed longitudinally (within subjects), strong individual median correlations were found in some studies (Janssen, et al., 1999; Janssen, et al., 2005; Janssen, et al., 2000), moderate correlations were observed in other (Ebelt, et al., 2000; Janssen, et al., 1998), whereas minimal longitudinal correlations were reported by (Adgate, et al., 2003). Removing ETS-exposed subjects improved the longitudinal correlation coefficients in (Janssen, et al., 1998; Janssen, et al., 1999; Janssen, et al., 2000), but not in (Adgate, et al., 2003). Seasonal variations have been reported, with stronger within-subject correlations in summer than in winter (Brown, et al., 2008; Sarnat, et al., 2000). In studies reporting both measures, longitudinal correlation coefficients were stronger than cross-sectional (Ebelt, et al., 2000; Janssen, et al., 1999; Sarnat, et al., 2000). A meta-analysis of studies investigating longitudinal associations between personal and ambient $PM_{2.5}$ found a wide range in individual correlation coefficients (Avery, et al., 2010). Similar results were found for a meta-analysis of personal versus home outdoor concentrations (Avery, et al., 2010). Previous studies have shown that associations between personal exposure and ambient levels were affected by personal activities, exposure to indoor generated particles, season, air exchange rate, open window etc., (Brown, et al., 2009; Nerriere, et al., 2005; Rojas-Bracho, et al., 2004; Sarnat, et al., 2000).

In the present study, the cross-sectional correlation between personal $BS_{2.5}$ and urban background $BS_{2.5}$ was similar to that found for $PM_{2.5}$ mass (**Paper I**). BS is correlated with elemental carbon or soot and, accordingly, serves as a marker for combustion-related air pollutants, mainly with outdoor origin (Gotschi, et al., 2002; Kinney, et al., 2000). For trace elements with mainly outdoor sources such as S, V and Pb, slightly stronger associations between personal and urban background levels were found than for $PM_{2.5}$ (**Paper II**), however, results for S and V should be interpreted with caution since several of the personal samples were below LOD. In Basel, personal exposure to S was strongly correlated with outdoor S concentrations (despite the lack of correlation for $PM_{2.5}$), and a moderate correlation was found for Pb, whereas no correlation was found for Ca (Oglesby, et al., 2000). Also for longitudinal associations, stronger correlations for S (or SO_4^{2-}) than for $PM_{2.5}$ have been reported (Ebelt, et al., 2000; Janssen, et al., 2005; Sarnat, et al., 2000), and the same was found for BS (Janssen, et al., 2005).

In general, the higher the spatial variability of a PM constituent or source-specific pollutant, the less likely it is that a fixed-site monitor will reflect the personal exposure of the population (Schlesinger, et al., 2006). It has been demonstrated that individuals may have markedly different exposure to trace

elements when their exposures are dominated by different microenvironments (Edwards and Jantunen, 2009). The fixed-site outdoor monitoring stations tended to underestimate personal exposure to several trace elements in $PM_{2.5}$, also after adjusting for season and community (Adgate, et al., 2007). Heterogeneity between cities in the relationship between personal and ambient PM concentrations was observed in France (Nerriere, et al., 2005). The heterogeneity was attributable to the influence of activity patterns and indoor sources on personal exposure as well as the location of the fixed-site monitor and calls for some caution and maybe also site-specific analyses. It should also be emphasized that an association between personal and ambient levels presented as a correlation coefficient measures the relationship between the two variables. However, there might still be a difference in exposure levels.

5.3   Air mass origin

The impact of air mass origin on the measured air pollutant levels in **Paper I** and **II** was investigated by computing air mass trajectories. The air mass in Gothenburg during the time of monitoring was traced back to see wherefrom it originated. It was shown that the origin of air masses had a large impact, mainly on the outdoor levels. Air masses originating from Central Europe (Continental trajectories) increased the urban background levels of $PM_{2.5}$, BS and some trace elements derived from industry and combustion processes (S, Br and Pb). Seasonal differences in the results when analyzing air mass origin may be expected, since an increased demand for heating during the cold season will result in increased emissions of, for example, BS and S from coal burning, which is common in other parts of Europe. The large impact of long-distance transport of PM on levels in Sweden has been described by Forsberg, et al., (2005) and colleagues. Also, a large impact of air mass origin on measured outdoor levels of S during winter was found in the small town Hagfors in Sweden, with higher levels for air masses originating from Central and Eastern Europe (Continental trajectories) than air masses from the North Atlantic (Marine trajectory) (Molnar, et al., 2005).

