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Fine and ultrafine particle exposure: Health effects and biomarkers

YIYI XU

DEPARTMENT OF LABORATORY MEDICINE | LUND UNIVERSITY 2017



Fine and ultrafine particle exposure: Health effects and biomarkers

Yiyi Xu



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DOCTORAL DISSERTATION

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<p>Abstract</p> <p>Particle, especially fine and ultrafine particle exposure has been linked to lower airway infections, asthma, chronic obstructive pulmonary disease (COPD), ischemic heart disease, stroke and cancer. The underlying mechanisms are under active investigation, and are still not fully understood. Several mechanisms have been proposed such as oxidative stress, inflammatory response and genotoxicity. Different biomarkers have been developed to investigate the mechanisms. This thesis includes exposure to fine and ultrafine particles from three different exposure sources: diesel exhaust, asphalt fumes and welding fumes. The main aim is to investigate adverse health effects caused by airborne fine and ultrafine particle exposure, and to analyze biomarkers that are hypothesized to be in the causal pathway from exposure to pulmonary and cardiovascular disease. The thesis is based on three studies (four papers): i) a human experimental exposure study with 18 volunteers; ii) a field study with 167 asphalt workers and 100 controls; iii) a field study with 101 welders and 127 controls. We investigated airway symptoms and lung function as health outcomes, and measured different biomarkers: cytokines (as biomarkers for inflammatory response), mitochondrial DNA copy number (as biomarker for oxidative stress) and telomere length (as biomarker for genotoxicity) to explore mechanisms.</p> <p>The exposure levels in our studies were lower than the current occupational exposure limits. However, we still found exposure related eyes and airway irritation and transient decrease in lung function. Changes in cytokines after exposures were not statistically clear, but may indicate a mild inflammatory response. Higher mitochondrial DNA copy number together with lower methylation suggests possible exposure related oxidative stress. No difference in telomere length was found between exposure groups and controls, but telomere length was positively associated with PAH metabolites, indicating more PAH exposure was associated with longer telomere length.</p> <p>This thesis shows health effects and change of biomarkers under low to moderate exposure to particles. Although the effects seem to be in the compensatory stage, reconsideration is still called for regarding current occupational exposure limits.</p>		
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Fine and ultrafine particle exposure: Health effects and biomarkers

Yiyi Xu



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路漫漫其修远兮,吾将上下而求索

The road ahead will be long. The climb will be steep.

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List of papers included in the thesis

I. **Xu Y.**, Barregard L., Nielsen J., Gudmundsson A., Wierzbicka A., Axmon A., Jönsson B., Kåredal M., Albin M. Effects of diesel exposure on lung function and inflammation biomarkers from airway and peripheral blood of healthy volunteers in a chamber study.

Particle and Fibre Toxicology 2013, 10:60.

II. **Xu Y.**, Kåredal M., Nielsen J., Bergendorf U., Strandberg B., Antonsson AB., Tinnerberg H., Albin M. Exposure, respiratory symptoms, lung function and inflammation response of road paving asphalt workers.

Manuscript

III. **Xu Y.**, Lindh C., Jönsson B., Broberg K., Albin M. Occupational exposure to low level of polyaromatic hydrocarbons may relate to increased mitochondria DNA copy number and telomere length.

Submitted

IV. **Xu Y.**, Li H., Hedmer M., Hossain MB., Tinnerberg H., Broberg K., Albin M. Occupational exposure to particles and mitochondrial DNA - relevance for blood pressure.

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Introduction

Air pollution in and from big cities and industrial areas is a continuous hot topic in global environment and health. In the past decades, clear associations between air pollution and adverse health outcomes have been demonstrated by multitudinous studies. Moreover, the research indicates that fine respirable particles are a major risk factor since stronger associations were found with particles than with gaseous pollutants. Compared to the general population, workers in some industries are generally exposed to fine respirable dust at higher concentrations, and they usually have higher risk of exposure related health outcomes. A numbers of studies have been performed regarding particle exposure in occupational settings. Currently, attention is being focused on the size and composition of different respirable particles, and on the understanding of pathophysiological mechanisms.

This thesis includes investigations of particles from three different exposure sources (mainly occupational): diesel exhaust, asphalt fumes and welding fumes. Main focuses are exposure related airway response, and alternation in biomarkers for possible mechanisms linking exposure and adverse health effects.

Occupational and environmental particle exposure: historical overview

An understanding of the hazards of occupational dusts and chemicals exposure has a long history. As long ago as the 15th and 16th centuries, it was well understood that airborne dusts and chemicals can cause illness and injury among workers, e.g. among miners. But it was not clear at that time what concentrations levels and exposure periods could lead to illness. Although in the 18th century, Bernardino Ramazzini, the father of occupational medicine, mentioned providing good ventilation in dusty trades and protective clothing for workers, little was done until the 19th and early 20th centuries [1, 2]. The earliest efforts to set an occupational exposure limits (OELs) was carried out by Max Gruber in Germany and the first OEL for carbon monoxide was set in 1883. In 1916, the exposure limit for dust with 80-90% quartz content was set. In the 1940s, the American Conference of Governmental Industrial Hygienists (ACGIH) published the first list of maximum

allowable concentrations, later known as Threshold Limit Values (TLVs). Many countries subsequently developed and adopted their own values [3].

On the other hand, the public awareness of the impacts of air pollution on the general population was not raised until after 1952, the year when the catastrophic London smog occurred, which led to about 4,000 premature deaths [4]. Clean air regulations, thereafter, were introduced in the 1960s and 1970s in many industrialized countries and successfully reduced air pollution levels. However, the problem of particle exposure had not been faced squarely during that time. It was not until the late 20th century that researchers and governments realized that particle exposure affects more people than any other pollutant. WHO announced guideline limits for particulate matter in 2005, aimed at achieving the lowest possible concentrations of particles: Annual mean concentration of PM_{2.5} (particulate matter with an aerodynamic diameter less than 2.5µm) should be lower than 10µg/m³, and PM₁₀ (particulate matter with an aerodynamic diameter less than 10µm) should be lower than 20µg/m³. Correspondingly, the 24 hour mean concentration should be lower than 25µg/m³ and 50µg/m³, respectively [5].

In recent years, researchers and governments have shown an increased interest in smaller particles, i.e. fine and ultrafine particles (fine particles refer to PM_{2.5}, ultrafine particles refer to PM_{0.1}). Researchers suggested that these fine and ultrafine particles, which can be high in number, but contribute little to particle mass, may result in more adverse effects [6, 7], since they can penetrate into the small airways and alveolar region (figure 1), where they may exist for weeks or months, or even longer than coarse particles (PM₁₀) [8]. Meanwhile, governments are discussing how to regulate particle exposure levels by using PM₁ and PM_{0.1}, since many sources (like combustion engines) generate higher amounts of PM₁ or PM_{0.1}.

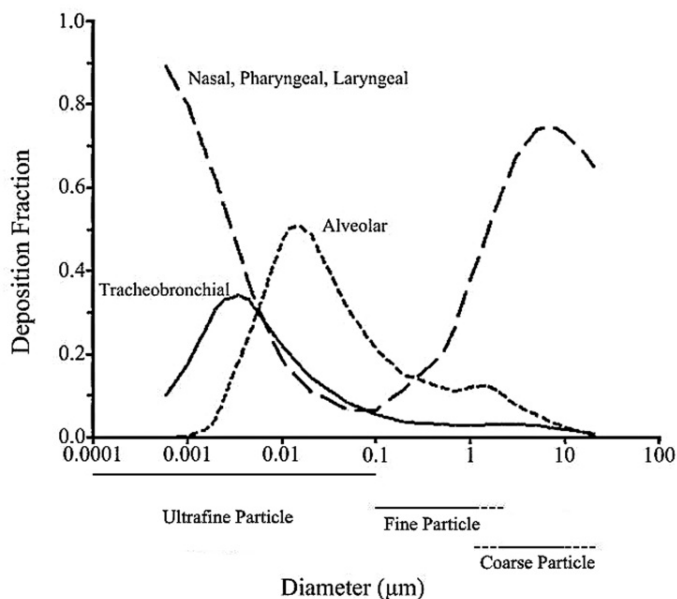


Figure 1: Typical particle size fractionation and airway deposition of inhaled particle.

Fine and ultrafine particles are mainly deposited in small airways and the alveolar region, while coarse particles are mainly deposited in the nasal and upper airway passages. Modified figure from ICRP model 1994 [9])

Health effects of exposure to fine and ultrafine particles

After deposition in the lung, fine and ultrafine particles may be cleared or retained in the lung compartment and induce local pulmonary responses. They may also translocate to the capillary system and cause systemic effects like cardiovascular diseases. Plenty of studies have associated particle exposure with increases in airway symptoms and acute lower airway infections, exacerbation of asthma and chronic obstructive pulmonary disease (COPD), impaired lung growth in children, and increased risk of cardiovascular diseases including ischemic heart disease and strokes, as well as cancer [7]. Some particle characteristics have been proposed to be possibly responsible for adverse health effects, such as particle size and concentration, particle surface area, transition metals, organic compounds, sulfate and nitrate compounds, peroxides and free radicals, soot (elemental carbon and associated PAH), and correlated gaseous pollutants [10]. Table 1 summarizes the particle characteristics and exposure related health effects from three different exposure circumstances included in this thesis. Details are presented separately below.

Table 1.

Particle size, composition and exposure related health effects of particles from diesel exhaust, asphalt fumes and welding fumes.

Particle source	Particle size (CMD) ^a	Particle compositions	Exposure groups	Health effects
Diesel exhaust	55-89nm [11, 12]	Sulfate, metals, organic compounds including PAHs	General population (from traffic) Workers in mining, railroad, construction and transportation (from diesel engines)	Short term: airway symptoms, inflammatory response, acute myocardial infarction Long term: allergy, COPD, ischemic heart disease, heart failure, arrhythmia, cancer (IARC group I)
Asphalt fumes	50-80nm [13]	Various organic compounds including PAHs. Metals like iron, nickel, vanadium.	Mainly asphalt workers	Lung function decrease, cough, bronchitis, inflammatory response, asthma, COPD, cancer (IARC group 2B)
Welding fumes	149-240nm [14, 15]	Metals like iron, manganese, chromium, nickel	Mainly welders	Metal fume fever, lung function decrease, chronic bronchitis, airway irritation and infection, arrhythmia, ischemic heart disease, cancer (IARC group 2B)

a. CMD: count median diameter, i.e. 50% of all particles are less than this diameter. Listed CMDs were reported in different studies.

Diesel exhaust

Physical and chemical characteristics

Diesel engines are widely used for on-road traffic such as passenger cars, buses and heavy goods vehicles, and off-road transport like trains and ships. They are also common in industries such as mining and construction. This has been of great concern in past years because diesel engines emit relatively high amounts of fine and ultrafine particles. Although the complex composition of diesel emission may vary widely due to different engine types, emission control systems, fuel and so on, the emissions can be grouped into gaseous and particulate phase. In the gas phase, it usually contains carbon monoxide, oxygen, nitrogen oxides and various volatile organic compounds like benzene and formaldehyde. In the particulate phase, it contains elemental and organic carbon (soot), ash, sulfate and metals [16]. Typical diesel exhaust particles are formed from primary particles (like carbon black) of about 15-40 nm in diameter, and then agglomerate with organic compounds and other adsorbed materials [17]. Our chamber study showed that the average count median diameter (CMD) of diesel exhaust particles was 89 nm (i.e. 50% of particles had a diameter < 89nm) [11]. Other studies suggested that about 80% of diesel exhaust particles have a size less than 100 nm (PM_{0.1}) [18].

