

# **The Role of Particle Size and Chemical Composition for Health Risks of Exposure to Traffic Related Aerosols - A Review of the Current Literature**

Maria Sehlstedt<sup>1</sup>

Bertil Forsberg<sup>2</sup>

Roger Westerholm<sup>3</sup>

Christoffer Boman<sup>4</sup>

Thomas Sandström<sup>1</sup>

<sup>1</sup> Department of Respiratory Medicine and Allergy, Umeå University Hospital, SE-901 85 Umeå, Sweden

<sup>2</sup> Department of Public Health and Clinical Medicine, Occupational and Environmental Medicine, Umeå University, SE-901 85 Umeå, Sweden

<sup>3</sup> Department of Analytical Chemistry, Arrhenius Laboratory, Stockholm University, SE-106 91 Stockholm, Sweden

<sup>4</sup> Energy Technology and Thermal Process Chemistry, Umeå University, SE-901 87 Umeå, Sweden

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## **1. INTRODUCTION**

Over one hundred epidemiological studies world-wide have demonstrated exposure to ambient PM in urban concentrations to be associated with considerable adverse health effects (WHO, 2006). These include increase in respiratory symptoms, worsening of asthma, chronic obstructive pulmonary disease (COPD), airway infections including pneumonias as well as worsening of cardiovascular diseases like heart attacks and stroke [1]. Additionally, exposure to ambient air pollution has been associated with substantial increases in death from respiratory and cardiovascular causes, which have not been restricted to elderly age groups but may also be observed in children. An increase in infant mortality with particulate matter (PM) air pollution has been demonstrated from several continents [2, 3].

Ambient air pollution levels and proximity to traffic exposure has also been associated with impaired lung growth in children [4, 5].

The European Union has through the European framework program CAFE (Clean Air for Europe) estimated ambient particulate air pollution to be responsible for approximately 350 000 excess deaths in EU annually [6]. A national study estimated the premature deaths in Sweden related to long-range transported anthropogenic particles to be about 3500 deaths per year, and the influence of local particle sources more uncertain due to lack of matching exposure data and risk coefficients, but probably about 1800 deaths or more per year [7]. This means that particles, largely originating from traffic, may cause many more deaths per year in Sweden than traffic accidents do.

Traffic generated air pollution has come much into focus and it is indicated that exposure in traffic situations and its proximity may result in reduced lung growth in children, increase in asthma as well as worsening of respiratory and cardiovascular conditions. Heart attacks have elegantly been shown to occur in substantially increased frequency within an eight hours following traffic exposure [8]. Long distance transport of traffic generated pollution may also contribute to adverse health effects. A major problem has so far been to dissect and evaluate the different air

pollution components in traffic situations. Diesel exhaust has long been indicated to be of importance, but the contribution from the many separate components of traffic related air pollutants have so far not been conclusively addressed. This is a challenge in terms of gaseous components but even more so for the particulate components which range from large and heavy crustal components from sand, windblown dust and road surface down to nano meter sized particles in enormous numbers but relatively low contribution to mass amounts. An inherent difficulty lies in the rough mass measurements in terms of  $PM_{10}$  and the slightly more refined  $PM_{2.5}$ , which have obvious limitations in determining source contributions and relationship to toxic potential in relationship to health effects. A recent EU study “Health Effects of Particles from Motor Engine Exhaust and Ambient Air Pollution” (HEPMEAP) and a systematic review [9] have indicated both fine ( $PM_{2.5}$ ) and coarse particles ( $PM_{2.5}$ - $PM_{10}$ ) to carry potential for causing adverse health effects, with smaller particles appearing slightly more prone for cardiovascular effects and larger more for respiratory.

Major questions arising both within the scientific and stakeholder communities, industry as well as authorities is what risk contributions exist from the different sources in traffic generated air pollution and what detailed components are important. This review is therefore aimed to describe “The State of the Art” concerning the different sources and chemical properties of road traffic related particle emissions and their implications to human health.

## INTRODUKTION

Hundratals studier över världen har uppmärksammat att exponering för luftföroreningspartiklar i omgivningsluften leder till en rad olika hälsoeffekter. Dessa spänner från milda effekter som övergående slemhinnesymtom, via försämring av astmatiker och andra lungsjuka, till uppkomst av hjärtinfarkter, slaganfall och förtida dödsfall. Även uppkomst av kroniska lungsjukdomar och effekter på barn har visats. Lungorna tillväxer sämre hos barn som växer upp i områden med höga halter luftföroreningar och närhet till stark trafik, och det förekommer även en ökad spädbarnsdöd.

Inom EU:s luftvårdsstrategi Clean Air for Europe (CAFÉ) har det beräknats att 350 000 förtida dödsfall per år sker i Europa. I en motsvarande svensk studie beräknar det årliga antalet förtida dödsfall på grund av långdistanstransporterade partiklar till cirka 3500 personer. Partiklar genererade i trafiken har uppskattats leda till cirka 1800 dödsfall per år. Detta innebär att många fler svenskar dör till följd av trafikens föroreningar än i trafikolyckor. Hittills har det varit svårt att bedöma hur partiklar med olika ursprung i trafikmiljön leder till hälsoeffekter på grund av att man i huvudsak endast haft enkla masskoncentrationsmätningar. Det har dock indikerats att effekterna tycks skilja sig bland annat mellan fina avgaspartiklar och grövre vägdamm.

Syftet med denna översikt är därför att beskriva dagens kunskapsläge beträffande olika slags partiklar från vägtrafik, särskilt deras fysikaliska och kemiska egenskaper och betydelse ur hälsorisksynpunkt.

## **2. PM PROPERTIES AND DEFINITIONS WITH RELEVANCE FOR TRAFFIC POLLUTION**

When discussing health effects caused by particulate matter the physical (e.g. particle size and shape) and chemical (e.g. composition, solubility and redox capacity) properties are of vital importance. Particulate pollution from traffic includes the whole particle size range that an atmospheric aerosol normally comprises, i.e. particles sizes from a few nanometers up to approximately 100  $\mu\text{m}$ . Size is the most obvious and important physical particle parameter that governs their behaviour, fate and effects in the environment. All specific properties of aerosol particles depend on particle size, although to a very varying degree [10]. Particle size controls processes like dry deposition in the atmosphere and penetration to indoor environments as well as deposition on surfaces, for example within the respiratory system. Linked to the size is the shape of the particles. Liquid particles are almost always spherical (i.e. droplets) while solid particles may have a more or less complex shape. The shape may be of importance for toxicological responses. Agglomeration of particles may differ in effects as compared with single particles of the same size and there are indications that the physical surface and crystalline structure can account for some of the reactive potential for instance for mineral particles.

Ambient aerosol particles can generally be described in terms of a trimodal size distribution that is related to different formation processes and characteristics (Figure 1). Most important is the difference between particles larger and smaller than 1  $\mu\text{m}$ , since it in principle divides particles formed from gas-to-particle processes and mechanically generated particles. Based on fundamental physical principles, gas-to-particle conversion primarily generates fine (<1  $\mu\text{m}$ ) particles while different kinds of mechanical wear processes generally generate coarse (>1  $\mu\text{m}$ ) particles. Rather often, especially concerning ambient air measurements, the term “fine particles” is used for the size fraction of  $\text{PM}_{2.5}$  (particulate matter with aerodynamic diameter less than 2.5  $\mu\text{m}$ ). The term “ultrafine particles” refers to particles smaller than 0.1  $\mu\text{m}$ .

Combustion engines in cars, trucks etc. are today major sources of fine particle emissions to the atmosphere, often in heavily populated urban environments. In principal all exhaust particles emitted by present fossil fuel driven vehicles are fine particles, in mass dominated by accumulation mode particles but in number often dominated by nanometer sized particles in the ultrafine (or nucleation) mode [11]. Diesel engines have generally been associated with higher particle mass emissions than gasoline fuelled spark ignition engines, as a result of more incomplete operation of the engine with considerable production of carbonaceous soot particles. Considerable reduction of the PM emissions from diesel vehicles, due to optimized engine performance and after treatment measures (i.e. exhaust gas particle filters), have been seen, and the relative importance of gasoline exhaust concerning present air pollution issues has gained increased focus lately. Beside soot particles, traffic exhaust PM generally consists of a vast number of organic compounds that might range from a few percent up to 90% by mass, as well as minor amounts of sulphuric acid and metals [11, 12]. Only a very small fraction (some %) of coarse PM may exist in the exhaust, originating from reentrained agglomerated fine mode particles of soot or unburned fuel and lube oil residues. However, traffic is still a major source also of coarse particles, mainly originating from wear of vehicle components (e.g. brakes and tires) as well as resuspension of road dust [13, 14]. These inorganic coarse particles of crustal material from pavement abrasion are often rich in minerals containing for example silicon (Si), aluminium (Al), potassium (K), sodium (Na) and calcium (Ca) [15, 16], while brake and tire wear particles may contain metals such as copper (Cu), antimony (Sb), lead (Pb), cadmium (Cd) and zinc (Zn) [17-20]. The contribution of coarse particles to ambient PM is however season dependent, especially in countries with cold winter climate. High levels are often found during spring time when studded tyres are wearing off the roadway at the same time as road dust levels are high due to the fact that the gritting used on icy roadways are drying up and thereby is whirling up from the streets [21-25]. Further, it was recently shown by Dahl et al [26] that abrasion from the pavement-tyre interface, can generate also significant amounts of ultrafine particles and it was concluded that these particles presumably originates from the rubber tyre material in the form of mineral oils from softening filler and fragments of carbon-reinforcing filler material (soot agglomerates).