Air mass origin did not impact the personal exposure or the indoor concentrations of $PM_{2.5}$ and BS in **Paper I**, indicating that personal exposure and indoor concentrations are strongly influenced by other factors, such as indoor sources and personal activities. Also, the study subjects spent over

90% of the sampling time indoors. Air mass origin influenced personal exposure to the trace elements S, V, Ni, Pb and Cl (**Paper II**), which have mostly or mainly outdoor sources. With its limited indoor sources, S has been shown to be a suitable tracer of outdoor $PM_{2.5}$, especially for the smaller size fractions (<0.5 µm) (Sarnat, et al., 2002). The results showed that analysis of air mass origin could provide valuable information about the PM levels and its content of BS and trace elements, and pointed to the contribution of long-range transported air pollution in Gothenburg. Air mass origin is therefore important to taken into account in the description and interpretation of time-series studies of air pollution and health. The use of back trajectory models to facilitate the linkage of acute health outcomes with specific pollution sources has been recommended (WHO, 2007).

In the present study five out of the 42 urban background measurements of $PM_{2.5}$ were above 25 µg/m^3, the guideline from WHO (99th percentile, 3 days/year). It is therefore likely that this guideline would have been exceeded if we had performed continuous measurements over a year. During four of these days the air masses came from Central Europe, and on the fifth day the air had passed the UK on its way to Gothenburg. A publication merging data from 60 European sites, found the highest $PM_{2.5}$ concentrations at near-city and urban background sites in southern Europe (Putaud, et al., 2010). Lower levels in Europe overall would presumably lead to lower levels also in Gothenburg.

5.4 Exposure variability

The within-person variance component was found to dominate the total exposure variability for most of the measured particulate air pollutants (Chapter 4.3, Table 5). Variance components have been reported in a limited number of studies investigating environmental personal exposure to various air pollutants. However, our finding of a higher within- than between-person variance is in agreement with the results reported for personal exposure to $PM_{2.5}$ and BS within the ULTRA-study (Lanki, et al., 2007) and in Copenhagen (Sorensen, et al., 2005). It is also in agreement with results for personal exposure to NO_2 and SO_2 (Lee, et al., 2004), nine different volatile organic compounds (VOCs) (Rappaport and Kupper, 2004), lead, phenanthrene and chlorpyrifos (Egeghy, et al., 2005), and for sulfate (SO_4^{2-}) (Sarnat, et al., 2009). In a paper by Lin and colleagues (Lin, et al., 2005),

variance components were estimated for 33 datasets of environmental exposures (air samples) to various air contaminants (mainly metals, VOCs and pesticides), and the median within-person variance was found to outweigh the median between-variance component. Also for particulate exposures in occupational settings, larger within- than between-person variances have been reported (e.g. (Hagstrom, et al., 2008; Kromhout, et al., 1993; Symanski, et al., 2006)). Furthermore, when distributions of the corresponding fold-ranges for the within- and between-person variances ($_wR_{0.95}$ and $_bR_{0.95}$, respectively) were compared, a substantially larger within-person fold-range was found for environmental exposures than for occupational settings (Lin, et al., 2005; Rappaport and Kupper, 2004; Rappaport and Kupper, 2008).