Exposed population

The general population is exposed to diesel exhaust mainly from traffic in daily life. No matter whether living or working close to roadways, or driving or commuting in vehicles, people are exposed to traffic related particles, of which, diesel exhaust could be an important component [19]. According to estimations from the US, 4-16% of total traffic particles are contributed by diesel emissions in rural and urban areas [20, 21]. In addition to vehicle traffic, ship emissions in the port cities are another source of diesel exhaust. People living or working close to the harbor are exposed. In developing countries, another major source is small to medium-sized stationary diesel electricity generators. Occupational exposure to diesel exhaust occurs among truck drivers, miners, construction workers and so on. In Europe, about three million workers in the mining, railroad, construction and transportation sectors are occupationally exposed to diesel exhaust (estimated by International Agency for Research on Cancer (IARC) in 2010) [22]. The exposure levels vary among industries, as well as different working tasks within the same industry. Generally, tunnel construction workers and underground workers including miners are exposed to relatively higher level of diesel exhaust ($100\text{-}300\mu\text{g}/\text{m}^3$ measured as element carbon) [23-25], while workers working outdoors or in open areas, such as drivers or railway workers are exposed to relatively lower concentrations (element carbon concentrations around $10\text{-}20\mu\text{g}/\text{m}^3$) [26, 27].

Exposure related health effects

Numerous epidemiological and human experimental studies have shown associations between exposure to diesel exhaust particles and various adverse health effects. Short term exposure can cause upper airway symptoms, acute transient decrement in lung function and inflammatory responses [28, 29], as well as an increased risk of acute myocardial infarction [30, 31]. People living or working close to major roads with long term, accumulated exposure are more likely to experience a permanent reduction in baseline lung function [32], and to develop respiratory diseases such as allergies and COPD [33-35], as well as cardiovascular disease such as ischemic heart disease, heart failure and arrhythmia [36-38]. Small children, elderly people and patients with pre-existing cardiopulmonary diseases are susceptible groups and may experience more adverse effects [39-42]. Moreover, studies also found associations between exposure to diesel exhaust particles and an elevated risk of developing lung [43-45] and bladder cancer [46], as well as cancer at other sites including the colon, rectum and kidney [47]. Recently, diesel exhaust has been categorized as 'carcinogenic to humans' (Group I) by IARC [16].

Asphalt fumes

Physical and chemical characteristics

Asphalt, also known as bitumen in Europe, is a black, sticky and semisolid or highly viscous liquid form of petroleum. Sometimes 'asphalt' is also used as an abbreviation for 'asphalt concrete', a mixture of gravels, sand and asphalt/bitumen, which is widely used in road construction and roofing. There are various classes of asphalt: basic and most commonly used 'straight-run' asphalt for road paving, oxidized asphalt for roofing, and other modified and produced asphalts to achieve specific physical characteristics such as modified asphalt with special additives, fluxed asphalt, asphalt emulsions and thermally-cracked asphalt [48]. When asphalt is heated, it vaporizes and condenses and emits a complex mixture of vapors, fumes and solid particles containing hundreds of different compounds and carcinogens including PAHs [48]. There are also traces of metals like iron, nickel and vanadium. Although many studies have been carried out to define the physical and chemical characteristics of asphalt fumes [13, 49], the compositions have not been well characterized due to the high variability, depending on many factors, such as manufacturing process, handling temperature, and presence of additives and modifiers. Despite the broad variations in asphalt fumes composition, some studies suggested that 50% of all particles in asphalt fumes are within a size of around 80nm (CMD=80nm) [13, 50].

Exposed population

Exposure to asphalt fumes occurs primarily within the occupational context, while the frequency and concentration of potential asphalt exposure is much lower in the general population. According to WHO estimates approximately 100,000 people working in road crews engage in asphalt road paving in Western Europe [51]. In Sweden, about 2,800 workers apply asphalt mixtures to road surfaces [52]. Several air measurement methods have been adopted to measure the airborne exposure levels among asphalt workers, such as measuring total particle concentration, benzene-soluble particle and total organic content [52]. However, none of them are specific and have the capability to describe total exposure to asphalt fumes. Previous studies showed a wide range of exposure levels for different working tasks (i.e. paver operators, screedmen, rakers, roller drivers and etc.) as shown by measuring different exposure parameters. For instance, total particle exposure ranged from 400 to 1400 $\mu\text{g}/\text{m}^3$ [13], benzene-soluble particle exposure was around 300 $\mu\text{g}/\text{m}^3$ [53], total polycyclic aromatic compounds exposure ranged from 10-200 $\mu\text{g}/\text{m}^3$ [53], and airborne PAHs exposure (measured as pyrene) ranged from 100-600 $\mu\text{g}/\text{m}^3$ [54]. In addition, since asphalt fumes contain a complex mixture of organic substances and compounds such as PAHs can be absorbed through both the airways and dermally, measurements of PAHs metabolites in urine are also

widely used to determine the internal dose of PAHs exposure. However, the reported excretion of PAH metabolites (mostly measured as 1-hydroxypyrene) was highly variable (from <0.1 up to 38 $\mu\text{mol/mol}$ creatinine) between studies [54-56].

Exposure related health effects

A number of reports have shown that road paving asphalt workers are more likely to experience acute eyes, nose and throat irritation and cough, though these effects appear to be mild and transient [51, 57]. Chronic exposure is related to a reduction in lung function, coughing, bronchitis and mild inflammatory responses [58-61]. A higher incidence of asthma and COPD among asphalt workers has also been reported in some studies [62]. However, the possible adverse cardiovascular effects of asphalt fume exposure are less clear, although some inflammatory markers related to coronary heart disease have been found to be elevated after asphalt paving. One recent cohort study showed an association between exposure to benzo(a)pyrene in asphalt workers and elevated mortality due to ischemic heart disease [63]. Studies of lung cancer risk among workers engaged in asphalt paving have yielded contradictory results [64-66], which may possibly be due to variability in critical exposures, such as use of recycled old asphalt containing coal-tar layers [52, 67]. Similarly, elevated risks of bladder cancer and stomach cancer among asphalt workers have been reported, but the correlations with asphalt exposure are not definite [68-70]. Recently, IARC classified occupational exposures to conventional asphalt and its emissions during road paving as Group 2B (possibly carcinogenic to humans) [71]. In addition to airway and cardiovascular effects, dermal contact with asphalt fumes can cause skin irritation, rashes, dermatitis and sometimes skin burns [51, 72, 73], which can be reduced by using personal protection and keeping equipment clean.

Welding fumes

Physical and chemical characteristics

There are a large variety of welding methods, among which, gas metal arc welding is the most common in today's industries. During the process, work-piece metals melt and join after heating up by a formed electric arc between consumable wire electrode and the workpiece metals. Shielding gases are additionally added to reduce oxidation during the welding process. Welding fumes are formed in the thermal process and have both gaseous and particle phases. In the gas phase, it usually contains ozone, nitrogen oxides and carbon oxides due to added shielding gases. In the particle phase, they contain elements that are in their pure or oxide forms, but the composition varies depending on the material used [74]. Mild steel welding mainly generates particles of iron and manganese, while chromium and nickel can be additionally found in the particle phase from stainless steel welding

[75]. The enrichment with metal oxides makes the welding fume particles potentially toxic. In addition, the particle size of welding fumes is also important in determining the potential risk of exposure. Previous studies showed that the mean size of generated particles in gas metal arc welding is in the fine and ultrafine size range (about 100-200 nm) [76, 77].

Exposed population

Circumstances leading to exposure to welding fumes in the general population are rare. However, welding is an important source of fine and ultrafine particles in working environments. In 1990, IARC estimated that around three million workers worldwide had jobs in which welding was involved to some extent [78], and the number is likely to rise. In Europe, the estimated number in 2011 was around 1.8 million including both full-time and part-time welders [77]. The exposure levels vary widely and are influenced by factors such as ventilation, the general contamination of the working environment and current welding activity. Intensive welding activity can dramatically increase the background particle concentration from $100\mu\text{g}/\text{m}^3$ to $3000\mu\text{g}/\text{m}^3$ [79]. The typical particle concentrations of the personal breathing zone are as high as $5\text{mg}/\text{m}^3$ throughout the industry [14], which corresponds to the current Threshold Limit Value-Time Weighted Average (TLV-TWA) of welders ($5\text{mg}/\text{m}^3$ for total particles).

Exposure related health effects

The most common health concern of the welders is metal fume fever, a nonspecific transient flu-like condition, due to exposure to metal oxides through inhalation [80]. Also, occupational exposure to welding fumes has been linked to an increase in airway symptom occurrence, a decrease in lung function, chronic bronchitis and airway irritation and infection [81, 82]. The association between welding fume exposure and lung cancer is less clear, but recent studies consistently showed that welders had an increased risk of lung cancer [83, 84]. IARC has classified welding fumes as ‘possible human carcinogen’ (Group 2B) [78]. Recently, concern regarding the adverse health effects of welding fumes has shifted from pulmonary to cardiovascular effects since fine and ultrafine particles are considered to be hazardous to the cardiovascular system. Welders were found to be more likely to have higher blood pressure [85], experience alterations in cardiac autonomic function [86, 87], increased risk of arrhythmias [88] and ischemic heart disease including acute myocardial infarction [89, 90]. Recently, several reports have also suggested that welding can influence the central nervous system because of its enrichment of manganese, lead and aluminum, and is a risk factor for parkinsonism [91, 92], but the conclusion may suffer from some limitations [93].

Possible mechanisms and biomarkers of interests

Although epidemiological studies have demonstrated clear associations between exposure to particles and adverse effects in the respiratory and cardiovascular systems, the underlying mechanisms are under active investigation, but still not fully understood. Several mechanisms have been proposed to explain the adverse health effects, among which the most studied and important mechanism is oxidative stress. Moreover, inflammation response and genotoxicity are also involved. Recently, particles have been shown to irritate pulmonary nerve endings directly and cause changes in heart rate variability, an indicator and prognostic factor for cardiovascular disease. While investigating mechanisms underlying pathophysiological pathways, different biomarkers were developed. Figure 2 lists a summary of proposed mechanisms which will be discussed in detail below.