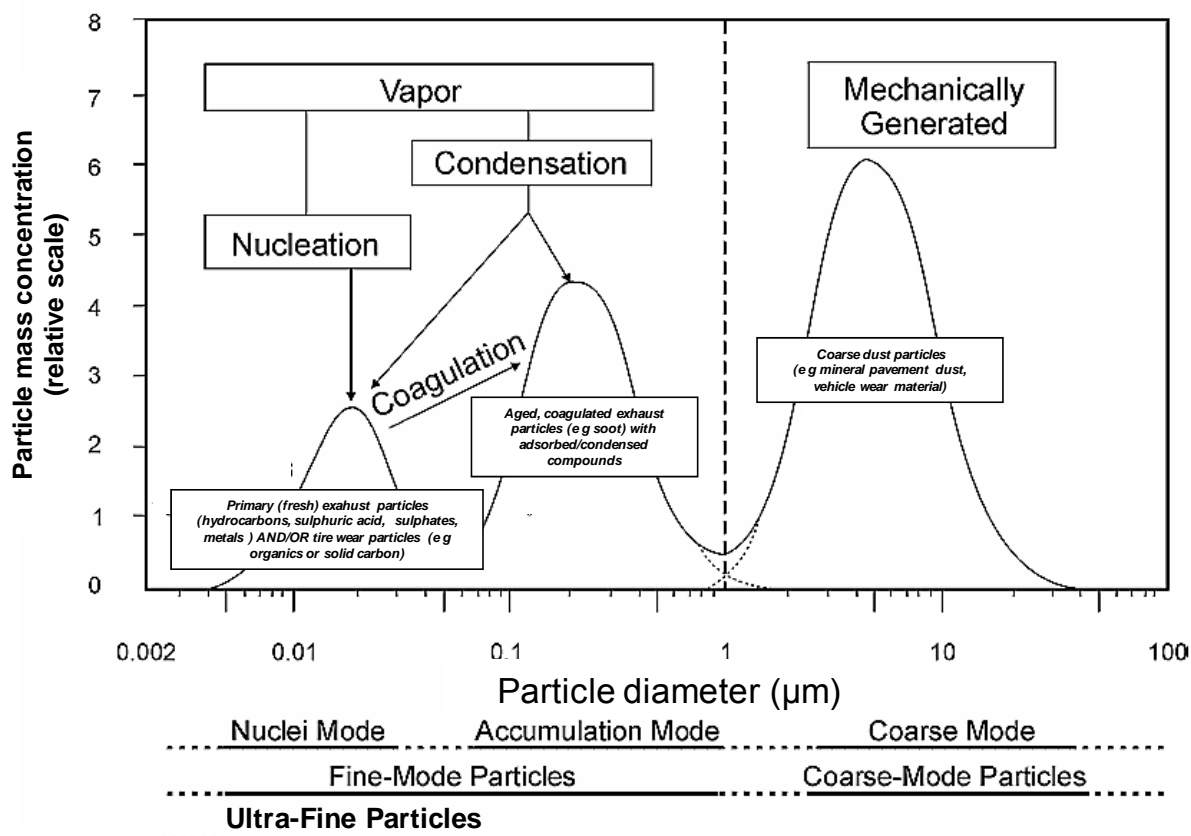


Figure 1. Typical particle size fractionation, formation/transformation processes for ambient aerosols in general from Baron and Willeke (2001) [27], with some additional information from the authors related to sources and composition of motor vehicle traffic related PM emissions.

Thus, traffic aerosol particles do comprise a large variety of physical and chemical characteristics with different behaviour and, most certainly, biological effects during exposure. Several different particle characteristics or specific properties are considered to be important to health responses, i.e. size, mass concentration, number concentration, acidity, solubility, surface area, metals, organic compounds [28, 29]. There are indications of ultrafine particles tending to possess a higher mutagenic and toxic potential than larger particles, which might be partially explained by the enhanced ability of translocation from the respiratory tract to extra pulmonary sites, together with their high number concentration and large surface area [28, 30-33]. However, the comparison of the adverse potential of different size classes is, for real world aerosols, always associated with differences in particle composition, as also stressed in the recent review by [28].

Further, the biological response is often correlated, or at least dependent on the actual dose delivered to the specific target tissue within the respiratory system. In general, smaller particles are more likely to induce adverse health effects than larger ones since they are more prone to be inhaled and also reach deeper into the tissue [33, 34]. Particles larger than 10  $\mu\text{m}$  in diameter are filtered out in the upper airways while inhaled particles that are smaller than 10  $\mu\text{m}$  in diameter, have the ability to penetrate the lung. Particles between approximately 5-8  $\mu\text{m}$  are most likely deposited in the tracheobronchial tree while those between 1-5  $\mu\text{m}$  often are deposited in the respiratory bronchioles and the alveoli [35]. Particles smaller than 1  $\mu\text{m}$  in general, and ultrafine particles more specifically, behaves similar to gas molecules and will therefore penetrate down to the alveoli, deposited by diffusion forces and potentially also translocate further into the cell tissue and circulation system. Their small size, chemical composition, charge and media interactions, high number concentration, and relatively large surface area per unit mass, contributes to their unique characteristics [36-39]. Studies have shown ultrafine particles to have a high capacity to absorb organic molecules and possess the ability to penetrate cellular targets in the lung and systemic circulation.

Concerning the deposition of particles in the respiratory system, the ability of particles to absorb water, i.e. hygroscopicity, in the humid environment of the lungs (RH 99.5%), has a major influence on the deposition pattern during inhalation. For identical dry size distributions, the water vapour uptake, govern by the chemical composition, can result in considerable changed particle deposition (actual dose). It has, for example, been shown by Löndahl et al. [40] that the respiratory-tract-deposited dose of different types of ultra-fine particles can vary substantially, depending on hygroscopicity, exercise level and intersubject variability. A newly developed experimental set-up for respiratory-tract deposition measurements of submicron particles [41] has recently been used for residential biomass combustion aerosols and real traffic pollution related particles [42].

The respiratory tract is working in a number of ways to clear the site from inhaled particles and substances, in order to minimize the damage. Inhaled particles may be

cleared by exhalation, mucociliary clearance and swallowing. If not, ingestion by macrophages which transports the inhaled particles to other areas is another way to clear the respiratory tract, together with dissolution directly into the surrounding tissues and fluids [33].

Thereby, solubility is an important factor of the particles that has to be concerned, since it is known that material that is solved in the respiratory tract have the ability to be transported via the bloodstream to other tissues in the body, and there give rise to different effects [33]. For traffic related aerosols, the question of solubility is mainly related to the presence of submicron particulate sulphuric acid and metal content, since soot and organics in the fine PM as well as mineral matter in the coarse PM have none or very low solubility. There has been a large focus, including a vast number of toxicological and a minor number of epidemiological studies, concerning the influence of chemical form on the toxicity and adverse health potential of particle associated metals. Although some indistinctions and inconsistencies exist, there seem to be a consensus that transition metals (e g Fe, Ni, Cu, V and Zn) can play an important role for some toxicological responses, where the effect is related to their potential for oxidative activity and production of reactive oxygen species (ROS) [28].

As discussed earlier, the particle size is related to the source of generation (i.e. formation processes), the chemical composition is often very different between different size classes. It is therefore rather difficult to discuss the relative effects seen of particles sampled in different environments, only with respect to differences in size. Instead, the composition (i.e. chemical speciation) as well as the particles behaviour and fate, might be more relevant to consider when discussing health effects and toxicology of particulate air pollution. A more detailed discussion of the chemistry of traffic related particle pollution is therefore given in chapter 3 and 4, in the context of toxicology and health effect implications.

### **3. SOURCES, COMPOSITION AND HEALTH RISK IMPLICATIONS**

In this chapter different sources are discussed in relation to their size and chemical properties. Their toxicological potential is described based in experimental cell, animal and human studies. The epidemiological knowledge is given with notions that it has often been difficult to separate health effects related to specific sources in traffic environment.

#### **3.1 VEHICLE EXHAUST**

##### **3.1.1 Exhaust particle characteristics**

Combustion of fuels like diesel, gasoline and biofuels generates mainly CO<sub>2</sub> and H<sub>2</sub>O when oxidized by air within the engine. These are the end products in an ideal combustion process, however very seldom completely achieved in reality. In practical situations, although to a very varying degree, a complex mixture of gaseous and particles is produced, including products of incomplete combustion, like CO, gaseous hydrocarbons, polycyclic aromatic compounds and soot particles. Also elements like nitrogen and sulphur are more or less oxidized during combustion, mainly to NO<sub>x</sub> (NO and NO<sub>2</sub>) and SO<sub>2</sub>. In liquid fuels, only a small fraction consists of incombustible ash forming matter in the form of trace metals, either from the fuel it self or by the lubricating oils or additives used.