Knowledge about exposure variability is important both for designing and interpreting studies (Nieuwenhuijsen, 2003). The sources of random variation complicate the quantitative characterization of exposure levels, and can lead to attenuated estimates of exposure-response relationships. In an individual-based study, bias increases with the variance components ratio (λ). With knowledge of the variance components, the number of samples per subject (n) that would be needed to restrict bias to a fixed level can be estimated. Using the estimated variance components ratios in our study, values of n needed to limit bias to 20% varied between 3 and 39 (Chapter 4.3, Table 5). For PM$_{2.5}$, four samples per subject would be needed. However, this number was based on the range of exposure levels measured within the study group with smokers included. Excluding the three smokers lowered the range of exposure concentrations and caused a reduction in the estimated σ^2_{bY} which led to a much larger λ (λ=9.5), for which the corresponding estimate of n would be 38 samples per person. Consequently, if the exposure contrast is small, increase in the number of samples per subject and/or the number of subjects will be needed in order to prevent attenuation of the exposure-response relationship. Hence, it is important to seek as wide a range of exposure levels as possible in a study population (increase σ^2_{bY}) in order to decrease the variance components ratio and thereby reduce the biasing effect of exposure measurement errors in epidemiological studies. Environmental studies with many repeated samples per subject are, however, difficult to perform, since they would be very costly and labor intensive. Another way to reduce attenuation bias is to perform group-based study designs, which has been suggested to be useful for environmental studies due to their typically large within-person variances (Rappaport and Kupper, 2008).

It may be noted that the number of samples per subject required to limit bias to 20% was estimated to be 19 in **Paper I** and 38 in **Paper III**, for the 26

non-smokers. A possible explanation for the differing results is that the different procedures in SAS can give different results when a dataset is more unbalanced (Proc Nested was used in **Paper I** and Proc Mixed in **Paper III**). In this case a lower between-person variance was estimated with Proc Mixed (0.021) than with Proc Nested (0.035), thus yielding a higher lambda-estimate.

5.4.1   Determinants of exposure

The time-activity diaries distributed to the study participants included questions regarding personal activities that may have an impact on exposure to PM, and time spent in different microenvironments. Variables derived from these items were introduced in the mixed-effects models to identify possible determinants of exposure. Since monitoring was carried out during either spring or fall, season was tested in the model as a possible determinant. Gender was also added as a covariate, even though we did not expect exposure to differ solely depending on gender. The intention was not to develop a model that could estimate the total personal exposure. Instead, the aim was to identify factors that influence personal exposure based on information that could fairly easily be gathered through questionnaires and time-activity diaries.

Season was found to be a determinant for personal exposure to $PM_{2.5}$ and the elements Fe and Pb (monitoring during fall lowered the personal exposure compared with spring). Seasonal effects on personal exposure to $PM_{2.5}$ have also been estimated using mixed-effects models (Rojas-Bracho, et al., 2004). The number of smoked cigarettes predicted personal exposure to $PM_{2.5}$, BS, K and Ti (**Paper III**). It is well-known that smoking substantially increases exposure to fine particles (e.g. Koistinen, et al., 2001; Pellizzari, et al., 1999), and the elements K and Ti have both been detected in cigarette smoke (Chang, et al., 2003; Mishra, et al., 1986).

The urban background concentration was a significant determinant for personal exposure to $PM_{2.5}$, BS and the trace elements Cl, Zn and Pb. The influence of outdoor levels of $PM_{2.5}$ on personal exposure has been shown by several other studies (e.g. Brown, et al., 2009; Ebelt, et al., 2000; Janssen, et al., 1998; Janssen, et al., 2005; Koistinen, et al., 2001; Lanki, et al., 2007; Liu, et al., 2003; Rojas-Bracho, et al., 2004; Sorensen, et al., 2005). Urban background BS was a significant determinant for personal exposure, also

found by (Lanki, et al., 2007; Sorensen, et al., 2005), in accordance with the hypothesis that indoor BS originates from outdoor sources (Gotschi, et al., 2002). Pb is an element with mainly outdoor sources, such as industrial combustion processes (e.g. refuse incineration) (Molnar, et al., 2006; Vallius, et al., 2003), and since Gothenburg is a coastal city, exposure to Cl is likely to originate from airborne sea salt. Moreover, for Zn tire wear is an outdoor source (Molnar, et al., 2006; Swietlicki, et al., 1996).