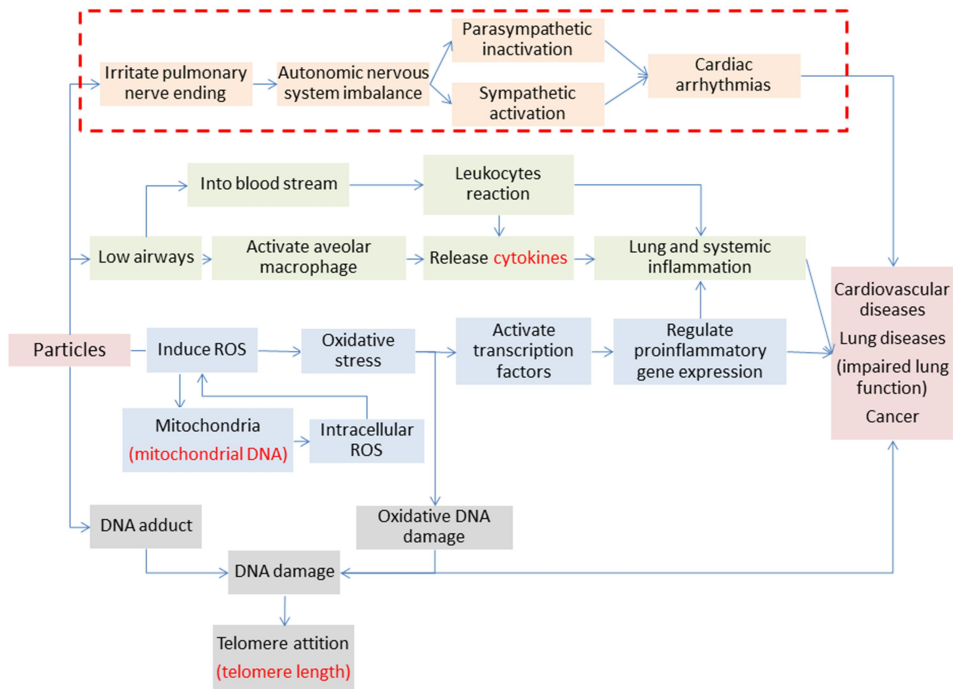


Figure 2 : Proposed schematic of possible mechanisms and biomarkers of interests in the thesis.

Four possible mechanisms (oxidative stress, inflammatory response, genotoxicity and irritating pulmonary nerve ending) linking particle exposure and health impacts are listed. Mechanisms regarding cardiac arrhythmias (in red dashed box) was not studied in this thesis and will not be discussed in detail below. Biomarkers of interest included in the thesis are highlighted in red.

Oxidative stress and mitochondrial DNA

Oxidative stress has been suggested as one of the most important pathophysiological mechanisms of health effects induced by particle exposure, which reflects the imbalance of redox and is mainly caused by the generation of reactive oxygen species (ROS). The exogenous ROS can be produced from particle's metal composition, which can catalyze Fenton-type reactions and generate ROS (hydroxyl radical, hydrogen peroxide, superoxide radical) [94, 95]. Absorbed PAHs of particles may be metabolically converted to redox-active quinones and some of them can yield ROS by redox cycling [96]. Endogenous excess ROS can also be produced under stress through multiple mechanisms in which mitochondria are involved. The generated ROS can disturb redox homeostasis of biological systems, thereby causing oxidative damage [97, 98]. It has also been suggested that PM such as diesel exhaust particles which have a carbonaceous core, also have an oxidative capacity merely by presenting a large surface area (meaning more surface free radical activity) even without reactive species (i.e. metals or PAHs) on its surface [99]. Oxidative stress induced by particle exposure can be stratified into three phases (Figure 3). Phase one is low and mild oxidative stress which occurs when the exposure level is relatively low or exposure has just started. Several studies showed that under mild oxidative stress, nuclear factor-erythroid 2-related factor 2 (Nrf2) is activated and binds to antioxidant response elements (ARE). Through transcriptional induction of ARE-driven genes that encode antioxidant-detoxifying enzymes, Nrf2 initiates ROS elimination and activates cellular rescue against oxidative damage [100, 101]. When oxidative stress is exacerbated by increasing ROS production, the protective antioxidant response becomes inadequate (phase two). A series of redox-sensitive transcription factors and kinases which mediate the expression of various cytokines and chemokines, are activated, and in succession, induce a proinflammatory response and causes airway and systemic inflammation [102, 103]. Phase three is the one with a high level of oxidative stress but overwhelmed and defeated antioxidant defense. Severe cell toxicity and oxidative DNA damage takes place, which causes cell death and contributes to the carcinogenicity of particle exposure.

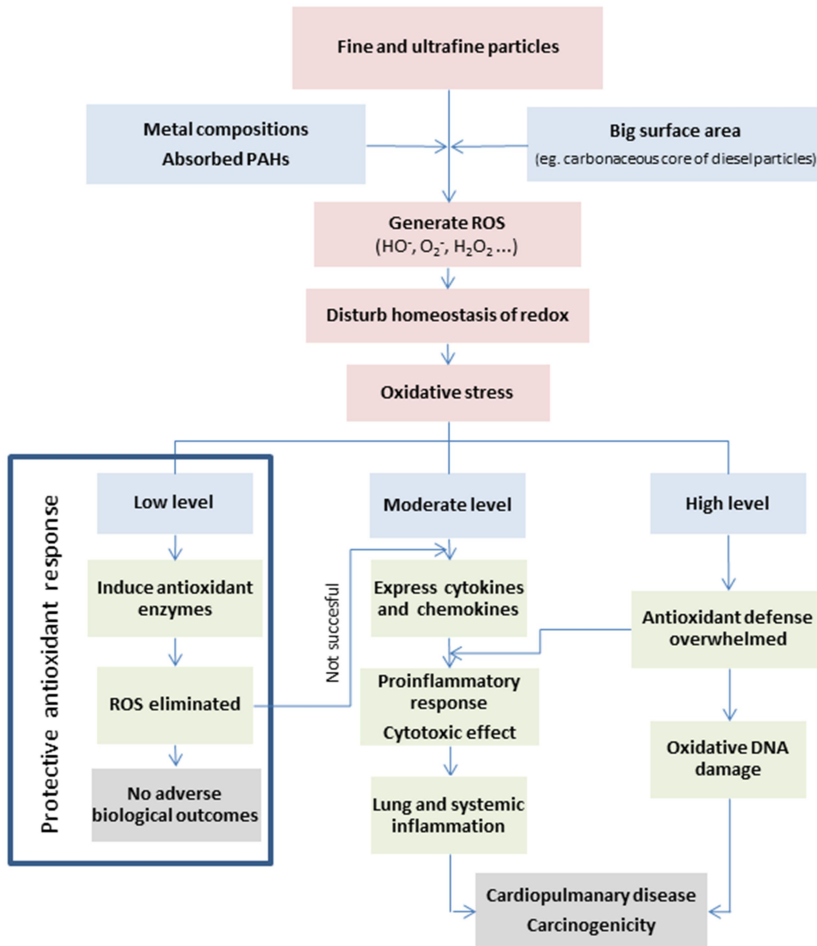


Figure 3: Proposed schematic of three phases of oxidative stress induced by particle exposure.

Many different biomarkers have been developed to monitor oxidative stress in environmental studies, such as biomarkers for lipid peroxidation (malondialdehyde formation), a biomarker for oxidative DNA damage (8-oxo-dG), and measurements of antioxidant enzymes [104]. In our field studies, we analyzed mitochondrial DNA, another biomarker of oxidative stress of interest due to its high susceptibility to oxidative stress and its relevance to diseases.

Mitochondria have been described as “the powerhouses of the cell” because their primary role is to convert energy for the cell into ATP through oxidative phosphorylation. Their second major function in regulating cell death through

generating ROS and releasing proteins related to different modes of cell death is now well established [105]. It has been suggested that mitochondria may be involved in some or all of the three phases of oxidative stress by generating energy for cells and ROS as a byproduct [106]. Mitochondria carry their own extra-nuclear DNA (Figure 4), so-called mitochondrial DNA. It is more susceptible to oxidative stress than nuclear DNA due to its limited capability for DNA repair and lack of protection by histones [107]. The copy numbers of mitochondrial DNA vary in each mitochondrion, as well as in different cells, different tissues and individuals. Mitochondrial DNA has two strands. The heavy strand (guanine-rich strand) encodes 9 genes and the light strand (cytosine-rich strand) encodes 28 genes. These genes include 13 respiratory chain polypeptides, 22 transfer RNAs and 2 ribosomal RNAs. It also has a non-coding control region called the displacement loop (D-loop) [108]. The presence of mtDNA methylation has been debated for decades. Recently, Bellizzi *et al.* confirmed that mtDNA is indeed methylated, particularly in the D-loop region [109]. This progress in understanding mitochondrial DNA features provides an opportunity to study its function in relation to diseases and exposure. Moreover, the ongoing development of methods with which the mitochondrial genome can be analyzed, and the availability of a consensus human sequence, support the methodology.

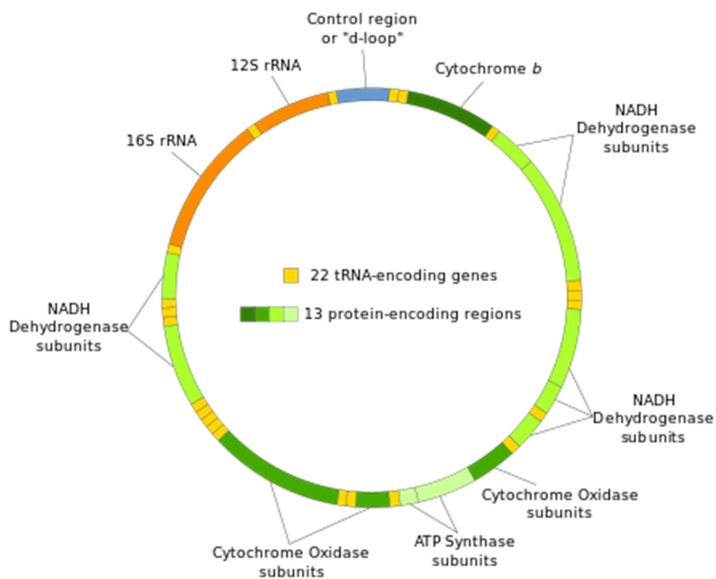


Figure 4: Human mitochondrial DNA and encoding genes. Derivative work: Shanel (talk) Mitochondrial DNA de.svg: translation by Knopfkind; layout by jhc - Mitochondrial DNA de.svg. (from wikipedia https://en.wikipedia.org/wiki/Mitochondrial_DNA#/media/File:Mitochondrial_DNA_en.svg)

In recent years, considerable progress has been made in understanding mitochondrial DNA damage in aging [110]. Studies also pointed out that age-related mitochondrial DNA damage is a potential contributing factor to diseases including cardiovascular disease, particularly ischemic heart disease, a condition characterized by an increase in oxidative stress [111, 112]. However, the relations between exposure and mitochondrial DNA alteration, and the link to diseases, have not been studied sufficiently. Animal studies suggest that some risk factors, such as second-hand smoke exposure and hypercholesterolemia, may induce mitochondrial DNA damage in cardiac tissues in mouse models [113]. Recently, several human studies found associations between alteration in mitochondrial DNA (content and/or methylation level) in peripheral blood and various exposures, such as elemental carbon, PAHs and benzene [114-117], but the results are inconclusive. More studies are needed to develop knowledge about this.