It is today well known that exhaust from gasoline and diesel fuelled vehicles are significant sources to inhalable particles in urban environments [13, 43, 44]. These particle emissions are very chemically complex and comprise different particle sizes as well as a wide range of organic and inorganic compounds [12, 44]. Exhaust emissions for vehicles emanating from combustion engines are divided into two groups of exhaust emissions i.e. regulated and un-regulated exhaust emissions. Regulated (by law) exhaust emissions from vehicle are; HC (hydrocarbons), CO, NO<sub>x</sub> and particulates [45]. Unregulated exhaust emissions are defined as

compounds that are not specified by law, consisting of thousands of chemical compounds. However, these may well belong to the group of HC but not as an individual compound. The compounds emitted from vehicles range from gaseous compounds to heavy molecular weight compounds associated to emitted particles.

The focus concerning exhaust particles have so far been on diesel engines since particulate mass emissions from diesel typically are 10-100 times higher than those from gasoline (Otto) engines [11]. From a *qualitative chemical perspective* (chemical identity of emitted compounds), they have relatively similar tailpipe emissions [46], but from a *quantitative perspective* the relative amounts of organic compounds may differ substantially.

Diesel exhaust particles (DEP) mainly consist of submicron fine particles which includes a subgroup of a large number of ultrafine particles (<0.1  $\mu\text{m}$ ). As described earlier and illustrated in Figure 2, the PM mass is often dominated by soot particles in the accumulation mode with typical particle sizes of 50-300 nm, while the number distribution is dominated by ultrafine (primary) particles below 50 nm [11, 44]. The production of soot particles is enhanced during heavy load with fuel rich engine conditions. Soot particles consist of fractal-like agglomerates of primary spherical carbon particles (<20 nm) with more or less adsorbed organic compounds, as well as small amounts of sulphate, nitrate, metals, and other trace elements. A fraction of fuel and lube oil generally escape oxidation in the engine and exist as exhaust PM consisting of volatile or soluble organic compounds. This soluble organic fraction (SOF), which includes polycyclic aromatic compounds (PAH) containing oxygen, nitrogen and sulphur [47], may range from 10 to 90% of the PM mass depending of fuel, engine design and operation. During low load and idling conditions the production of soot is low and the fraction of volatile unburned fuel and lube oil often dominates the exhaust PM mass [11].

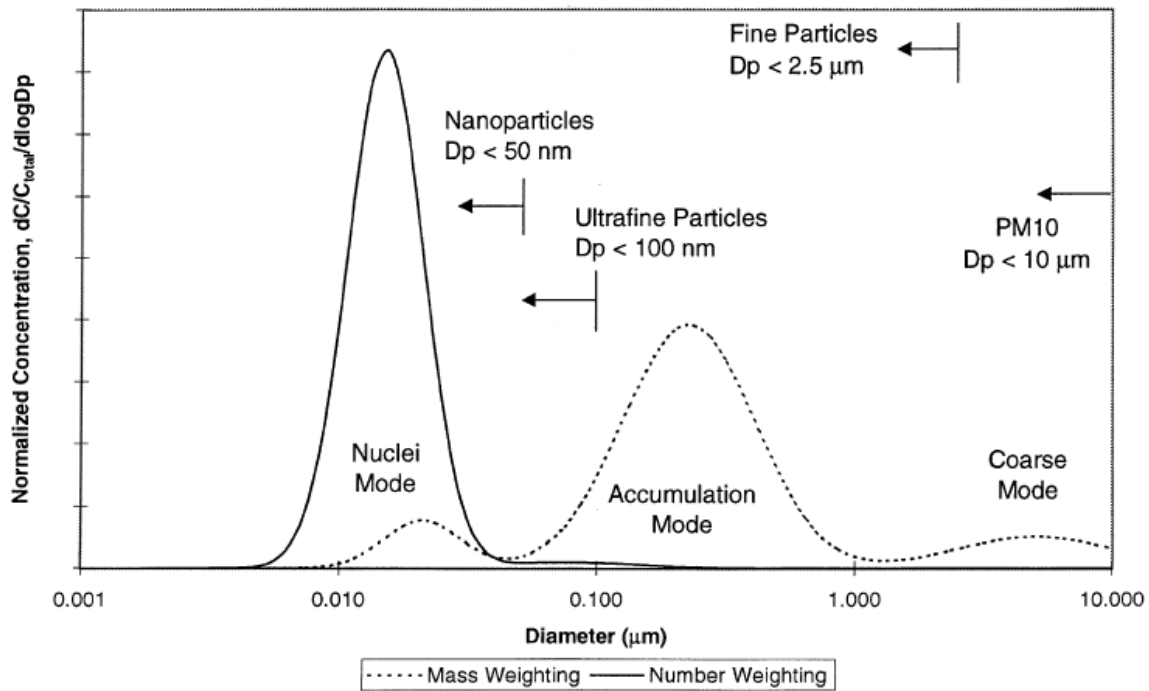


Figure 2. Idealized diesel exhaust particle number and mass weighted size distributions, published by Kittelson (1998) [11].

The ultrafine particles within the nucleation mode mainly comprise volatile organic material and sulphur compounds, either as solid particles or droplets up to 50 nm in size. Since measurements in this mode are very sensitive to temperature, and dilution repeatability in PM size measurements could be challenging. The sulphur content in the fuel is of vital importance for the formation and emission of these ultrafine particles. During combustion, most of the sulphur in the fuel is oxidized to  $\text{SO}_2$  but a small fraction is further oxidized to  $\text{SO}_3$ . During cooling of the exhaust the  $\text{SO}_3$  can react further with water vapour that leads to formation of sulphuric acid that, in supersaturated conditions during cooling, subsequently can form new nanometer-sized particles very rich in number [11, 12] Accordingly, the sulphur content is of great importance, and it was for example shown recently that low sulphur fuels (< 10 ppm S) did not generate such ultrafine sulphuric acid particles during medium and high speed runs with a diesel passenger car [48]. The details of the dilution and cooling processes of the exhaust determine the relative amounts of material that adsorb or condense onto existing particles and nucleate to form new particles. A schematic illustration of the condensation and nucleation processes during diesel exhaust dilution and cooling, given by Sheer et al, 2005 [49], is shown in Figure 3.

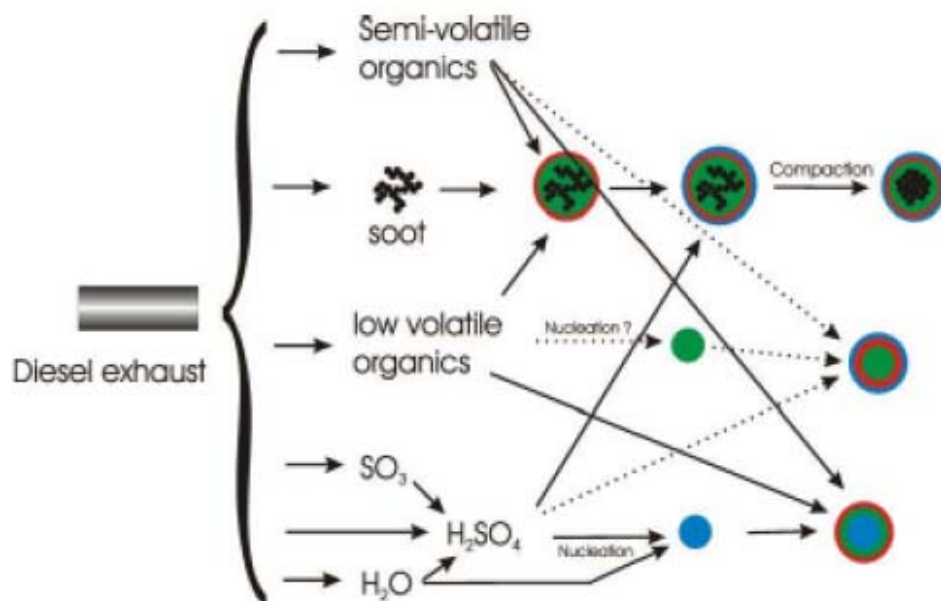


Figure 3. Schematic illustration of the condensation and nucleation processes during diesel exhaust dilution and cooling taken from [49]. Blue: Volatile (H<sub>2</sub>SO<sub>4</sub>); Red: Semi-volatile organics (unburned fuel); Green: Low-volatile organics (lubricating oil).

The relative intensity (amount emitted per driving distance) between the individual chemical compounds are strongly dependent on parameters such as type of combustion engines (Diesel or Otto) [46, 50], engine torque and load, engine wear [51], engine lubrication oil, engine development [52], exhaust after treatment technology [46, 50, 52], fuel additives and selection of fuel combusted (diesel, gasoline and alternative fuels) [46, 50, 53, 54], ambient starting temperatures [55]. Due to this fact, it is very difficult to identify specific individual marker compounds for specific engine/fuel combustion concepts in ambient air.

As mentioned earlier, the content of organics in the PM may range significantly. Many of these semivolatile or particle bound compounds are known to have mutagenic and carcinogenic properties. For example, PAHs, nitro-PAHs, and oxidized PAH derivatives are present on the diesel particles, with the PAHs and their derivatives comprising about 1% or less of the diesel exhaust particle mass. Further, it has been shown that the emission characteristics differ between on-road and off road engines, since off road engines generally are of older technology. The mass of particles and organics emitted from on-road diesel engines have been reduced over the years.