By estimating the between-person and within-person variance components while taking into account the significant fixed effects in the final models, a reduction in either the between- or within-person variance component (or both) may be obtained (chapter 4.3, Table 4). For K, the fixed effect of smoking reduced the between-person variance by as much as 91%, i.e. the difference in mean exposure levels of K between subjects was almost entirely due to smoking. For $PM_{2.5}$, including the fixed effects season, smoking and urban background caused a reduction of the between-person variance component by 69%, for which smoking habits and seasonal effects accounted for the major part. Consequently, these two variables could in part explain the difference in mean exposure to $PM_{2.5}$ between the study subjects in the group.

5.5 Effects on blood biomarkers

The study in **Paper IV** was designed to reflect the real-life situation for a group of healthy adults exposed to varying levels of air pollution within Gothenburg. Blood sampling was performed after days with either high or low ambient levels of air pollutants (PM_{10} and NO_2), with the purpose of obtaining the largest, but feasible, contrast in exposure. The results did not, however, support the hypothesis of increased levels after days with elevated air pollution for any of the biomarkers. The significant increase in sVCAM-1 levels found only for the follow-up samplings was likely due to chance since it was not found in the first samplings, nor was it found for any of the other biomarkers. The negative associations found for CRP, SAA and SP-D did not remain when the exposure was classified by the NOx concentrations, indicating that these negative associations were not real effects resulting from air pollution exposure.

Associations between exposure to urban air pollution and biomarkers in blood have been investigated in several panel studies, but results have been

somewhat inconsistent when it comes to results for the specific biomarkers. The acute-phase proteins CRP and fibrinogen, as markers of a systemic inflammatory response, and fibrinogen as a determinant for blood viscosity, are the biomarkers that have been most commonly used as outcomes in previous panel studies. In studies investigating healthy subjects, no associations between the main ambient air pollutants and the biomarkers CRP and fibrinogen were reported from a study conducted in Rotterdam (Rudez, et al., 2009) or between $PM_{2.5}$ and CRP, SAA and fibrinogen in the Utah Valley, US (O'Toole, et al., 2010). Furthermore, no consistent changes in CRP or fibrinogen were associated with changes in air pollutant levels in Beijing, China, (Rich, et al., 2012). Consequently, our findings for CRP, fibrinogen and SAA are in agreement with these previous panel studies. In contrast to our findings are results from Taiwan, where positive associations were found between increases in ambient PM_{10} and CRP, fibrinogen and PAI-1 in healthy college students (Chuang, et al., 2007). Several panel studies have instead focused on subpopulations with a potentially increased susceptibility to PM-related health effects, often people with pre-existing cardiovascular or pulmonary diseases (Delfino, et al., 2008; Hildebrandt, et al., 2009; Huttunen, et al., 2012; Ruckerl, et al., 2006; Ruckerl, et al., 2007; Sullivan, et al., 2007). In summary, it seems like the majority of these studies have shown positive associations for at least one investigated biomarker.

This study examined effects in a group consisting of healthy study subjects of working-age. However, people with preexisting cardiovascular or respiratory diseases may be more susceptible to PM-related health effects (Sacks, et al., 2011). On the other hand, chronic diseases often involve medication, which could blur a possible association between exposure and outcome, and these medicines have to be taken into consideration in the statistical analysis.

Several of the biomarkers used in the present study show a considerable between-person variance. This study was the first panel study in Sweden investigating intra-individual associations between air pollution and blood biomarkers in healthy subjects. A previous study from Stockholm involved healthy, middle-aged subjects, but relied on only one blood sample per subject (Panasevich, et al., 2009). By comparing the levels of the biomarker within each subject (i.e. each subject served as his or her own control), individual factors that might influence each subject's base levels are controlled for. To account for diurnal variations, blood samples were taken at the same time of the day (in the morning). On some occasions, our study subjects reported intake of inflammatory medicine (for example due to headache, menstrual cramps, etc.) during the week prior to blood sampling, and medicine was therefore included as a variable in the statistical analysis.

In addition, the dataset was analyzed without these samples to see if results were changed (which they weren't). However, our attempts to control for possible confounding factors could not rule out the possibility that physiological within-subject variability of the blood biomarkers, and also effects due to personal behavior have acted on our results. These possible confounders are hard to control for in studies involving humans. It could also be that increased levels of these biomarkers cannot be seen in healthy adults living in a city like Gothenburg with moderate levels of urban air pollution.