Inflammatory responses and cytokines

There is a link and vicious circle between oxidative stress and inflammatory response. The redox changes caused by oxidative stress can activate redox-sensitive transcription factors like nuclear factor-kappa B (NF- κ B) and mitogen-activated protein kinase, which are involved in regulating proinflammatory gene expression, and then cause inflammation in the lungs [102]. On the other hand, recruited leukocytes and released cytokines as an expression of inflammatory response could generate intracellular oxidants, which in turn augment oxidative stress [118]. In addition to oxidative stress, the inflammatory response following particle exposure can also be induced by particles per se, through interacting with alveolar macrophages. Although the potential pathophysiological impact of PM on alveolar macrophages is not fully understood, it has been suggested that upon contact with PM, alveolar macrophages are activated to phagocytose the particles, and at the same time produce and release acute response cytokines such as tumor necrosis factor alpha (TNF- α), interleukin (IL)-6, IL-8 and granulocyte macrophage colony stimulating factor (GM-CSF). They can interact with airway epithelial cells and induce neutrophil chemotactic activity to recruit leukocytes to assist in clearing PM. Alveolar macrophages also play a key role in initiating systemic inflammatory responses by generating the cytokines, particularly IL-6 and GM-CSF, and these systemic mediators translocate from lung tissues to circulation [119, 120].

A broad range of cytokines has been investigated in the studies of particle exposure. Increased release of proinflammatory cytokines (IL-6, IL-8 and TNF- α , etc.) by activated alveolar macrophages and blood mononuclear cells, as mentioned before, have been found in both bronchioalveolar lavage fluid and serum/plasma following particle exposure [121, 122]. IL-6 can initiate hepatic

synthesis of acute-phase proteins, including the C reactive protein (CRP), serum amyloid A (SAA) and fibrinogen. Positive associations have been demonstrated between these acute phase proteins and increased particle exposure [123, 124]. Also, some anti-inflammatory proteins such as the Clara cell protein (CC16) and surfactant protein D (SP-D) have recently become of interest and have been used as biomarkers of inflammation in some studies [125, 126].

Genotoxicity and telomere length

Particles are well recognized as causing genotoxicity. In addition to the pathways of inducing oxidative stress and inflammatory response as described above, particles often containing various types of genotoxic/mutagenic chemical substances, such as organic compounds and metals, can cause DNA damage.

Telomeres are DNA-protein complexes which are located at the ends of eukaryotic DNA strands as a protective 'cap'. Human telomeres consist of hundreds to thousands of tandem 'TTAGGG' repeats [127]. Telomeres play a key role in maintaining chromosomal stability [128]. However, telomeres experience progressive shortening with successive cell replications due to the 'end-replication problem'. In general, on average, 50-100 base pair of telomeres are deleted from the ends of chromosome during each cell replication; and when telomeres reach a critically short length, the cell goes into senescence and an apoptosis process (figure 5). Telomere-driven replicative senescence is one of the important factors determining biological aging [129]. Shorter telomere length has been reported to be responsible for age-related diseases, such as cardiovascular disease [130], pulmonary fibrosis [131], COPD [132] and cancer of the lung as well as at several other sites in the body [133].

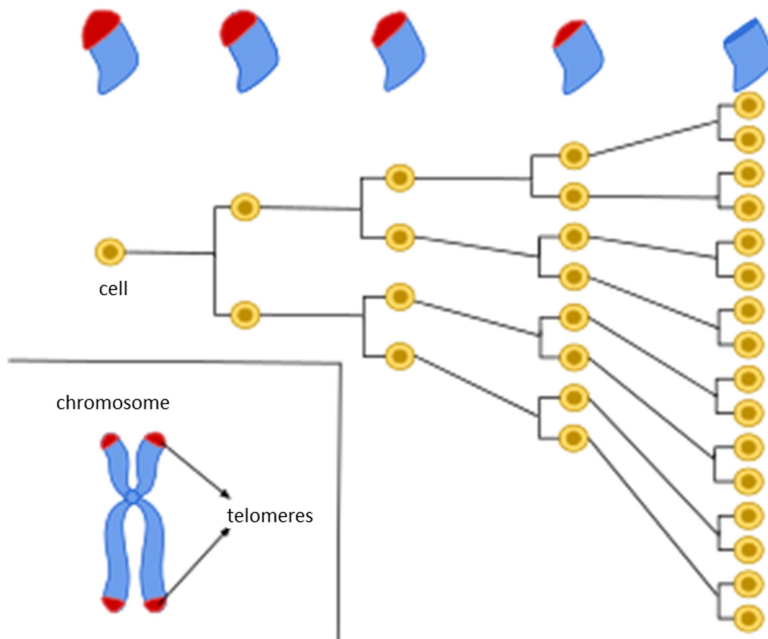


Figure 5: The telomeres on the end of the chromosome get smaller during each cell replication.
 (from wikipedia [https://en.wikipedia.org/wiki/Telomere#/media/File:Hayflick_Limit_\(1\).svg](https://en.wikipedia.org/wiki/Telomere#/media/File:Hayflick_Limit_(1).svg))

Apart from the naturally occurring telomere attrition, telomere shortening can be accelerated by exposure to various chemicals and physical factors [134]. One of the mechanisms for accelerated telomere shortening could be oxidative stress [135]. Since the telomere sequence is rich in guanine, it is susceptible to oxidative modifications of guanine. In addition, the DNA repair capacity in the telomere region is relatively lower, hence the damages tend to remain unrepaired, and cause telomere loss during DNA replication [136]. However, studies investigating the associations between particle exposure and telomere length showed inconsistent results. There are studies reporting positive associations between particle exposure and TL [137], inverse association [138], and no association [139] in the respective research settings. The associations between PAH and telomere length were mostly reported to be inverse [140, 141].

Aims

The overall aim of the thesis was:

To investigate adverse health effects caused by airborne fine and ultrafine particle exposure, and to analyze biomarkers that are hypothesized to be in the causal pathway from particle exposure to pulmonary and cardiovascular diseases.

Specific aims were:

To investigate the effects of diesel exhaust on airway symptoms, lung function, airway and systemic inflammation in healthy human subjects; and to monitor the pattern of changes in lung function and inflammatory cytokines during diesel exposure.

To assess occupational exposure from asphalt fumes; and to investigate the adverse effects on airway symptoms, lung function and systemic inflammatory response in the workers paving with conventional asphalt and with crumb rubber modified asphalt.

To assess occupational exposure to PAH from asphalt fumes by measuring PAH metabolites in urine; and to investigate the adverse effects by measuring mitochondrial DNA copy number, a marker of oxidative stress; and telomere length, a marker for genotoxicity.

To investigate the effects of occupational exposure to welding fumes on mitochondrial DNA copy number and methylation. Furthermore, we aimed to explore if these mitochondrial markers can modify the association between welding fumes and cardiovascular response, measured as blood pressure.

Materials and methods

Overall study design

The studies encompassed different designs and recruitments of voluntary participants to investigate specific study aims (Figure 6). All studies were approved by the Regional Ethical Review Board, and study participants provided written consent.

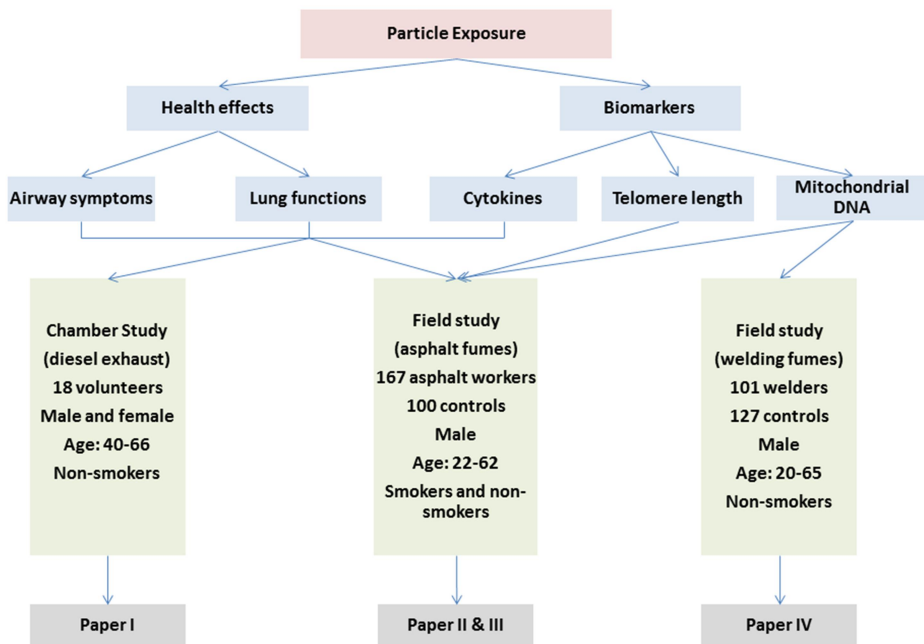


Figure 6: Study designs and participants for the studies included in this thesis.

Study of diesel exhaust exposure (Paper I)

This was a chamber experimental study on healthy non-smoking adults, which was a part of the DINO study, “Health Effects of Combined Exposure to Diesel and Noise”. We recruited 18 volunteers (nine males and nine females) of age 40 to 66 (mean age: 51). Inclusion criteria were no symptoms or diagnosis of asthma, normal chest auscultation, normal lung function and a negative skin prick test for a standard panel of common aeroallergens.

The volunteers underwent four different exposure scenarios (table 2). Each exposure lasted for three hours and took place in a specially built exposure chamber (22 m², suitable for three people simultaneously) on different occasions in a randomized sequence at least one week apart. The volunteers were asked to sit quietly during the entire exposure and went through a series of examinations such as questionnaires, medical examinations, lung function tests, and nasal lavage as well as blood sampling (details see section ‘health outcome measurements’).

Table 2.

Four exposure scenarios in the DINO chamber experimental study

		Diesel exhaust (measured as PM ₁₀)	
		Low (~2µg/m ³)	High (~300µg/m ³)
Traffic noise	Low (46 dB(A))	Reference exposure	Diesel exposure
	High (75 dB(A))	Noise exposure	Combined exposure

Study of asphalt fumes exposure (Paper II & III)

In this field study, we recruited 167 male asphalt workers and 100 male controls. The asphalt workers mainly came from four large road construction and maintenance companies. 116 of the asphalt workers were primarily applying conventional asphalt and 51 of them primarily rubber asphalt. The control subjects came from the same companies or municipal department and primarily worked with green area maintenance (i.e. performing manual outdoor work with no known occupational exposure to asphalt fumes). There were 31 asphalt workers who paved with both types of asphalt (i.e. conventional and CRM asphalt) in different periods, thus they were included in a nested self-controlled study to investigate the exposure and health effects of CRM asphalt exposure compared to conventional asphalt.

The participants were investigated twice during four consecutive working days: pre-exposure on Monday morning (after a 72-hour exposure-free period); and post-exposure on Thursday afternoon. At each investigation, they filled in the

questionnaires and left urine samples. They also went through the lung function test and blood sampling.