Available data for on-road engines indicate that toxicologically relevant organic components of the emissions (e.g. PAHs and nitro-PAHs) emitted from older vehicle engines are still present in emissions from newer engines, though the amounts have decreased [44]. The US Environmental Protection Agency (EPA) has estimated that approximately more than 20 000 individual chemical compounds are emitted from diesel fuelled vehicles. Of those compounds are approximately 500 positively/tentatively identified in the scientific literature [56]. This means that more than 97 % of the estimated number of compounds emitted from diesel vehicles still remains unknown. Much work has been performed concerning exhaust PM formation and characteristics and the main formation processes as well as physical and chemical particle characteristics are today rather well known. Since most focus has been on diesel engines, the knowledge of gasoline engine particle emissions as well as new engine technology and alternative fuels is scarcer. In many aspects, a detailed and fundamental understanding of the formation mechanisms and properties of exhaust particles still remain to be elucidated, not at least important in order to be able to assess implications on human health of future technology and fuels.

### **3.1.2 Diesel exhaust and health risks**

#### ***3.1.2.1 Toxicology***

##### ***Lung effects***

Health effects of diesel engine exhaust has been investigated in a series of studies in Sweden, where human subjects have been exposed in an experimental chamber. This has been achieved by use of a validated exposure chamber system which allows for controlled and steady concentrations of diluted diesel exhaust [57]. The range of health effects investigated range from effects from the airways and lungs, the blood, blood vessels, the heart and the brain [57-68]. Following inhalation diesel engine exhaust causes a bronchoconstrictive response reflected in increased airway resistance in both healthy and asthmatic subjects [63]. This is associated with a pronounced airway inflammatory response which is mainly located to the conducting

airways and to lesser degrees the alveolar compartment, as reflected by bronchial mucosal biopsies and analysis of different aliquots of sampled bronchoalveolar lavage fluid. It has been indicated that the oxidative potential by the diesel exhaust may trigger inflammatory events. This is mediated by oxidative stress sensitive transcription factors such as NF $\kappa$ B and AP-1 and involves mitogen activated protein-kinases (MAPK). This controls a range of neutrophil chemo attractants such as IL-8 and Gro- $\alpha$  resulting in a neutrophilic infiltration into the airway mucosa and airway lumen. Neutrophils secrete the secondary oxidative enzyme myeloperoxidase (MPO), which may add to tissue damage. Other inflammatory cells involved are CD-4 and CD-8 + T-cells and mast cells.

Time course investigations studying effects from a PM<sub>10</sub> concentration of 100 and 300  $\mu\text{g}/\text{m}^3$  suggest a highly established airway inflammation in human airways six hours after a higher dose of diesel engine exhaust particles. Exposure to lower concentrations results in a slower onset of inflammation where the extent and amplitude at 18 hours correspond to that at six hours after the higher (300  $\mu\text{g}/\text{m}^3$ ) concentration. This notion may be of importance for epidemiological studies if time course of events may be highly influenced by the triggering diesel concentrations [64, 68, 69].

A vast number of animal investigations have studied whether diesel particle exposure of the lungs would enhance allergic responses. Most of the studies have investigated intratracheally instilled particles but some have used models where the animals have been inhaling the particles which are the more normal route. Sensitization has often been done with ovalbumin injections but in some studies local allergens like Red cedar has been used. A considerable series of studies have demonstrated diesel engine exhaust particles to result in increased sensitization to allergen in various allergen models such as mice and rats. Increased levels of Th:2 cytokines like IL-4, IL-5, GM-CSF have been demonstrated in association with increased IgE-levels. The majority of the studies have been performed with stored and aged diesel particles rather than fresh exhaust [70]. Many of the early studies were able to demonstrate an increased anaphylactic response after administration of diesel in diesel exhaust particles in sensitized animals. More recent studies have often investigated airway inflammation and bronchial hyperresponsiveness which are features more in line with

asthma. Together, a vast number of studies have indicated diesel exhaust particles to enhance allergic responses [70-72]. An interesting set of nasal instillation studies with diesel particles have been performed by Diaz Sanchez and co-workers in the US [73, 74]. They have been able to demonstrate that diesel particles instilled in high doses in the nose enhance the allergic responses, both as regards enhancing the allergic response per se but also considering primary sensitization. This indicates that diesel particles may actually turn individuals from non-allergic states to an allergic. Recently the diesel inhalation experiments in human subjects in Umeå have given complementary data by demonstrating perfectly non-atopic individuals to actually demonstrate a Th2 response in the airways after diesel exposure in terms of enhanced expression of IL-13, a key cytokine involved in allergic responses [62]. Furthermore asthmatic individuals have been investigated. Certain differences had been displayed as compared to the inflammatory response in non-asthmatic health individuals in terms of cytokine response. A four-fold increase in IL-10 was demonstrated in bronchial mucosal biopsies of asthmatic subjects as compared with the reduction by half in biopsies from healthy volunteers [69]. No enhancement of the pre-existing asthmatic inflammation involving increased numbers of eosinophils and mast cells was seen. Most interesting is the physiological endpoint reached the day after exposure. In a group of asthmatic individuals treated with the anti-inflammatory mainstay treatment of inhaled corticosteroids (average 1200 µg per day) in Swedish asthmatic subjects, this treatment was inefficient in preventing the subjects from experiencing a worsening of their bronchial hyperresponsiveness, a cardinal symptom in asthma [61]. The metacholine PC<sub>20</sub> of the individuals decreased from 3.4 mg to 1.7 mg representing a doubling of bronchial hyperresponsiveness, which is a clinically significant affect. This may well explain why asthmatic subjects experience exacerbations after exposure to traffic related air pollution. The local inflammatory events and functional change in the airway smooth muscle that are responsible for these asthmatic responses demand further studies to clarify.

It has been shown that repeated low-dose exposure to DEP may result in down regulation of T cell-mediated immune responses, while a single high-dose exposure has the ability to aggravate bacterial infection and trigger T cell-mediated immunity. This might indicate that the inflammatory response following diesel exposure can affect the susceptibility to respiratory viral and bacterial infections [75, 76].

### **Cardiovascular effects**

There are now a considerable number of animal studies which support the notion from epidemiological studies that air pollution causes adverse cardiovascular effects. Increased thrombotic events have been demonstrated in hamsters 24 hours after intratracheal instillation of diesel exhaust particles [77]. The events were accompanied by a bronchoalveolar inflammation with increase in histamine and it was indicated that pre-treatment with systemic corticosteroids inhibited the airway inflammation thrombogenicity and histamine release. Diesel exhaust particles have been demonstrated to increase adverse cardiovascular events. Intratracheal instillation of diesel exhaust particles has been shown to increase the risk for arrhythmias in the aftermath of coronary ischemia-reperfusion when investigated 24 and 48 hours after DEP-instillation. Serious ventricular arrhythmias also occurred [78].

While ventricular arrhythmias have so far only been reported in one study following intratracheal instillation of diesel exhaust particle in rats [78], this has more commonly been associated with exposure with particulates including metals such as residual oil fly ash (ROFA). Wellenius and co-workers demonstrated ROFA to increase ventricular arrhythmias in myocardial and function model in rat associated with the increase in heart rate variability. In another model Kang et al demonstrated intratracheal instillation by PM 2.5 to increase serum endothelin concentrations and type A myocardial endothelin receptors in rats in an experimental myocardial infarction model [79, 80]. The relationship between ventricular arrhythmias and decrease in heart rate variability (HRV) has recently been investigated in an animal model. Healthy and chronic ischemic heart failure Wistar rats were exposed to diesel exhaust during three hours. A decrease in heart rate variability returned to baseline within 2.5 hours after exposure start. The ventricular pro-arrhythmic effects persisted as late as five hours after end of the exposure. At least in this animal model, reduced HRV was not indicated to be predictive of ventricular arrhythmia [81].

Enhanced ischemia – reperfusion injury in rodents is not restricted to diesel exhaust but also enhanced by other pollutants such as ultrafine particulate matter to which

diesel exhaust is an important contributor [82]. Cozzi and co-workers demonstrated intratracheal instillation of ultrafine particles to double the size of myocardial infarction and also induce an inflammatory response associated with increased oxidative stress also in the heart tissue.

The genetic variant in lipoprotein metabolism, the ApoE<sup>-/-</sup> mouse, has been demonstrated to be sensitive for development of cardiovascular disease. The animal model has been used for demonstrating ECG abnormalities during exposure to whole diesel exhaust emissions related to coronary ischemia [83]. Interestingly the effects remained after filtering of the diesel exhaust with a non-catalytic ceramic particle filter, and were therefore interpreted to be related to the nonparticulate components of the exhaust.