Limitations of the study include the long time frame, and that levels from a stationary urban background monitoring station were used to assess the participant's exposure. Personal exposure measurements would have given a more precise measure of each subjects' exposure, however, it would not have been feasible since it would have required continuous personal monitoring of the study subjects over a very long time period.

5.6 Validity

5.6.1 Validity aspects in Paper I-III

Study subjects

The study group in **Paper I-III** consisted of 20 randomly selected subjects and 10 volunteers from the Department of Occupational and Environmental Medicine. The reason for recruiting the staff members was the duplicate personal samplings, involving the carrying of two pumps, which was thought to be more conveniently done on volunteers from the department. The question must, however, be raised if these staff volunteers differed from the randomly selected subjects and, if incorporating data from the staff volunteers did change the overall results?

Answers from the daily diaries were compared between the 20 randomly selected subjects and the 10 volunteers. The percentages of time spent in different environments were similar, except for time spent at work which was higher for the staff volunteers than for the randomly selected subjects (median: 34% and 25%, respectively), but the difference was not statistically significant (Wilcoxon rank sum test). The volunteers were in the same range

of age (24-50 years) as the randomly selected subjects (23-51 years). Moreover, statistical analyses were performed for only the randomly selected subjects (**Paper I**), and the results for $PM_{2.5}$ and PM_1 were in general similar to the results for the total group. For $BS_{2.5}$ and BS_1, however, a few of the observed differences or associations were weaker or did not reach statistical significance.

Exclusion of subjects

Study participants had to have the possibility to perform the residential outdoor measurements. Due to this prerequisite, four subjects who had agreed to participate had to be excluded from the study group. Nevertheless, efforts were made to accomplish monitoring, e.g. for one participant a neighbor's balcony was used for setting up the pumps, and for another the cyclones could be attached outside a window in the stairwell.

The intention was to investigate environmental exposure to PM, therefore the study subject who reported to have been heavily exposed to dust and paint during the workday was excluded in all statistical analyses. The collected filter was however weighed, and the personal exposure to $PM_{2.5}$ for this subject was 79 $\mu g/m^3$. This mass concentration was about twice the exposure to $PM_{2.5}$ measured for the smoker who smoked the most (13-15 cigarettes per day), and it therefore seems reasonable that this subject was excluded from the data set.

Smoking habits

According to the Swedish National Environmental Health Survey, 2007; 14% of the Swedish population smoke daily (Swedish National Board of Health and Welfare, 2009). Applying this percentage on our study group would imply four smokers among 30 subjects. Consequently, it seems that the number of smokers (3) in our study group was fairly representative for the general Swedish population. None of the 10 staff volunteers were smokers, however, the question whether subjects were smokers or not was not asked during the recruitment. Most of the statistical analyses in **Paper I** and **II** were performed with and without smokers included, see chapter 4.1 and 4.2.

Gender aspects

The distribution according to gender was uneven for the study group, with more female than male participants (22 and 8, respectively). Due to the small size of the group, no statistical comparison regarding personal exposure for men and women separately was performed in **Paper I** or **II**. However, in **Paper III** gender was introduced as a covariate in the mixed-effects models for each compound, but was not found to be a significant determinate for any of the substances. In the European EXPOLIS-study, it was in general easier to get women and higher educated individuals to participate in the survey (Rotko, et al., 2000).

Socioeconomic status and ethnic structure within the study group

Two questions related to socioeconomic status were asked in the questionnaires; education and current employment/studies. People of higher education have been shown more likely to participate in surveys (Rotko, et al., 2000). The possible effect of socioeconomic status was however not evaluated due to the small study group. Despite the lack of information about the distribution with regard to ethnicity within the adult population (age 20-50 years) of Gothenburg in the years 2002-2003, it seems likely that the number of participants with immigrant background was too few to reflect the ethnic constitution of the target population. Difficulties in speaking and understanding Swedish are likely reasons for a lower participation rate among people with other native languages than Swedish.