Study of welding fume exposure (Paper IV)

In this cross-sectional field study, we recruited 101 welders and 127 control subjects. They were all males and currently nonsmokers (at least for the last 12 months). The welders came from 10 different companies in southern Sweden, using the same gas metal arc welding method, and were therefore exposed to relatively homogenous compositions of welding fumes. The control subjects came from seven companies that handled grocery goods and mainly worked in storage rooms (i.e. loading and unloading products), and thus, their physical activity load was comparable to the welders in our study.

All participants were investigated only once during their work day. They went through a structured face-to-face interview with a qualified occupational health nurse. After the interviews, the participants' blood pressures were measured and blood samples were collected.

Exposure measurements and characteristics

Exposure setup for the chamber study of diesel exhaust exposure (Paper I)

The diesel exhaust was generated from a passenger car (Volkswagen Passat TDI, -98, 1900cm³, 81kW) running in idle mode placed outside the laboratory. Swedish Environmental Class 1 (EC1) diesel fuel with sulfur content less than 10ppm was used. Dilution was controlled to supply the desired concentration of particles to the chamber for the different exposure scenarios. The particle number concentration and size distribution (10-500nm) was determined with a scanning mobility particle sizer consisting of a differential mobility analyzer (DMA, Vienna-type, 28 cm long) and a condensation particle counter (TSI Inc. 3010). PM₁ mass concentration was determined with a Tapered Element Oscillating Microbalance (TEOM, R & P Inc., model 1400a). The chemical composition of particles was also measured using another approach [11]. The characteristics of high and low diesel exhaust exposures are presented in Table 3.

Table 3.

Summary of particle characteristics during high diesel exhaust exposure (Diesel exposure and Combined exposure) and low diesel exhaust exposure (Reference exposure and Noise exposure)

	High diesel exhaust (Diesel & Combined exposure)	Low diesel exhaust (Reference & Noise exposure)
PM ₁ mass concentration ($\mu\text{g}/\text{m}^3$)	276 \pm 27	2 \pm 2
PM ₁ number concentration (particles/cm ³)	3.9 $\times 10^5$ \pm 0.5 $\times 10^5$	14 \pm 16
Elemental carbon fraction (%)	82 \pm 3	--
Organic carbon fraction (%)	18 \pm 3	--
Particle phase PAHs (ng/m ³)	60 \pm 1.2	--

Traffic noise was recorded at a street crossing and it was played from two loudspeakers reproducing each channel in a stereo recording. The noise had a continuous, natural change of noise level. The temperature and the relative humidity in the chamber were maintained at 23°C and between 30% and 40%, respectively.

Air sampling for the field studies of asphalt fumes (Paper II & III) and welding fumes exposure (Paper IV)

Full-shift personal-breathing-zone sampling was used to measure the airborne particle exposure in two field studies. The workers carried air sampling equipment with a pump and filters to collect dust for various endpoints analysis: respirable dust (for both studies), total dust, total PAH (gas phase and particle phase), nitrosamine and benzothiazole (only for asphalt fumes study). Respirable dust was collected on 37 mm filters, pore size 0.8 μm fitted to cyclones. Total dust and airborne particulate PAHs were simultaneously collected on 37 mm filters fitted in conductive filter cassettes. Adsorption tubes (XAD-2, SKC, USA) were used downstream the total dust filters to collect gaseous PAHs and benzothiazole. The flow rate was 2.2 L/min for respirable dust and 1.5 L/min for total dust and total airborne PAHs.

In the asphalt fume study, air sampling was conducted in around 20 conventional asphalt workers and 20 CRM asphalt workers; however, we could not estimate the exposure levels for the rest of asphalt workers due to complicated and highly variable exposure situations outdoors. In the welding fumes study, air sampling was performed in 70 welders (53 welders were included in the study and 17 were not). For the welders who used powered air purifying respirators (PAPRs) during welding, the samples were collected outside the PAPRs and results were reduced by a correction factor of three to get an estimation of the exposure inside PAPRs.

The other 48 welders included in the study with no personal sampling data were assigned estimated exposure levels to respirable dust based on the exposure data from the 70 measured welders.

Biological sampling methods

Urine sampling for PAH exposure in the study of asphalt fumes exposure (Paper III)

Two urine samples from each participant were collected to assess the total exposure to PAHs from both dermal contact and inhalation: First morning urine sample (pre-exposure) on Monday morning and spot urine sample (post-exposure) on Thursday afternoon. All urine samples were transported to the laboratory at room temperature and stored at -20°C for further analysis. The metabolites: 1-hydroxypyrene (1-OH-PYR), 2-hydroxyphenanthrene (2-OH-PH), 3-hydroxybenzo[a]pyrene (3-OH-BaP) and 3-hydroxy benzo[a]anthracene (3-OH-BaA) were measured. All samples were prepared in duplicates and the average concentrations were used. The concentrations of 3-OH-BaP and 3-OH-BaA were not evaluated in further analysis since more than 95% of the samples were below the limit of detection. Creatinine was analyzed using an enzymatic method and used for adjustment of urinary dilution (as μmol per mol creatinine).

Blood sampling for biomarkers analysis in all studies (Paper I - IV)

In the chamber study, peripheral blood samples were taken before exposure, after exposure and 20 h post-exposure. Serum and plasma were separated from blood samples and stored at -80°C until analysis of cytokines was conducted. The rest of the peripheral blood samples were stored at -20°C until DNA extraction. In the field studies, peripheral blood samples were obtained onsite and transported to the laboratory in dry ice. The same procedures were used to get serum, plasma and DNA as in the chamber study.

Nasal lavage for biomarkers analysis in the chamber study (Paper I)

Nasal lavage was collected before, after, and 20 h post-exposure. 18ml 37°C 0.9% NaCl solution was instilled with a syringe into one nostril until the liquid appeared in the opposite one. The liquid was sucked back and instilled again. The procedure was repeated three times in each nostril. The lavage liquid was immediately

chilled and the cells were separated by centrifugation (Centrifuge 5702R, Eppendorf AG, Hamburg, Germany). The supernatant was separated from the pellet and stored at -80°C until the analysis of cytokines.

Health outcome measurements

Symptoms scoring and medical examinations

In the diesel exhaust chamber study, a visual analogue scale (VAS) (range 0 to 100 millimeter) was used to record the symptoms including eye irritation, runny nose, nasal congestion, throat irritation and chest tightness before exposure, and at 15, 75 and 135 min into exposure during each exposure session. Signs of eye, nose and throat irritation (redness/secretion/swelling) were also recorded (normal/slight/moderate/pronounced) together with auscultation of the lungs by a physician before and after exposure (Paper I).

In the asphalt fumes exposure study, health questionnaires were used to report the symptoms of eye, nose, or throat irritation, cough, shortness of breath, wheezing pre-working (Monday morning) and post-working (Thursday afternoon) (Paper II).

Lung function

A spirometry test was performed using a computerized spirometer in both the chamber study and the asphalt study. Forced vital capacity (FVC), forced expiratory volume in one second (FEV_1) and percent of predicted values of FVC [FVC (% predicted)] and FEV_1 [FEV_1 (% predicted)] were measured. Each participant repeated the test at least three times to ensure test reliability and the 'best' record was included in the statistical analysis. Additionally, peak expiratory flow (PEF) and rhinometry test were performed in the chamber study. PEF was measured using a Mini-Wright Peak Flow Meter (MWPFM) with a measuring range of 60–800 L/min. Rhinometry test was measured using acoustic rhinometry and the minimal cross-sectional area between 0–22 mm and between 22–54 mm into the nasal opening, and the volume of the nasal cavity between these distances were recorded (Paper I and II).

Cytokine analysis

Different cytokines were analyzed in our studies: In the chamber study, IL-6, IL-8, intercellular adhesion molecule-1 (ICAM-1) and TNF- α were assessed in both serum and nasal lavage. In the asphalt study, only serum IL-6 and IL-8 were analyzed. Multiplexed magnetic bead-based immunoassays using Luminex XMAP technology on a Bio-plex 200 platform was adopted for analysis according to the instructions from the manufacturer. The results were evaluated in Bio-Plex manager 6.0. Plasma CRP and serum SAA were measured by immunoturbidimetry at the Department of Clinical Chemistry in Lund University Hospital, according to standard protocols. In the chamber study, serum CC16 and SP-D were additionally analyzed in Gothenburg University using commercial ELISA kits from Biovendor, according to the protocols (Paper I and II).

Mitochondrial DNA copy number and Telomere length

DNA was extracted from peripheral blood samples using the Qiagen DNA Blood Midi kit (Qiagen, Heidelberg, Germany). Real-time quantitative PCR was used to determine the relative mitochondrial DNA copy number and telomere length [142, 143]. One reference DNA sample was diluted serially by twofold per dilution to produce five concentrations of 1 – 16 ng/ μ L for the standard curve. The standard curve, samples and one blank (sample with no template) were run in triplicates with 2.5 μ l DNA (4 ng/ μ L) in each reaction. R^2 for each standard curve was >0.99 . Standard deviations of triplicates <0.1 were accepted for the C_t values. SDS 2.4.1 software (Thermo Fisher Scientific) calculated the quantity of the mitochondrial DNA copy number, telomere length and *HBB* for each sample based on the standard curve. The relative mitochondrial DNA copy number was the quotient of the quantity of the mitochondrial DNA copy number and *HBB*. Likewise, the relative telomere length was the quotient of the quantity of telomere length and *HBB*. Both the relative mitochondrial DNA copy number and telomere length are arbitrary values (Paper III and IV).

Mitochondrial DNA methylation

Bisulfite modification was performed on 500 ng of peripheral blood DNA with EZ-96 DNA Methylation-Gold kit (Catalogue number D5008; Zymoresearch, Irvine, CA) according to the manufacturer's instructions. We used 0.6 μ l bisulfite-treated DNA in a 15 μ l PCR reaction using the Pyromark PCR kit (Qiagen) by PyroMark PCR reagents (Qiagen, catalog nr 972807) [31]. Pyrosequencing was performed using PSQ HS96 Pyrosequencing System (Qiagen). The degree of

methylation was expressed as the percentage of methylated cytosines over the sum of methylated and unmethylated cytosines. Three CpG sites from the D-loop region and 1 CpG site from the *MT-TF* gene were measured. An average percentage of the methylation of the D-loop was used for the analysis since the three sites were highly correlated (r_s range 0.71-0.78) (Paper IV).

Statistics

When dependent variables were a continuous variable (i.e. health outcomes such as lung function, cytokines, mtDNAcn and methylation, TL), they were first tested for normal or symmetric distribution. If the distribution was skewed, natural logarithm transformation was performed to approach symmetric distribution. Spearman's correlations were then investigated among various variables including health outcomes and subjects' characteristics and exposure status for data exploration.

The general linear models were used to investigate associations between health outcomes and exposure status. Potential confounders (e.g. age, BMI, variables regarding smoking history, disease history, potential particle exposure, daily diet, and physical activity) were first chosen based on published studies and general knowledge, as well as the results from Spearman's correlations. Then, they were tested one by one in the models. The inclusion criterion of confounders was that only the confounders which changed the β -estimate of exposure status by more than 10% remained in further analyses. In Paper IV, to explore the modifying effects of mtDNA function, interaction terms of mtDNA function markers and exposure status were introduced into the general linear models. Then, data was stratified into subgroups and general linear models were performed in separated subgroups. In Paper I and the nested self-controlled analysis in Paper II and III, repeated measurements were performed in subjects. Absolute changes from baseline were calculated for each individual and each exposure. Then, generalized estimating equation (Paper I) and linear mixed models (Paper II and III) were adopted to analyze the associations.