During the last few years a series of experiments using the Umeå exposure chamber have explored the effects of diesel engine exhaust on the cardiovascular system. The onset and time course have been determined in relationship to the cardiovascular effects described in certain well designed epidemiological studies. Peters and colleagues presented a much cited study where the exposure to traffic during the hours preceding a myocardial infarction was evaluated [8]. It was demonstrated that 1-2 and 6-8 hours before arriving to the emergency room with the myocardial infarction there was a high frequency of exposure to traffic situations such as walking or spending time curbside, cycling or being exposed in a bus or a car. With this as a background together with the data from the human bronchoscopy studies the first investigation set out to explore cardiovascular events during the first six hours. Using a bilateral forearm plethysmography with arterial needles in the brachial arteries and infusion of phase active drugs followed by venous sampling in healthy subjects the mechanisms behind the vascular effects started to appear. First of all a disturbance in the vasodilatory response was demonstrated indicating that the blood vessels were not able to dilate in demand of a stress, which could be crucial in the situation of a highly constricted artery especially if a plaque rupture have occurred. In such a situation the main factor preventing a thrombus formation which may totally clog an artery and lead to a stroke or myocardial infarction, would be the release of tissue plasminogen activator (t-PA), the main component from the endothelium which prevents a clot into occur. There is a continuous release of t-PA from the endothelium

all through the life which balances the activity of the coagulation in the blood. In this first study it was demonstrated that a mean reduction in t-PA was present six hours after diesel exhaust exposure [59]. A subsequent study investigated the time course of the vascular effects by dilute diesel engine exhaust in healthy subjects. It was demonstrated that the disturbed vasomotor response with reduced dilatory ability had occurred as early as 1.5 hours after exposure. While the majority of the effects had ceased at 24 hours it still remained a certain disturbance in vasodilatory response as reflected in reduced dilatation to infusion of acetylcholine [66].

Most recently, subjects with established cardiovascular disease were investigated. The individuals were treated with percutaneous intervention (PCI, "balloon-dilatation") against coronary stenosis and were on full preventive medication [58]. All were asymptomatic with a totally normal treadmill ECG:s and fully asymptomatic. It was demonstrated that those individuals that already have an advanced vascular disease had blood vessels which would not further dilate during infusion of vasal active drugs. In similarity with the healthy subjects, they experienced a substantially reduced release from the endothelium of the anti thrombotic component t-PA. Furthermore they had a systematic suppression of the ST segment during exercise indicating an increased ischemic burden. The data are in full support of the mentioned epidemiological findings by Peters and co-workers with several factors and biological events occurring during and after exposure to diesel exhaust in the traffic situation.

### ***Mutagenic/ carcinogenic effects***

It is clear that exposure to diesel exhaust produces lung tumours in rats and it is classified as probable carcinogenic to humans. These neoplasms may be caused by the particle fraction of the exhaust [84-86]. Due to the findings that TiO<sub>2</sub> and carbon black particles, with no genotoxic compounds adsorbed on them (i.e. "clean" particles only) give rise to lung cancer in rats [87-89] the interest in particles as particles only has increased. Important particle parameters in general are size, number, surface area and chemical composition. The experiments of Heinrich and co-workers [90] indicate that particle size is a very important parameter. The Institute of Environmental Medicine (Karolinska Institute, Sweden) has published a report which concludes that it was not possible to decide if it is a non-specific particle factor or a

direct genotoxic effect of material adsorbed on the particles, which is responsible for causing lung cancer [86].

### **3.1.2.2. Epidemiology**

There are indications from numerous studies that traffic air pollution gives adverse respiratory and cardiovascular effects, as earlier delineated. The composition of the vehicle fleet is often not well known in epidemiological studies, and they may include 10-20 years of observations. In addition, the measured indicators are not totally specific for either diesel or gasoline exhaust. PM<sub>2.5</sub> has at least for mortality often given stronger associations than PM<sub>10</sub> which also includes larger particle components like road dust and wind blown dust with soil, sand and biological material. NO<sub>2</sub> is often regarded as a marker of combustion from traffic and is highly correlated to particle exhaust emissions. Elemental carbon has in some studies been used as indirect indicators for diesel emissions since they emit more of fine and ultrafine combustion particles. This parameter is, however, often lacking and it may thus be difficult in epidemiological studies to separate diesel from gasoline effects. Some studies have been designed to investigate health effects in situations where diesel trucks have been abundant and this approach has given some important findings. The data are consistent with earlier studies where effects of diesel exhaust in confined spaces, such as ferries and roll-on roll-off ships was studied [91]. Less has been done to try to separate traffic situations to which diesel engines would not be a major contributor to exhaust particle mass and particle numbers.

Below are some of the studies which have tried to study effects in traffic where diesel trucks have been especially common.

There is first of all several European time-series studies that indicate stronger effects on mortality from particles when traffic is a major source, according to the levels of NO<sub>2</sub> in 29 European cities [92, 93] or stronger effects among those living along busy streets [94].

In a Dutch study lung function was associated with truck traffic density (diesel vehicles) but had a lesser association with automobile traffic density [95]. This relation was stronger in children living close (< 300 m) to the motorways. Lung function was also associated with the concentration of black smoke levels inside the schools, seen as a proxy for diesel exhaust exposure. These results indicate that exposure to traffic-related air pollution, in particular diesel exhaust particles, may lead to reduced lung function in children living near major motorways. Chronic respiratory symptoms reported a questionnaire were as well as doctor-diagnosed asthma significantly more often reported for children living within 100 m from the freeway [96]. Truck traffic intensity and the concentration of black smoke measured in schools were found to be significantly associated with chronic respiratory symptoms.

In a related Dutch study respiratory symptoms were collected by parent-completed questionnaires [97]. Sensitization to common allergens was measured by skin prick tests and serum immunoglobulin E. Bronchial hyperresponsiveness was also measured. Symptom prevalence was increased near motorways with high truck but not high car traffic counts. They were also related to air pollutants that increased near motorways with high truck traffic counts. Sensitization to pollen increased in relation to truck traffic counts but not car traffic counts. Elemental carbon (EC), an indicator of combustion particles and especially diesel exhaust, was as many other indicators significantly associated with cardiovascular mortality but not total mortality in a time-series study from Phoenix [98].

In a recent investigation from the Children's Health Study in California it was demonstrated that children living in the close vicinity of major roads had much worse growth of their lungs as compared with children living further away, even when tobacco smoking and socioeconomic status in the households was taken into account. Living close to a major road was a stronger factor than ambient air pollution in the area. Furthermore, elemental carbon was more closely related to impaired lung growth than PM<sub>2.5</sub> and PM<sub>10</sub> which was interpreted by the authors as diesel exhaust potentially being a major driver behind the effects [4].

### **3.1.3 Gasoline exhaust and health risks**

#### **3.1.3.1 Toxicology**

The effect of gasoline exhaust has been little studied so far. No human experimental studies have been reported and only most recently scientists from Lovelace Respiratory Research Institute in Albuquerque (New Mexico, USA) have reported on effects in animal models. The investigators studied effects of gasoline engine emissions with resuspended paved road dust in a mouse model of coronary insufficiency. Apolipoprotein E (ApoE)<sup>-/-</sup> mice were exposed to gasoline emissions or paved road dust for six hours per day for three days. Electrocardiograms demonstrated the T-wave area to significantly deviate from basal conditions after exposure to gasoline exhausted particles, but not after exposure to road dust or filtered gasoline emissions. The gasoline exposed mice demonstrated elevated plasma endothelin-1, but no systemic inflammation. The study shows interesting similarities with the ischemic effects shown in human subjects exposed to fresh diesel engine exhaust [99]. In a more recent animal study, ApoE<sup>-/-</sup> mice were exposed to either whole gasoline exhaust, including particulate matter, or filtered exhaust with only gases remaining. The authors were able to demonstrate and perform detailed characterisation of the vascular events involved in arteriosclerosis by analysing mice aorta samples. Increased levels of endothelin1, hemoxygenase-1, MMP3, 7, 9 and TIMP2 were seen. Important to note is that the effects demonstrated were not due to the particulate matter in the emissions, since animal exposed to filtered exhaust demonstrated similar effects.

A recent engine study investigated the pro-inflammatory and anti-inflammatory cytokine balance in healthy male Swiss mice exposed to unleaded gasoline exhaust. The animals were exposed in an inhalation exposure chamber for 7, 14 and 21 days. TNF- $\alpha$  and IL-6 were increased in bronchoalveolar lavage fluid with IL-1 $\beta$  and IL-10 unaffected. Gasoline exhaust increased alkaline phosphatase and lactate dehydrogenase, the latter indicating tissue damage. Total protein increased after 14 and 21 days indicated increased alveolar capillary permeability. The histopathological changes after gasoline exhaust included perivascular and peribronchial accumulation

of mononuclear cells, polymorphonuclear cells, alveolar thickening and mild alveolar oedematous changes indicating inflammation.

### **3.1.3.2 Epidemiology**

As mentioned above it is often difficult to separate diesel and gasoline exhaust effects. Studies which especially focused on diesel rich environments are given above. Below is an overview of epidemiological studies which commonly contain mixed traffic situations.

In a study of persons from Los Angeles County [100] health data from the famous ACS Cancer Prevention II survey was used only for metropolitan LA at the zip code-area scale. The authors interpolated PM<sub>2.5</sub> data from 23 state and local district monitoring stations in the LA basin for the year 2000 to derive exposure assessments. They were able to assign exposure to 267 zip code areas with a total of 22 905 persons. Among these subjects enrolled in 1982, 5856 had died during the follow up to 2000. For all-cause mortality they found with adjustments for 44 individual confounders a relative risk of 1.17 per 10 µg/m<sup>3</sup> (95% CI = 1.05–1.30). These results suggest that the chronic health effects associated with intraurban gradients in exposure to PM<sub>2.5</sub> are larger than previously reported associations across metropolitan areas in the ACS cohort. A direct comparison with the previous ACS results show effects that are nearly 3 times larger than in models relying on between-community exposure contrasts. It appears likely that the within-city contrast are more dependent on traffic emissions than the previously used between-community gradients to a large extent driven by sulphates related to SO<sub>2</sub> emissions from coal and oil burning. The findings from this study from Los Angeles are in line with recent evidence suggesting that intraurban exposure gradients may be associated with even larger health effects than reported in interurban studies. For example, in the Netherlands a doubling of cardiopulmonary mortality (RR =1.95; 95% CI 1.09 –3.52) was found for persons living near major roads [101].