The size of the study and power

A study group of 30 subjects may seem rather small in size. However, due to limited budget and labor capacity, a choice between monitoring a larger number of subjects and performing repeated samples had to be made, and we chose the latter.

The mean exposure level, μ_X ($\mu_X = \exp(\mu_Y + \sigma^2_Y/2)$, estimated under model (1), was 12 $\mu g/m^3$ (95% CI 9.6-14 $\mu g/m^3$), based on the 49 samples from 29 subjects. That is, the mean exposure lies (with 95% confidence) between 9.6 and 14 $\mu g/m^3$. The relatively narrow confidence interval suggests that the mean personal exposure could be estimated with a fairly good accuracy.

In **Paper I**, differences between personal exposure and indoor, residential outdoor and urban background levels were assessed. Personal exposure to $PM_{2.5}$ was found to be significantly higher than the indoor, residential outdoor and urban background levels for the study group of 29 subjects

(smokers included). However, after removing the three smokers, only the difference between personal exposure and the residential outdoor concentrations was significant.

In order to give 80% power to detect a significant difference between personal exposure and the corresponding indoor, residential outdoor and urban background levels, mean differences of at least 2.1, 4.2 and 4.7 µg/m^3, respectively, would have to be obtained within our study group of 29 subjects. For the non-smoking sub-group, the corresponding mean differences would have had to be 1.7, 2.3 and 3.3 µg/m^3, respectively. These mean differences do not seem unrealistic to obtain from a sample of the general population.

Generalizability

A study group has to be randomly selected from the target population, if the intention is to apply information from the sample on the target population. This is referred to as probability sampling, which means that members of a population are selected at random and each person has an equal chance of being selected for the sample (WHO, 1992). Furthermore, the study group should be representative for the entire target population and a satisfactory participation rate is required.

One of the aims for the study presented in **Papers I-III** was to characterize the environmental personal exposure to fine particles, and compare it with the simultaneous measures of indoor and outdoor concentrations. Our target population was adults of working age living within the city of Gothenburg. The study subjects were randomly selected from the Swedish Population Register (Statens Personadressregister, SPAR). The random selection was restricted with regard to age (subjects should be between 20 and 50 years of age). The participation rate was high (80%), possibly because subjects were contacted over telephone and asked to participate. In addition, an economic compensation was paid to each participant after completed sampling. Results from a randomly selected study group may, however, never be applied on individuals not part of the target population (WHO, 1992). Therefore, results from this study are not to be applied on other cities, nor can it be applied on the entire population of Gothenburg, which also includes children and elderly.

Among studies reporting personal PM$_{2.5}$ exposure, there are few that have used a randomly selected study group. The PTEAM-study from California was the first large-scale study using a stratified probability-based sampling design for measurements of personal exposure to particles (PM$_{10}$) (Ozkaynak,

et al., 1996) It was followed by the Toronto study using a similar approach for measuring $PM_{2.5}$ (Pellizzari, et al., 1999). Furthermore, the multi-city study EXPOLIS was the first large-scale European study using a random population sample (adults 25-55 years of age), and a similar design was applied in Oxford (Lai, et al., 2004).

Achieving a high participation rate is crucial in order to reduce effect of bias in population based studies. Including demanding personal exposure measurements and/or extensive questionnaires in epidemiological studies may imply difficulties when recruiting study participants. This was acknowledged in a publication from the multi-city study EXPOLIS, with highly variable participations rates among the cities (Oglesby, et al., 2000). However, no matter how careful a study sample is drawn, that is, despite a randomly selected study group and a high participation rate, the results may be biased (WHO, 1992). Ideally, a follow-up of subjects who did not want to or did not have the possibility to participate in the study should be carried out to obtain information about these subjects. This has, however, not been done in the study presented in **Paper I-III**.

5.6.2 Validity aspects in Paper IV

Study subjects

The study group in **Paper IV** consisted of 16 volunteers, eight men and eight women. The recruitment process aimed at an even distribution with regard to gender within the study group. Smoking was not an issue, since all subjects were non-smokers, a prerequisite for participation in the study. Only health volunteers were allowed, thus none of the participants had any severe chronic disease. Ethnicity is not likely to have influenced the results of the study (with each subject serving as his or her own control).