The residuals from each linear regression model were examined and all showed symmetric distribution. All statistical analyses were completed by SPSS (Version 22.0 or 23.0; IBM SPSS Statistics for Windows, NY, USA).

Main Results

Particle exposure levels (Paper I ~ IV)

In the diesel exhaust chamber study, the exposure level of PM₁ was maintained at around 300µg/m³. Such levels may occur in occupational settings [144], as well as at rush hours in large city centers. In the two field studies, we measured different exposure metrics (table 4). It seemed that respirable dust exposure was higher among the welders than the asphalt workers, which could be due to the working environment. Welders mainly work indoors while asphalt workers work outdoors in large open areas. In the asphalt fumes exposure studies, we additionally measured total airborne PAHs. The concentrations we got from our samples (2.8µg/m³ for conventional asphalt and 2.6µg/m³ for rubber asphalt) were relatively lower than other studies, but much higher than the background exposure (in the general population). The exposure to PAHs from asphalt fumes were also measured as PAH metabolites (1-OH-PYP and 2-OH-PH) in the urine samples. The asphalt workers showed higher pre-working PAH metabolites than the controls, with further increments after working for four days (table 5), but the concentrations were lower than in other studies. Considering the fact that some PAHs are non-threshold carcinogens, the relatively lower exposure may still be associated with adverse effects.

Table 4.

Concentrations (presented as median and maximum in brackets) of various airborne exposures in two field studies included in this thesis.

Paper	Study subjects	Measured exposure metrics	Concentrations
II and III ^a	Asphalt workers	Respirable dust	0.19 (1.33) mg/m ³
		Total dust	0.20 (3.07) mg/m ³
		Total airborne PAHs	2.75 (9.81) µg/m ³
		Nitrosamine	0.07 (3.25) µg/m ³
IV ^b	Welders	Respirable dust	1.1 (8.8) mg/m ³

a. Exposure was only measured in around 40 to 50 paving workers, the exposure concentrations were calculated based upon those workers

b. Exposure were measured in 70 welders and the rest had estimated values. The exposure concentration was calculated based on all welders.

Table 5.

Pre- and post-working concentrations of urinary 1-OH-PYR^a and 2-OH-PH^a in three occupational groups.

Variables	Occupational group	Pre-working (Monday morning)			Post-working (Thursday afternoon)		
		Adjusted mean (5-95% CI) ^c	β (5-95% CI) ^c	p ^c	Adjusted mean (5-95% CI) ^c	β (5-95% CI) ^c	p ^d
1-OH-PYR ($\mu\text{mol/mol}$ creatinine)	Conventional asphalt workers	0.058 (0.049, 0.068)	0.40 (0.21, 0.58)	<0.001	0.10 (0.084, 0.12)	0.96 (0.76, 1.16)	<0.001
	CRM asphalt workers ^b	0.050 (0.040, 0.064)	0.26 (0.01, 0.52)	0.041	0.13 (0.10, 0.16)	1.20 (0.93, 1.47)	<0.001
	Controls	0.039 (0.033, 0.045)	--	--	0.038 (0.032, 0.045)	--	--
2-OH-PH ($\mu\text{mol/mol}$ creatinine)	Conventional asphalt workers	0.17 (0.15, 0.20)	0.40 (0.20, 0.59)	<0.001	0.27 (0.23, 0.32)	0.82 (0.64, 1.00)	<0.001
	CRM asphalt workers ^b	0.17 (0.13, 0.21)	0.35 (0.088, 0.62)	0.009	0.28 (0.22, 0.35)	0.84 (0.59, 1.09)	<0.001
	Controls	0.12 (0.10, 0.14)	--	--	0.12 (0.10, 0.14)	--	--

a 1-OH-PYR: 1-hydroxypyrene; 2-OH-PH: 2-hydroxyphenanthrene.

b CRM asphalt workers: crumb rubber modified asphalt workers

c Adjusted mean, β estimates and p values were derived from general linear regression after adjusting for age, BMI, smoking and snus status, cigarette pack-year.

d P values were derived from related-samples Wilcoxon test by comparing pre- and post-working concentrations in each occupational group.

Particle exposure and symptoms (Paper I and II)

In the chamber study, study subjects reported eye and throat irritation during exposure to diesel exhaust but not during exposure to filtered air. Moreover, we also found that clinical signs of irritation in upper airways (indicated by redness/secretion/swelling in airways) were more common during medical examinations after the diesel exhaust exposure.

Similar to the findings in the chamber study, the asphalt workers also reported discomfort, of which, eye irritation, nasal irritation and coughing were the most commonly reported symptoms. Unexpectedly, the control subjects also reported the same symptoms during our investigation; however, the causes of irritations were different: the control subjects reported irritation due to mowing, while the irritation among the asphalt workers was mainly attributed to the smell from hot-mixed asphalt fumes. Moreover, higher proportions of the asphalt workers than the controls reported that the eye irritation, and possibly also cough and wheeze, were symptoms with onset after entering the current job, indicating that these symptoms are more likely to be asphalt work related.

Particle exposure and change in lung function (Paper I and II)

In the chamber study, divergent trends in PEF changes during exposure to diesel exhaust and filtered air was noted, i.e. increased PEF during filtered air but decreased PEF during diesel exhaust (Figure 7a). The difference became significant at 75 min into exposure. However, this difference in PEF was not significant (but still close to significance threshold) after exposure. Other lung function tests, i.e. spirometry and rhinometry tests did not show any differences after filtered air and diesel exhaust exposure.

In the field study with asphalt workers, we only found decreased lung function after one week of paving in the crumb rubber modified asphalt workers, but not in the conventional asphalt workers (Figure 7b and c). Unexpectedly, a similar decrease in lung function was also found in the controls, thus, no difference of the lung function reduction was shown between the asphalt workers and the controls.

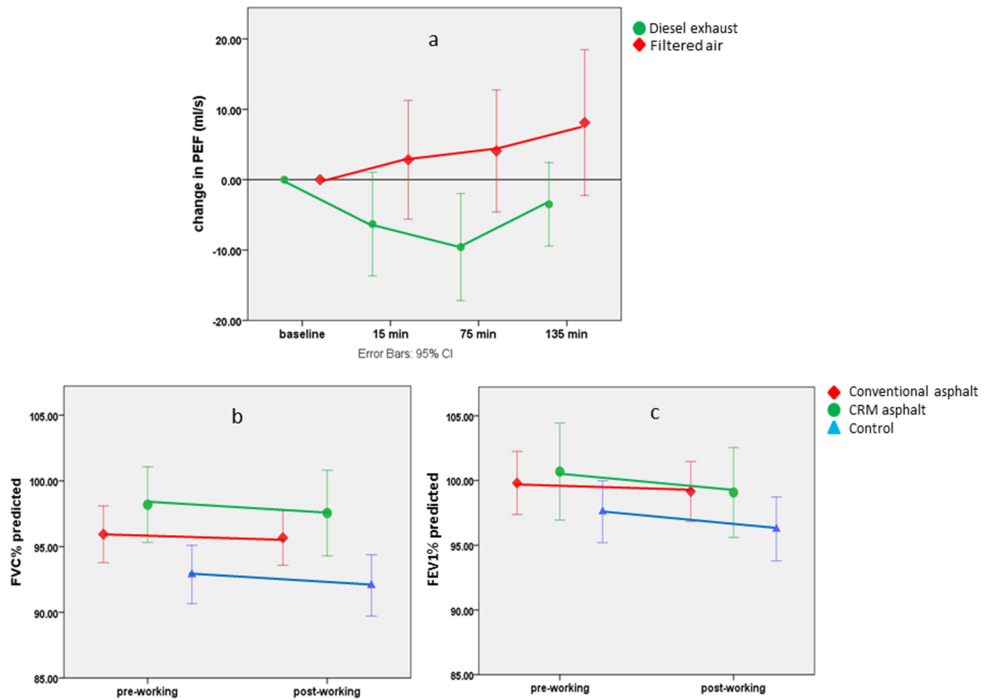


Figure 7: Lung function change in the chamber study (measured as PEF, a) and in the asphalt field study (measured as spirometry, b and c).

(a) PEF decreased in diesel exposure but increased in filtered air exposure with a statistically significantly difference at 75min into exposure. (b and c) FVC% predicted and FEV₁% predicted decreased in CRM asphalt workers and control subjects but not in the conventional asphalt workers after four working days.

Particle exposure and change in inflammatory cytokines (Paper I and II)

In the chamber study, serum IL-6 decreased slightly after diesel exposure but then increased 20 hours after diesel exposure. Together with higher counts in monocyte and leukocyte in peripheral blood 20 hours after diesel exposure, these could be evidence of mild inflammatory response after diesel exhaust exposure. No difference in the change of CRP was found between the two exposures.

In the field study with asphalt workers, the levels of serum IL-6 were too low to analyze. We found similar levels of CRP but higher levels of IL-8 pre-working on Monday morning in the asphalt workers. After four working days, CRP decreased in the control subjects but did not change in the two asphalt working groups; while

IL-8 decreased in both conventional and CRM asphalt workers but did not change in the control subjects.

Particle exposure and change in mitochondrial DNA and telomere length (Paper III and IV)

Mitochondrial DNA copy number was measured and analyzed in both of the two field studies and similar results were found: Both asphalt workers and welders showed higher mitochondrial DNA copy number than their control groups. Moreover, in paper IV, we also measured methylation levels in the D-loop and the *MT-TF* gene of mitochondrial DNA, and lower DNA methylation in both regions were noted in the welders than the control subjects. Higher copy number together with lower methylation levels of mitochondrial DNA in the exposed groups indicated a potential effect of fine and ultrafine particles from asphalt fumes and welding fumes on mitochondrial DNA. Moreover, the mitochondrial DNA copy number was also found to be associated with different exposure metrics. In paper III, it was found to be positively associated with 2-OH-PH in urine. In paper IV, it was found to be positively associated with personal respirable dust among the welders at a relatively moderate exposure level (personal respirable dust above 0.7mg/m^3). Thus, it seemed that not only particle compositions (i.e. PAHs here) but also particles per se are associated with change in the mitochondrial DNA copy number.