In similarity with the mentioned short-term studies from Europe, there are also several studies from the U.S. that in cities with mostly gasoline cars indicate larger

effects for PM originating from traffic than other sources. In a reanalysis of NMMAPS (National Morbidity, Mortality and Air Pollution Study) hierarchical modelling was used to deal with measurement error bias [102]. The greater effects then seen for CO may according to the authors reflect a true association with CO per se, or that CO is a surrogate for traffic particles, which suggests that attention should be focused on traffic pollution.

In a study from Atlanta emergency department cardiovascular visits from 1993-2000 were compiled from 31 hospitals and analyzed in relation to air quality data including criteria pollutants, as well as detailed measurements of mass concentrations for the fine and coarse fractions of PM and several physical and chemical characteristics of PM for the final 25 months of the study [103]. Cardiovascular disease (CVD) visits were associated with NO<sub>2</sub>, CO, PM<sub>2.5</sub>, organic carbon, elemental carbon, and oxygenated hydrocarbons. Secondary analyses suggested that these associations tended to be strongest with same-day pollution levels. CVD visits were associated with several particle measures (PM<sub>2.5</sub>, organic carbon, and elemental carbon) and gases (CO, NO<sub>2</sub>, and oxygenated hydrocarbons) that are characteristics of traffic-related pollution.

In a meta-analysis of daily mortality in “The Harvard Six Cities” the elemental composition of size-fractionated particles was used to identify source-related fractions of fine particles [104]. In the combined analysis, PM<sub>2.5</sub> from mobile sources had the strongest effect on daily mortality.

Daily mortality in three New Jersey cities was investigated after a factor-analysis had resolved source-related factors [105]. Statistically significant associations were found between mortality and the sources oil burning, industry (Zn/Cd), sulphate and motor vehicles, while PM from geological sources was not significant.

Mar et al (2000) [106] used gases and detailed PM composition data in a time-series analysis of daily mortality in Phoenix Total mortality was significantly associated with indicators for traffic exhaust (CO and NO<sub>2</sub>) and cardiovascular mortality with combustion-related pollutants in general and secondary aerosols (sulphates). In a later analysis of the apportioned anthropogenic PM<sub>2.5</sub> source categories the sources

with the largest cardiovascular mortality effect size were secondary sulphate and traffic [98]. For total mortality, the associations were weaker.

An examination of reported associations between daily mortality and source investigators' estimated source-apportioned PM<sub>2.5</sub> for Washington, DC for 1988-1997 found risk estimates for traffic-related PM<sub>2.5</sub> significant in some cases but more variable than the estimates for secondary sulphate and oil-burning [107].

Some epidemiological studies, including investigations from Stockholm, has linked traffic associated air pollution with cancer [108]. The major component correlating with cancer incidence was nitrogen dioxide, but since there is no toxicological support for this gaseous pollutant to be able to exhibit toxicological effects unless concentrations are extremely high, it is often viewed as a marker for other traffic related pollutants. The study was therefore not able to distinguish what specific components from traffic that was responsible for the carcinogenic effects. Toxicological data indicate organic compounds to be the most likely suspects.

Recently lung growth in Southern Californian children has been shown to be reduced for children living close to major roads [4]. The effect was linked to heavy vehicle traffic reflected by elemental carbon, and is estimated to predispose the children for future ill health.

In summary there is substantial evidence that traffic exhaust gives major health effects but it is often difficult to separate gasoline and diesel exhaust effects in epidemiological studies. Toxicological studies on the other hand give very strong evidence for toxic capacity for diesel exhaust, while only a handful studies have investigated and shown this for gasoline. It is therefore of apparent interest to study gasoline exhaust effects further.

### **3.1.4 Alternative fuels**

In the light of issues like *climate change* and *fuel supply security* the need for alternative and renewable fuels is an emerging challenge globally. In Sweden today, less than one percent of the fuel market is biofuels, although relatively high compared to the rest of Europe [109]. If the introduction of such alternative fuels in larger scale will be successful, they have to be competitive concerning production and distribution costs, reliable supply logistics and low emissions of environmental health damaging components. Fuels that today seem to be able to fulfil these criteria are methanol and ethanol for gasoline engines, as well as dimethyl ether (DME) and synthetic diesel (Fischer-Tropsch diesel) for diesel engines [109]. Ethanol can be produced by fermentation of biomass like sugar plants and wood based material, while the other alternative fuels can/will be produced via gasification and catalytic conversion to liquid fuels in so called Gas-To-Liquid (GTL) processes. In the public administration reference group recommendations [110] it is also concluded that it is important to have a wide spectrum of different solutions and allow “many flowers to bloom” until consensus can be reached on a European level for which systems should be applied. Hydrogen gas is the most promising fuel but requires a completely new production system, distribution and vehicle. Considerable development work is still needed, though, which is why hydrogen gas must be considered primarily as a long-term solution (20-30 years).

In addition, there are also a large number of other components that are or may be added to fuels, not at least different alternative fuels, in order to obtain certain technical properties and benefits. The area has so far been little examined from a particle physical, chemical and toxicological perspective. There are no epidemiological studies available and this is an area that certainly needs research attention. The available data are often in report formats rather than scientific publications

Biodiesel is the general term for biofuels that consist of different fatty acid methyl esters (FAME) like rape methyl ester (RME). RME are produced via transesterification of e.g. vegetable oil, and also includes a number of different

essential additives for proper function, e.g. cetane improvers, smoke suppressors, flow enhancers, cloud-point depressors, wax antissettling additives/detergents (to reduce injector nozzle fouling), antioxidants (for unsaturated oils and control of microbial growth). However, there are few emission data available from biodiesel in combination with such additives. This is therefore an issue that needs to be further investigated if RME and other biodiesels should be introduced in large scale since many of these additives may include components that most certainly can be associated with fine exhaust particles (e.g. metals) and thereby cause various health effects. The production standard may also affect the quality of the biodiesel. Poor post-transesterification refining has been shown to result in high emissions of aldehydes, which may result in negative health effects [110]. However, the future potential for RME is questioned and the Swedish Energy Agency and other national administration boards consider RME not to be prospective for the future, due to small potential production volumes, high production costs, influences on biodiversity during cultivation and none benefits during use as a fuel [109]. Still, the recommendation is that FAME should be utilised by using it as an additive in various “dry” fuels, due to its unique lubricating properties [110].

The effects of different alternative fuels in relationship to diesel fuel on mutagenicity have been reported only in a few recent studies. The results are however somewhat conflicting. Diesel engine emissions are classified as “probably carcinogenic to humans”, with a perception that considerably long exposure is needed and effects are only seen when large populations are investigated. Bünger et al proposed a lower mutagenic potency of RME compared to standard diesel fuel and suggested it to be due to lower emissions of PAH compounds [111]. However, in a study from 2007 the same authors investigated the mutagenic effect of diesel exhaust in relationship to rape seed oil (RSO), RME and natural gas derived synthetic (GTL) fuel [112]. A heavy duty truck diesel was running at the European stationary cycle. The mutagenicity of the particle extracts and condensate was tested using a *Salmonella typhimurium* microsomal assay. Two different RSO batches caused a significantly increase in the mutagenic effects. RME extracts also showed a significantly higher mutagenic response as compared with reference diesel fuel. GTL did not differ from diesel fuel [111].

Using similar techniques, the role of an oxidation catalytic converter (OCC) on the mutagenic effects of different fuels was investigated. Low sulphur diesel fuel caused less mutagenic effect than high sulphur fuel when investigated without the use of an oxidation catalytic converter. During low load, the OCC resulted in lower mutagenic effect but at high load the mutagenic effect significantly increased. The authors hypothesised that the oxidation catalytic converter could increase the formation of direct active mutagens by reaction of NO<sub>x</sub> with PAH resulting in formation of nitro-PAH which are powerful direct active mutagens [113].

Rather limited information is available in the open scientific literature concerning emissions from different alternative fuels. However, some GTL-fuels, like FT-diesel and DME, seems to have potential to reduce the emissions of CO, NO<sub>x</sub>, hydrocarbons and PM, compared to fossil based diesel fuels [114-116]. However, any detailed and generally applicable knowledge about the actual exhaust characteristics from the use of different alternative fuels in present and future engines is still missing and the relation to potential toxicological effects are even more unknown today.

Since 2003, ethanol is used as additive (5 %) to all gasoline fuel (octane 95) distributed in Sweden. However, whether the potential change in exhaust composition and characteristics from this action may cause less or more biomedical effects has not been properly addressed. A recent investigation from the US has considered the effects of ethanol (E85) and suggested no overall estimation of reduced cancer risks as compared with gasoline [117]. Worry was expressed over the potential for ethanol to increase ozone levels which may give additional adverse health effects, especially in combination with motor engine particle emissions [118, 119]. Ethanol combustion results in increased amounts of acetaldehyde and may also yield other aldehydes which may be of concern. The presence of oxygen in the fuel may give an increased potential for quinones to be produced which then could give increased potential toxicological effects.