The size of the study and power

The hypothesis was that exposure to elevated ambient levels of particulate air pollution would be associated with intra-individual increases in blood levels of the biomarkers. The results did, however, not support the hypothesis for any of the biomarkers. The power to detect an increase in levels of a biomarker was estimated for CRP and CC16, respectively. It was estimated that levels of CRP would have to increase by 23% (as group mean) in order to give 80% power to detect a significant effect. For CC16, blood levels would have to increase by 8% (as group mean). As a comparison, a

significant increase in serum CC16 by 17% was observed in a group of 13 healthy volunteers in an experimental study (exposure to wood smoke compared with clean air) (Barregard, et al., 2008). A subsequent study of wood smoke exposure showed a significant increase of 19% in CC16 levels, also in healthy study subjects (Stockfelt, et al., 2012).

Multiple significance testing increases the possibility of obtaining a significant finding just by chance. Each test has a 5% chance of a false positive result when there is no real difference (Type I error) (Altman, 1991). This is often referred to as "mass significance". In **Paper IV**, the mixed-effects model was applied on ten different biomarkers, thereby increasing the chance of finding a positive effect. However, no significant increase in blood levels of any of the biomarkers were seen after days with high levels compared with low levels. In this case, analyzing quit a large number of biomarkers may instead strengthen our findings of no association between exposure and outcome in our study group.

Generalizability

The study group in **Paper IV** consisted of healthy volunteers in working age, i.e. it was not a random sample of participants. The results from **Paper IV** cannot be applied on the general adult population of Gothenburg, which also includes elderly and people with cardiovascular- or respiratory diseases, all subpopulations with a possibly increased susceptibility to PM-related health effects.

5.7 Aspects on the measures of exposure

The primary measure of personal exposure in the study described in **Paper I-III** was $PM_{2.5}$. Fine PM has been linked to a wide range of health effects, and the current health based air quality guidelines are set for particle mass concentration (WHO, 2006), thus enabling the comparison between the measured exposure levels and the guideline values. Evidence is emerging for some components of PM being more toxic than others, and the collection of PM on filters enabled analysis of PM constituents.

Black smoke, measured with a reflectometer, is a metric that is based on the blackness of the PM collected on the filter. For BS, the amount of reflected light was transformed into absorption coefficients, according to the ISO Standard 9835. The amount of reflected light can be converted into mass units, however, the use of a constant conversion factor has been shown to be a major source of bias, and calls for a local calibration of the conversion factor on the basis of the OC/EC ratio in PM (WHO, 2012). Therefore, BS was expressed in absorption coefficients (a), and not converted to mass units in **Paper I** and **III**.

The PM samples were also analyzed for their content of particulate PAHs (18 different compounds). Benzo(a)pyrene (B(a)P) is the most widely investigated PAH-compound and is classified as a human carcinogen by the IARC (IARC, group 1). WHO has estimated a unit risk for inhalation of B(a)P, based on the risk for lung cancer, in which B(a)P represents the total carcinogenicity of the PAH mixture. The Swedish health-based guideline value for B(a)P is based on this unit risk estimate. Unfortunately, B(a)P was below the LOD in more than 50% percent of the personal samples, and the samples were not considered to provide usable data.

In **Paper IV**, exposure was assessed using ambient levels of PM_{10}. The preferred PM metric would have been $PM_{2.5}$, the same as in Paper I-III. However, on-line urban background concentrations of $PM_{2.5}$ were not available at the time of the study. Several time-series studies have found associations between ambient PM_{10} and biomarkers. The smaller size fraction $PM_{2.5}$ is incorporated in PM_{10}, and urban background levels of PM_{10} and $PM_{2.5}$ are usually highly correlated.

5.8 Risk assessment of exposure to environmental PM$_{2.5}$ in Gothenburg

There is no evidence of a safe level of exposure, or a threshold below which no adverse health effects of PM occur (WHO, 2011). Over 80% of the population in the European Region of WHO lives in cities with levels of particulate matter exceeding WHO Air Quality Guidelines. This pollution creates a substantial burden of disease, causing premature deaths and reducing life expectancy in all Europe. Since there is no safe level of PM exposure, the burden of air pollution to health is significant even at relatively low concentrations.