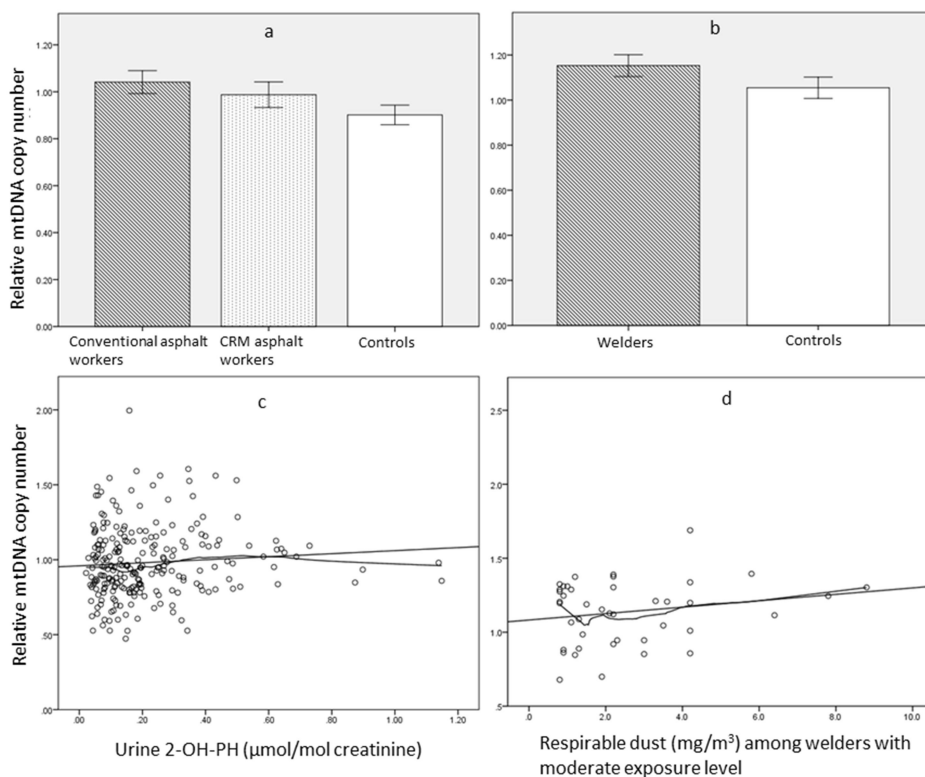


Figure 8: Relative mitochondrial DNA copy number in different occupational groups (a, b) and associations with exposure measurements (c, d)

The copy number was higher in asphalt workers (a) and welders (b) compared to their respective control groups. The copy number is positively associated with urinary 2-OH-PH in the asphalt study (c) and respirable dust in the welder study (d).

Telomere length was also measured in these two studies, however, unlike the mitochondrial DNA copy number, we did not find any differences in telomere length either in the asphalt workers (paper III) or in the welders [143]. We only found positive associations between telomere length and the urinary PAH metabolites 1-OH-PYR and 2-OH-PH among the asphalt workers.

In paper IV, we did an exploratory analysis of the modifying effect of mtDNA. The results showed that mtDNA function can modify the association between welding fumes exposure and an increase in blood pressure. To be more specific, welders with low mtDNA function had higher blood pressure than the control subjects, while no such difference was found in the group with high mtDNA function. Such modification may represent a mitochondria-environment interaction, and may indicate that mtDNA plays a critical role in the etiology of particle-related cardiovascular disease.

Discussion

Does particle exposure affect airway symptoms, lung function and inflammatory cytokines?

In our experimental chamber study with PM₁ exposure (diesel exhaust particles) at around 300µg/m³, clear evidence, both self-reported and from medical examination, has been found for particle induced airway irritation. In the field study with road paving asphalt workers involved, the exposure level of respirable dust (usually refer to PM_{2.5}) was around 190µg/m³ and the workers also reported eye and nasal irritation. Taking these findings together with previous reports [62, 145], it is clear that exposure to fine and ultrafine particles is related to airway symptoms. On the other hand, some co-exposures, especially gas phase pollutants can also irritate the mucous membranes. For instance, the NO_x, benzene and aldehydes from diesel exhaust are well-known respiratory irritants [146, 147].

Regarding the effects on lung function, PEF has been shown to decrease during exposure to diesel exhaust in our chamber study, however, such a decrease was small (but significant) and reversible as soon as the exposure was over. Because of the rapid recovery, we could not identify any changes in the spirometry tests from before and after exposure in this relative short term (3h) exposure. In the field study with continuous exposure, the asphalt workers showed decreased lung function (spirometry test) from pre-working on Monday morning to post-working on Thursday afternoon. Lung function decline in asphalt workers was also reported in other studies [58, 62]. Thus, it seems that short term exposure to particle can cause a transient lung function decline, and cumulative exposure may prolong this transient effect for a longer period and finally causes irreparable lung function damage.

However, neither the chamber study nor the field study found a clear association between particle exposure and inflammatory response. In the chamber study, a mild particle induced inflammatory response was shown by a higher leukocyte count and a slight increment in IL-6 (no change in IL-8). However, in the field study, a decrement in IL-8 after one week of exposure to asphalt fumes was found. The reason behind this disparity might be multi-faceted. First, the exposure levels in these two studies were different, and the asphalt exposure (as particles and

PAHs) was much lower than OELs. Second, the characteristics of diesel exhaust and asphalt fumes were not the same, and they might trigger different reactions in the body. Third, the different time point of measurements and the dynamic changes of inflammatory cytokines made it difficult to reach a conclusion.

Does particle exposure affect mitochondrial DNA function?

In the field studies with welders and asphalt workers involved, we measured mitochondrial DNA copy number as a biomarker for oxidative stress and mitochondrial DNA function. Our results showed that both the welders and the asphalt workers had a higher mitochondrial DNA copy number than their respective control subjects. Mitochondrial DNA has recently been studied in relation to environmental or occupational exposure to particles; however, the results were contradictory. In one study with 63 steel workers who were occupationally exposed to PM₁ at a time-weighted concentration around 9 μg/m³, a higher mitochondrial DNA copy number was noted [115]. However, a lower mitochondrial DNA copy number was found to be associated with exposure to elemental carbon and ambient PM₁₀ at around 120 μg/m³ in truck drivers in Beijing [114]. Furthermore, alteration in mitochondrial DNA content has also been linked to PAHs exposure. Pavanello S *et.al.* found a higher copy number of mitochondrial DNA among coke-oven workers occupationally exposed to PAHs [117]. Kim HY *et.al.* reported an increased mitochondrial DNA copy number in human leukemia-derived cell lines (K562, THP-1, MOLT-4, and HL-60 cells) and bone marrow-derived mesenchymal stem cells after a five-day treatment of pyrene [148].

On one hand, the inconclusive results between the studies might be due to the different size and compositions of particles. On the other hand, different exposure concentrations could be another explanation. A dual influence of oxidative stress on the mitochondrial proliferation has been suggested and illustrated in Figure 9 [115, 149]. At low to moderate oxidative stress, mitochondrial DNA synthesis is stimulated in order to produce more energy for damaged mitochondria disposal and cell survival. However, accumulated oxidative stress can then affect the efficiency of mitochondria, and further increase the rate of ROS production. The vicious circle, in the end, causes mitochondria defect and results in apoptosis and cell death.

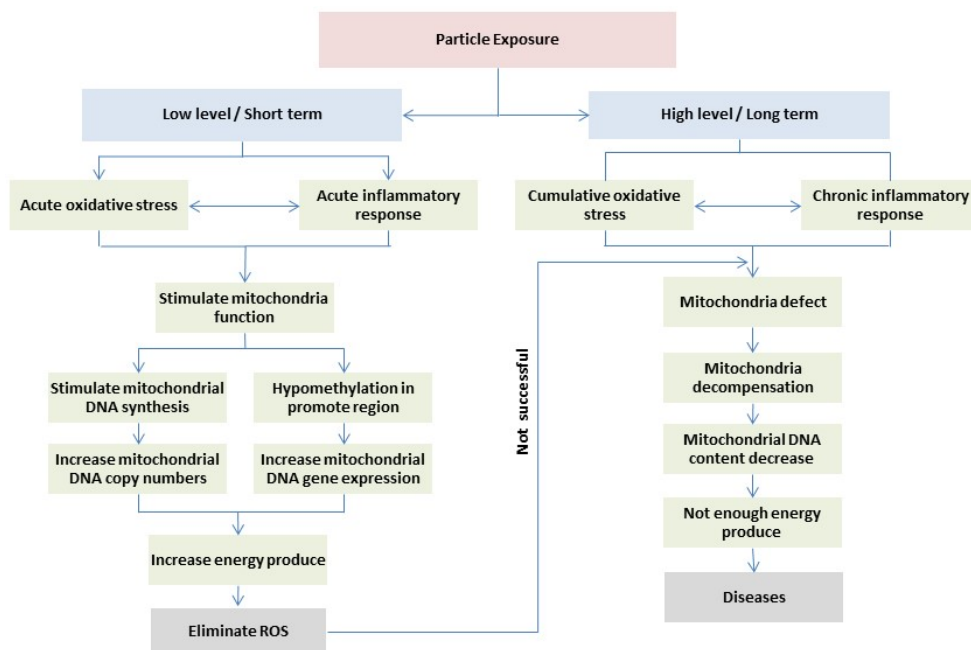


Figure 9: Hypothesized dual influence of oxidative stress on mitochondria

Besides the copy number, we also tried to measure mitochondrial DNA methylation in one field study involving welders and controls. We only picked two regions for measurement: D-loop region and *MT-TF* gene, since D-loop is the control region for the entire mitochondrial DNA transcription and *MT-TF* encodes tRNA, which is essential for protein synthesis. Our result showed lower methylation levels in the mitochondrial DNA D-loop region in the welders, which could be another molecular event related to particle exposure. Classic epigenetic theory suggests that hypermethylated DNA in the promoter region is generally transcriptional silent, while hypomethylated DNA is usually associated with active gene expression. The demethylation of D-loop induced by welding fume exposure might increase the gene expression to maintain normal cellular function to cope with the increased oxidative stress induced by particle exposure. Thinking of the moderate exposure level among the welders (1.1 mg/m^3), both the increase in copy number and the decrease in methylation level in welders can be interpreted as a compensatory response of mitochondrial DNA to particle exposure.

Does particle exposure affect telomere length?

The associations between occupational and environmental exposure and telomere length have been studied to some extent. However, no solid pattern has been shown. In literature, the associations with particle showed inconsistent results: some studies found positive association, while others found negative associations or even no association [137-139]. In our asphalt fumes study, we did not find any association between telomere length and particle exposure. However, we found a positive association between PAHs from asphalt fumes and telomere length. Our finding was inconsistent with other published studies, which mainly reported inverse associations between telomere length and PAHs exposure [140, 141].

The first explanation of the contradictory findings is the dynamic feature of telomere length in peripheral blood. The telomere length measured in blood reflects the mean telomere length of a number of blood cells (except red blood cells which have no nuclei), mostly composed of neutrophils and lymphocytes. These cells have different lifespans and their activity and proliferation rate are not constant, especially during inflammatory responses, which can be caused by external exposures. It has been indicated that telomere length in blood can change substantially within a short period of time (months), which creates a big challenge for cross-sectional studies to reveal a real association [150].

Secondly, aside from the fact that different compounds have different effects on telomere length, the exposure level and time period of exposure are also important in interpreting the effects. As shown in Figure 10, we think that cumulative oxidative stress induced by long term and/or a high level of exposure has a direct effect on DNA, which in turn, accelerates telomere attrition and leads to telomere shortening. Meanwhile, low level and/or short term exposure can increase telomere length in peripheral blood mainly due to an acute inflammatory response, which can induce cell proliferation and in turn increases ‘younger’ white blood cells with longer telomere length into the blood.