Overall, there is still a huge gap in the research literature over toxicological potential for alternative fuels as stated earlier in a Swedish report in 1999 [120]. Further concern has been given by the US Environmental Protection Agency scientists in a

recent research report [110]. They indicate the need to address certain research questions before the general population is exposed in large scale to combustion components from fuels not readily investigated from a scientific and toxicological perspective. While there is a potential for less health effects than for combustion of the classical fossil fuels, emissions of aldehydes and other oxygen containing organic components (e g quinones) as well as secondary produced ozone, still poses threat to human health. So far, only a very limited number of studies have been performed with alternative fuels, addressing detailed exhaust particle characteristics and its toxicological/health risk potential. While some studies have been reported for RME and ethanol, no such information seem to be available concerning GTL fuels like methanol, DME and FT-diesel. The future potential for these (and other) alternative fuels are rather difficult to speculate in, and beyond the scope of this report. However, the increasing interest for phasing out fossil fuels, has significantly increased the interest in alternative fuels, and will most certainly include also issues related to environmental health.

### 3.1.5 Summary of exhaust PM and health risks

#### ***Diesel and gasoline exhaust***

*There is clear evidence that diesel exhaust causes adverse health effects. This is first of all based on studies in animals and cell systems but there are also a substantial number of investigations in humans demonstrating adverse effects on lung function, airway inflammation and in particular worsening of asthma with increased bronchial hyperresponsiveness. During the last few years several studies have demonstrated adverse cardiovascular effects. Data have been shown that the blood vessels get an impaired ability to dilate and increase blood flow in case of increased demand, after exposure to diesel exhaust. Furthermore there is a lack of release of tissue plasminogen activator (t-PA) which should be constantly released to prevent blood clots from occurring in the blood vessels. These effects link strongly to heart attacks and strokes and are clearly impaired after diesel exhaust exposure. Most recently similar effects have been demonstrated in subjects with coronary disease and previous myocardial infarction despite full preventive medication [58]. In those individuals there was also myocardial ischemia demonstrated on ECG recordings during light exercise during diesel exhaust exposure. The cardiovascular effects may appear as early as after 15 minutes of exposure and some effects are maintained for at least for 24 hours. In this respect, the experimental findings fit exactly by the observational findings by Peters and coworkers report of traffic exposure in subjects before entering a hospital with a heart attack [8]. This is also in line with a range of other epidemiological.*

*From an epidemiological perspective, motor engine exhaust is clearly linked to adverse effects on lungs in asthmatic and other subjects, but also to impaired lung growth and asthma in children [4]. As regards the effects of children the indications are that diesel exhaust may be involved but also the proximity to highways. Living close to major roads appears to be unfavourable. Some indications also point more towards diesel exhaust than gasoline exhaust as major contributor for the cardiovascular events.*

*Very few studies have investigated effects of gasoline exhaust in experimental models. It appears that the characteristics of gasoline exhaust particles differs compared to diesel exhaust particles, e.g. it contains less elemental carbon (soot), are fewer in number for the smallest particles (“nanoparticles”) and the chemical constituents, mainly organic components, appear less toxic. A few animal studies have suggested gasoline exhaust particles to be able to cause an increase in atherosclerosis which potentially could lead to heart attacks and stroke in human subjects if the findings are transferable. Such effects have previously been outlined for diesel exhaust but it is not clear whether the magnitude is similar or less for gasoline exhaust.*

### ***Alternative fuels***

*There is a lack of literature on toxicological effects and health risk estimates of the use of alternative fuels such as ethanol, methanol, RME, DME, Fischer-Tropsch fuel, GTL and others. Some recent studies have suggested alternative fuels (RME) to be carcinogenic at a similar level as fossil diesel fuels. There are also indications of biofuels to produce more reactive aldehydes and increase ozone levels which may cause adverse health effects. There are also potentials for oxygen containing alternative fuels, such as ethanol, to lead to production of highly reactive oxidative components which have not yet been studied. With the high interest from societies worldwide, it is therefore important to determine the health potential adverse health effects of emissions from alternative fuels before fully implemented.*

## HÄLSOEFFEKTER AV MOTORAVGASER

### Diesel och bensin

Epidemiologiska studier har visat att trafikrelaterade partiklar och sot (i den fina partikelfraktionen  $PM_{2.5}$ ) har ett starkare samband med allvarliga hälsoeffekter som antal dödsfall och akut hjärt-kärlmorbidity än  $PM_{10}$  generellt och den grova fraktionen av partiklar i  $PM_{10}$ .

I befolkningsstudierna kan det svårt att separera effekterna av diesel- och bensinavgaser beroende att trafiken i studieområdena ofta har en varierande och vagt beskriven sammansättning, samt på grund av att man oftast använt tämligen ospecifika haltnivåer såsom  $PM_{10}$ ,  $PM_{2.5}$  och  $NO_2$ .

Flera nya studier har dock talat för att boende och skolgång nära stora trafikleder ger kraftigt ökad risk för hjärt-kärlsjukdom, försämrad tillväxt av barns lungor och ökad risk för astma. Detta har kopplats till markörer för motoravgaser snarare än den grova partikelfraktionen. Vissa studier har indikerat dieselavgaser som betydelsefulla, baserat på starka korrelationer med elementärt kol, eller boende i närhet av större vägar med tung dieseltrafik.

Det finns en ansenlig toxikologisk litteratur som visat att exponering för dieselavgaser leder till hälsoeffekter. Studier har gjorts i såväl djur, cellsystem, som människor. Bland de negativa hälsoeffekterna som dokumenterats experimentellt kan försämrad lungfunktion, inflammation och förvärrade astmasymtom med ökad hyperreaktivitet nämnas.

Det har nyligen publicerats en rad studier där evidens framkommit för att dieselavgaser ger mycket snabba akuta effekter på hjärtat, blodet och kärlsystemet hos såväl friska som personer med hjärtsjukdom. Blodkärlen styvnar mycket snabbt och kan inte längre vidga sig för att släppa fram mer blod om det behövs för tex ansträngning. Blodets tenderar att levra sig snabbare och risken för blodproppar och hjärtinfarkt ökar. Direkta tecken på syrebrist i hjärtat kan komma mycket snabbt hos

personer med hjärt-kärlsjukdom i samband med exponering för dieselavgaser och effekterna kan delvis kvarstå i 24 timmar, vilket tidsmässigt överensstämmer väl med undersökningar om när hjärtinfarkter kommer efter exponering i trafikmiljö.

Det finns endast ett fåtal experimentella studier som försökt separera effekterna från bensinavgaser från andra trafikkomponenter. Ett par djurstudier har indikerat att partiklarna från bensinavgaser kan öka förekomsten av arterioskleros, i likhet med dieselavgaser. Om resultaten är överförbara till människa skulle det kunna innebära en ökad risk för hjärtinfarkt och stroke. Generellt sett verkar det som att avgaspartiklar från bensinmotorer har en något annorlunda karakteristik jämfört med dieselpartiklar, t ex innehåller mindre mängd sot, färre nanopartiklar och dessutom med lägre toxisk kapacitet, men det vetenskapliga underlaget är sparsamt.

### **Alternativa bränslen**

Forskningen kring alternativa drivmedel är fortfarande väldigt tunn och har i princip endast omfattat kvantifiering och karakterisering av gaser och partiklar i avgaserna, utan specifika toxikologiska studier. I vissa fall tycks det finnas en potential till reducering av emissionerna, men det finns generellt sett inget stöd för att dessa bränslen skulle ge ofarliga utsläpp. Det finns dessutom indikationer från några studier att vissa alternativa bränslen (i detta fall RME) skulle ge lika eller mer carcinogena effekter som dieselavgaser. För etanol finns även en ökad sannolikhet för bildning av aldehyder och ozon, och det finns dessutom potential för bildning av olika reaktiva kolväteföreningar som skulle kunna ge oväntade hälsoeffekter.

Forskare vid amerikanska naturvårdsverket (EPA) har i en översiktsartikel från 2007 [110] pekat ut behovet att detaljstudera emissioner från alternativa bränslen och deras toxikologiska kapacitet för att inte hamna i en situation där oväntade hälsoeffekter uppkommer.

## **3.2. WEAR PARTICLES**

An important distinction should be made between road wear and road dust. Road dust includes any particle component that may be found in the road environment no matter whether it has been generated from wear of the road, tyres, brakes and other mechanical processes, or being added as sand or gravel to increase tyre grip on icy roads. Wind blown dust, which can include virtually any particle component such as crustal material with biological components, exhaust particles and secondary particles, adds to the mixture in road dust. This section is focused on wear particles of different origin.

### **3.2.1. Road wear**

#### **3.2.1.1. PM composition**

Vehicles may cause substantial wear on roads by tyres, especially if they are studded as is common in Nordic environments during winter time. The dust on roads also contain components from brakes and exhaust but also wind blown organic components, soil, gravel, salt and sand from antiskid treatment. Road dust is always a mixture of components. It is mainly of larger particle size in the range of 2.5-10  $\mu\text{m}$  (coarse particles) or larger. They may therefore contribute considerable to particle mass, even though the particle number may be low.