Applying the risk estimates from the American Cancer Society study (Pope, et al., 1995) for long-term cardiopulmonary mortality, it can be estimated that lowering the annual mean exposure to PM$_{2.5}$ by 2 μg/m^3 would imply that about 50 premature deaths would be saved annually within the municipality of Gothenburg.

A recent study from the US have shown associations between further reductions in PM$_{2.5}$ (between the years 2000 and 2007) and an increase in mean life expectancy (Correia, et al., 2013). The association was stronger in more urban and densely populated areas. The baseline PM$_{2.5}$ level appeared to have no role in the relation between PM$_{2.5}$ and life expectancy, which is in agreement with a previous study (Pope, et al., 2009). These findings indicate that there is no threshold below which further reductions in PM$_{2.5}$ levels provide no health benefits.

6 CONCLUSIONS

- The mean personal exposure to $PM_{2.5}$ was 12 µg/m^3. There was a strong correlation between personal exposure to $PM_{2.5}$ and indoor levels, and also a moderate correlation between personal exposure and the urban background concentrations. Personal exposure to $PM_{2.5}$ was significantly higher than the residential outdoor levels for non-smokers. Personal exposure to the trace elements Cl, K, Ca, Ti, Fe and Cu in $PM_{2.5}$ was significantly higher than the residential outdoor and urban background levels.

- The air mass origin affected the urban background levels of $PM_{2.5}$, BS and the trace elements S, V, Ni, Br and Pb (combustion processes and industry) and Cl (sea salt). For some of these elements (S, V and Pb), the impact of air mass origin was significant also on the personal exposure.

- The within-person variance component dominated the variability of personal exposure to $PM_{2.5}$, BS and the particulate trace elements for non-smokers. The relatively large within-person variance components point to the importance of performing repeated personal sampling when investigating environmental PM exposures.

- Determinants of personal exposure to $PM_{2.5}$ were season, smoking and the urban background concentration. Season and smoking were found to reduce the between-person variance, whereas urban background levels seemed to mainly affect the within-person variance component.

- Season was also a significant determinant of exposure to Fe and Pb, and smoking determined personal exposure to BS, K and Ti. The urban background levels were also a determinant for personal exposure to BS, Cl, Zn and Pb.

- Levels of biomarkers of inflammation and coagulation in blood were not found to be increased the mornings after days with elevated levels of ambient particulate matter compared with low levels when performing repeated samplings in a group of healthy volunteers living in Gothenburg.

7 FUTURE NEEDS

There is clear evidence that exposure to PM causes adverse health effects, but the specific physical and chemical properties that make PM more harmful are not known. Characterization of the personal exposure to PM constituents is one of the research fields needed in order to gain further knowledge. It is however unlikely that one single component in PM is responsible for all the health effects that have been linked to PM exposure.

Toxicological and epidemiological studies indicate that PM generated from combustion processes, e.g. vehicle emissions, biomass burning, energy production and industries, play a significant role in causing the adverse health effects. Future exposure studies would benefit from further analysis of PM components derived from various combustion processes. Analysis of BS and trace elements in the collected PM was in this thesis shown to be valuable and provided information about PM exposure from various sources. Further characterization of EC and BC in PM would be useful for assessing exposure to traffic exhausts. Also the potential effect of gaseous co-pollutants needs to be examined. Residential wood burning is a significant source of PM in Sweden and its contribution to personal PM exposures needs further investigation. Studies of personal exposure to PAHs are warranted.

Future studies of personal PM exposures should also include time-activity diaries that can provide information that can be used in mixed-effects models to identify important determinants of exposure to environmental PM and its' components.

Finally, there is no evidence of a safe level of exposure where no health effects of PM occur. Recent findings indicate that reductions in $PM_{2.5}$ levels generate health benefits regardless of the baseline level. A continuous reduction of PM exposure levels should be aimed at in Gothenburg as well as throughout Europe and the rest of the world.

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