Thirdly, the participants in Paper III and IV had a mixtures of exposure in which it is almost impossible to disentangle all the components such as fine particles. The welders were exposed to welding fumes, noise, strong lights, heat, etc. Even the welding fumes themselves contain numerous compounds in their gas and particle phase. The asphalt paving workers might face an even more complicated exposure scenario, where asphalt fumes, noise, diesel exhaust, heat, vibration, etc. can all exist simultaneously. This is one of the problems we faced when performing the studies, and we have to admit that this complexity hindered us from finding the true associations.

Thus, it is rather difficult to draw conclusions on the associations between PAHs or particles and telomere length from the results of our studies. There is still a long way to go to figure out the true effect of exposures on telomere length.

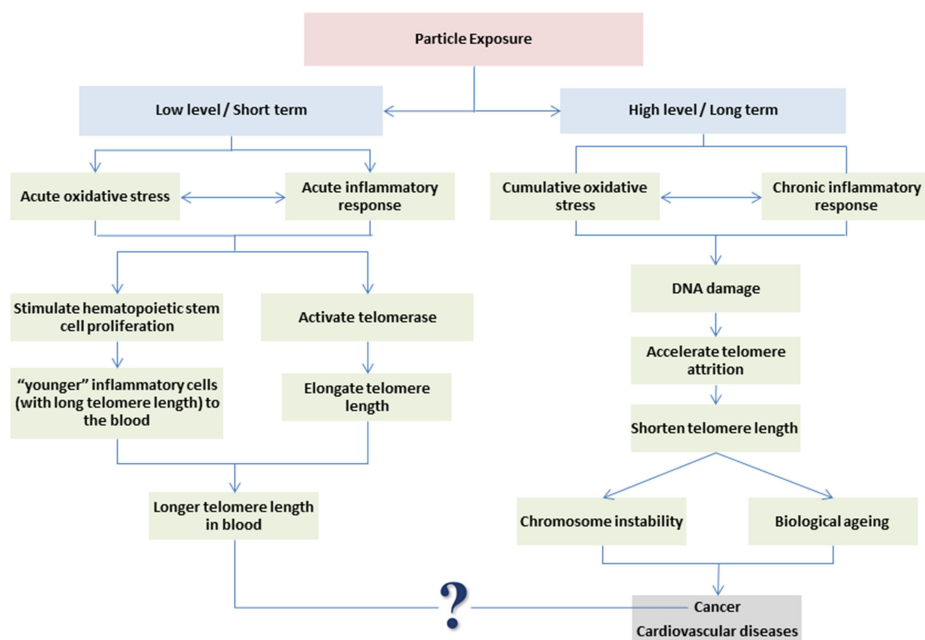


Figure 10: Hypothesized telomere length alternation related to particle exposure

Should we reconsider current OELs based on our findings?

In the chamber study, we carefully controlled dilution to supply; the desired concentration of particles (PM₁) to the chamber was at around 300µg/m³. In the field studies, different exposure metrics were measured according to the occupational exposure scenarios. Table 6 summarizes the exposure levels in the three studies involved in this thesis, and lists the available current exposure limit values accordingly. The exposure limits are mainly presented as threshold limit values (TLVs) set by ACGIH.

It is clear that most of our measured exposure levels in the field were much lower than the current OELs with only a few exceptions: 2 out of 43 asphalt workers had nitrosamine exposure exceeding the limit set in German, and 4 out of 101 welders

had respirable dust exposure exceeding the Swedish occupational exposure limit. These measured levels indicate that the current industries have the ability to keep the exposure lower than the current regulations. On the other hand, airway symptoms, decreased lung function, together with increased mitochondrial DNA content among the workers were observed in the field studies and in the chamber experimental study, where the set exposure levels were also lower than OELs. These findings point out possible airway effects and mild oxidative stress induced by different occupational exposures. Taken together, current occupational exposure levels need to be reconsidered and lowered, in order to protect the workers against the adverse effects. Moreover, proper education should be given to workers so that they realize that OELs do not mean ‘safety level’, and that precautionary measures, with personal protective equipment as the last solution, may be needed even though the exposure levels are below OELs.

Table 6.

The PM₁ and NO₂ concentrations (mean±SD) in the chamber study and concentrations (presented as median and maximum in brackets) of various exposure metrics in the field studies involved in this thesis.

Paper	Study	Measured exposure metrics	Concentrations	Current OELs
I	Chamber study (diesel exhaust)	PM ₁	276±27 µg/m ³ (3h exposure)	Respirable dust: 3mg/m ³ TWA ^c (ACGIH) 5mg/m ³ TWA (Sweden)
		NO ₂	1.3±0.4 ppm	3ppm TWA (ACGIH) 1ppm LLV ^d (Sweden)
II, III ^a	Field study (asphalt fumes)	Respirable dust	0.19 (1.33) mg/m ³	3mg/m ³ TWA (ACGIH) 5mg/m ³ TWA (Sweden)
		Total dust	0.20 (3.07) mg/m ³	10mg/m ³ (inhalable dust) TWA (ACGIH)
		Total PAHs	2.75 (9.81) µg/m ³	200µg/m ³ TWA (ACGIH)
		Nitrosamine	0.07 (3.25) µg/m ³	No OSHA Permissible Exposure Limits or ACGIH Threshold limit values available. 2.5µg/m ³ (German rubber industry)
IV ^b	Field study (welding fumes)	Respirable dust	1.1 (8.8) mg/m ³	3mg/m ³ TWA (ACGIH) 5mg/m ³ TWA (Sweden)

a. exposure were only measured in around 40 to 50 paving workers, the exposure concentration were calculated from those workers

b. exposure were measured in 70 welders and the rest had estimated values. The exposure concentration was calculated based on all welders.

c. TWA: time-weighted average.

d. LLV: level limit value.

What are the strengths and limitations of the thesis?

Exposure measurement

The occupational exposure in real life is complex. Workers are exposed to a mixture of various compounds. For instance, asphalt workers are exposed to not only particles, but also nitrosamines, benzo(a)pyrene, etc. Welders are exposed to metal oxides along with particles. In our study with asphalt workers, although we measured airborne respirable dust, airborne PAHs and nitrosamines in some of the workers, the exposure levels varied greatly due to the outdoor work nature, different types of asphalt mix, and paving temperatures used. Significant variations together with complex outdoor conditions (wind, rain, etc) created difficulties in estimating personal exposure levels for all workers. Thus, we could not draw clear associations between health outcomes and specific compounds. In the study with welders, there was no analysis of chemical composition and metal oxides. Thus, we could not rule out the possibility of contributions from other compounds to observed effects.

Biomarker selection

In our studies, we chose the biomarkers that are hypothesized to be in the causal pathway from particle exposure to cardiovascular/lung disease. The cytokines (IL-6, IL-8, CRP and SAA) are the most common choices when studying inflammatory responses. Mitochondrial DNA and telomere length can be considered as 'early biomarkers' for oxidative stress and DNA damage, because they are less stable than genomic DNA. Given the fact that the workers are in general healthy, the prevalence of DNA damage measured by other biomarkers (e.g. DNA-adducts, micronuclei, strand-break and chromosome aberration) could be too low to gain enough statistical power. Thus, these two biomarkers may be sensitive to relatively low exposure levels and reflect the early stage of body responses to these exposures. These biomarkers also have some drawbacks. The dynamic feature of the biomarkers here is a common issue. For instance, the change of cell proportion in the peripheral blood could influence the measurements of telomere length and mitochondrial DNA. Cytokine levels in serum or plasma may also change during the day independent of exposure. These dynamic patterns increase the difficulty of interpreting the associations, especially from a cross-sectional study design. In addition, mitochondrial DNA and telomere length are recently developed biomarkers, and the disadvantage of new markers is that it is more difficult to understand whether they are linked to a real health risk or not.

Control group selection

It is always critical to choose an appropriate control group. In our study with asphalt workers, we chose the employees working with lawn and garden maintenance as the control group due to similar working characteristic: outdoors, comparable physical workload, road traffic environment and possible exposure to motor exhaust. However, we unfortunately underestimated the irritative organic dust when mowing grass, which may also cause upper airway irritations and influence lung functions. This might, to some extent, obstruct finding a possible true difference between asphalt workers and the control subjects that we were aiming for. In addition, the healthy worker phenomenon is another common concern in studies dealing with occupational exposures. Since the healthy workers are more likely to stay in their current occupations, while the unhealthy ones are more likely to change their jobs; how much the “healthy worker effect” could explain results is usually difficult to assess.

What can we improve in future studies?

Research is a process of rethinking and improving. For the studies dealing with occupational exposure, cross-shift (workers being investigated twice one day) or similar study designs (workers being investigated twice during a continuous working period such as one week or one season) would be better than a cross-sectional study. These study designs offer the possibility to assess acute and/or sub-chronic health effects and have the advantage that workers serve as their own controls because an appropriate reference group is crucial to establish. We also need to be aware that biomarkers have their own pros and cons; no single biomarker is perfect enough and can explain everything. Caution should be taken in biomarker selection at the study design stage as well as during statistical analysis (i.e. adjusting for possible confounding) and in results interpretation. Future studies that include several biomarkers, especially traditional and new biomarkers together, will be interesting. Follow-up studies in experimental models are also needed to support the findings in epidemiological studies. Moreover, appropriate exposure assessments should be performed to truly assess the effects of exposures.

Conclusions

Current occupational exposures to fine and ultrafine particles in asphalt paving and welding industries were low to moderate, however, the relatively low exposure was also associated with airway symptoms, decrement in lung functions and mild inflammatory response.

- Short term exposure to diesel exhaust at PM_{10} concentration around $300\mu\text{g}/\text{m}^3$ induced eye and throat irritation, transient decrease in PEF, as well as mild systemic inflammation, shown as mild change in blood cell counts and IL-6 concentrations after diesel exposure.
- Occupational exposure to asphalt fumes was associated with complaints of airway symptoms, as well as a decrement in lung function after four days of paving. No change in cytokines was found.

Fine and ultrafine particle exposure was associated with mitochondrial DNA alternation, which may indicate a possible oxidative stress induced by such exposure.

- Asphalt workers showed a higher mitochondrial DNA copy number. Mitochondrial DNA copy number was positively associated with PAH metabolites.
- Welders showed a higher mitochondrial DNA copy number and lower mitochondrial DNA methylation, a possible compensatory response of mitochondria under oxidative stress.
- Mitochondrial function modified the effect of particle exposure on blood pressure, but more studies are needed to confirm such modification.

PAH exposure from asphalt fumes was positively associated with telomere length. The underlying mechanism need to be fully studies.

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Air, air, everywhere.

We can't see it, but it's there.

Air, air, why do we care?

Coz sometimes it's no more clear.

Air pollution, especially particulates in the air has been linked to various diseases. Workers in some occupations are at higher exposure than the general population. To better protect workers against adverse effects requires better understanding of health impacts and mechanisms of particle exposure. I hope this thesis can contribute to fill the knowledge gaps to certain extent.