The Swedish WearTox-project performed at the road simulator at the Road and Traffic Research Institute (VTI) in Linköping have demonstrated coarse ( $\text{PM}_{10}$ ) mineral particles from road wear of the studied asphalt/granite and asphalt/quartzite pavement, consisting mainly of silicon, oxygen, aluminium and potassium [121]

Of interest is that not only the size and mineral composition of particles may be of importance for toxic capacity but also shape, as demonstrated by Finnish researchers investigating mica mineral particles which are used for sanding of icy roads [122].

Mineral dust and crustal material generated by vehicle wear of the road surface is a major component of PM. The emissions associated with road wear are particularly high in the Nordic countries during winter time, especially where the use of studded tyres is common. Road wear PM contains a complex mixture of particles with different size, shape and chemistry depending on a number of factors, including the material of the pavement [123]. The main factor determining the PM structure is the mineral composition of the roadway, since 95% of the road surface is estimated to consist of stone material. The remaining part is mainly bitumen, which function as a binder and connect the stones together in the asphalt. It consists of a mixture of petroleum products with high concentration of high-molecular-weight paraffinic and naphthenic hydrocarbons [124, 125].

### **3.2.1.2. Toxicology**

During the last years a few studies have focused on investigating different minerals and highlighted certain toxicological aspects. PM<sub>10</sub> generated from the wear of studded tyres on two types of different pavements, asphalt/granite and asphalt/quartzite, have been investigated for their capability to induce inflammatory effects. BEAS-2B cells were exposed for PM<sub>10</sub> at different concentrations and an increase in the release of TNF- $\alpha$  was found, while the same exposure to human monocyte-derived macrophages showed an increase in TNF- $\alpha$ , IL-6 and IL-8. When comparing the two different types of roadway material it was found that the granite pavement had a significantly higher capacity to induce pro-inflammatory cytokines compared to the quartzite pavement. The wear particles were also compared with PM<sub>10</sub> collected at a traffic-intensive street, a subway station and DEP. The results showed that the granite particles were able to induce cytokine release to the same magnitude as the street particles, which was greater than both the DEP and subway particles. This was recently supported by an investigation using a macrophage cell line, and indicates the capacity of wear particles from studded tyres to contribute to the negative health effects seen in traffic-intensive areas [15, 126].

Schwarze and colleagues, at the Norwegian Institute of Public Health in Oslo, have in a series of studies investigated particle toxicology of a number of different mineral compounds. The particles' ability to induce changes in cytokine release, cell viability and apoptosis was evaluated in relation to their mineral and elemental composition. The investigations have been conducted both *in vitro* (lung epithelial cells and primary rat alveolar macrophages) and *in vivo* (rats) [127-132]. The results demonstrate that various mineral particles cause different inflammatory responses and that the characteristic of the particles were an important factor for the initiation of the investigated endpoints. In one of the studies it is suggested that when the bioactivity of mineral particles is to be predicted, analysis of elemental composition is insufficient. The particles have to be tested in biological systems.

An inherent contributor to effects in cell systems when road dust is investigated is contribution from endotoxin. Lipopolysaccharide components from the walls of gram-negative bacteria are strong inducers of cell responses through toll receptor 4 (TLR-4). In some studies these effects have not been possible to separate from those of other components and may account for an overestimation of effects from particles collected outdoors in certain traffic environments, as compared with laboratory generated road and tyre wear particles.

### **3.2.1.3. Epidemiology**

In a much cited systematic review article, Brunekreef & Forsberg (2005) explored epidemiological articles which had collected data on both fine and coarse particle amounts (PM<sub>2.5</sub> and PM<sub>10</sub>) in relationship to health effects of air pollution [9]. The review pointed out that the fine particle fraction, mainly reflecting exhaust particles, was associated with respiratory and cardiovascular deaths as well as symptoms and worsening of these conditions. The coarse fraction of PM (2.5-10 µm) was not found to be associated with deaths but with symptoms and worsening of respiratory diseases such as asthma. This article overturned some earlier misinterpretations based on selected papers, rather than being systematic and reviewing the whole literature.

Within the published epidemiological literature there are only a few studies of road dust or the coarse fraction when studded tyres are used and road dust has its greatest contribution to PM<sub>10</sub> [133]. However, a Swedish time-series study from Stockholm show a strong effect on respiratory admissions in Stockholm. Three Finnish panel studies have followed asthmatics during periods when increased levels of road dust have been reported, at least periodically. One of the studies, which was part of the EU funded PEACE (Pollution Effects on Asthmatic Children in Europe) study, followed 39 asthmatic children in Kuopio for 57 days. An effect on lung function (as morning PEF; Peak Expiratory Flow) was found and a greater effect was noted for PM<sub>10</sub> than for particle number or black smoke [134].

In a later panel study 49 children in Kuopio with chronic respiratory symptoms were studied [135]. The fraction of coarse particles were measured indirectly by subtracting PM<sub>2.5</sub> from PM<sub>10</sub>. Resuspended road dust (as PM<sub>10</sub>) was calculated using aluminium as a tracer. However, the very high correlation between the particle fractions made it impossible to distinguish between their effects. Cough reported in the diary had, with a few days delay, a significant relation to PM<sub>10</sub>, resuspended road dust, the coarse fraction and the fine fraction (PM<sub>2.5</sub>). For lung function (PEF) no significant relation were shown.

In a third Finnish panel study, 57 adults from Helsinki, were studied for 6 month. A certain reduction in lung function (PEF) correlated with increased levels of ultrafine particles [136]. Coarse PM had the opposite effect, but the author's believe this was due to problems with seasonal effect on the lung function. A later analysis of PM<sub>2.5</sub> from different sources found that both PM<sub>2.5</sub> attributable to local combustion and soil-derived PM<sub>2.5</sub> were negatively, associated with PEF [137]. No consistent associations were observed between source-specific PM<sub>2.5</sub> and respiratory symptoms or between individual chemical elements and any respiratory endpoints.

The mass of crustal particles (using Silicon as a tracer) was not associated with daily mortality in six eastern U.S. cities the Laden et al (2000) meta-analysis [104]. The elemental profile of the crustal factor in this study was qualitatively similar to published chemical analysis of road dust. Also a study of daily mortality in Phoenix found no increase in mortality with the identified soil factor, rather the opposite, but

the coarse fraction was positively associated with mortality [106]. Also in a later analysis fine particle soil was not associated with increased risks [98].

In the multi-centre PEACE panel study on asthmatic children silicon and iron concentrations tended to be negatively associated with peak expiratory flow and positively associated with the prevalence of phlegm [94]. The effects of silicon and iron could not be separated, which indicates that the crustal coarse PM<sub>10</sub> fraction was involved.

### **3.2.2. Brake wear**

#### **3.2.2.1. PM composition**

The use of brakes on vehicles leads to wear and emissions from brake pads, brake discs and brake cylinders. This leads to emissions of various metals which is discussed in this chapter, but also given in certain detail in the chapter 4 where specific metal aspects are given.

In a study from 2000 by Garg et al, a number of different brake pad formulations were investigated using a brake dynamometer, focusing on the production of brake wear [138]. Results showed that on average, 35% of the lost brake pad mass was emitted as airborne PM. On average, 86% of the airborne particles were smaller than 10 µm in diameter and 63% smaller than 2.5 µm. Mechanically generated particles are generally relatively large but a surprisingly high proportion of the emitted particles had an aerodynamic diameter < 0.1µm. This might be due to the high temperature produced during the brake process. It is possible that the heat enables brake pad and lining materials to volatilize during the braking event, which followed by condensation in the air stream, results in small particles. This process is however dependent on the dilution conditions, where an on-road situation can be difficult to mimic in an experimental environment. The results also revealed that the number of particles emitted in the brake process increases with temperature.

Elemental composition analysis showed that on average 18% of the PM mass was carbonaceous material, while the remaining mass consisted of various metals together with silicon, phosphorous, sulphur and chlorine.

### **3.2.2.2. Toxicology**

The majority of the particles produced from brake wear are generated from the brake lining and pads. The composition of these varies a lot between different manufacturers and a variety of particles produced. Cu, Fe, Cr, Ni, Pb and Zn have all been detected in brake wear and copper is often mentioned as indicator of choice on brake wear PM [124].

Most of the toxicology research regarding the health effects from traffic related air pollution has focused on combustion derived particles and not as much on wear particles, especially not the brake wear. Studies supporting the idea of brake wear particles causing negative health effects are largely those connecting the presence of metals to various biological effects. A number of studies have found metal content to be correlated to cytokine production, cellular stress, oxidative damage to DNA and radical generating capacity, which all might lead to negative health impacts. The main theory about the toxicity of metals, especially the transition metals, is suggested to be due to their high capacity to form reactive oxygen species (ROS). These in turn, are likely to initiate a variety of signalling pathways which might result in the induction of a number of cell and tissue damaging events (for details, see chapter 4.1 Metals). Metals are clearly able to elicit a variety of biological effects which may be related to health effects.

### **3.2.2.3. Epidemiology**

The role of brake wear particles has so far not been much investigated in epidemiological studies. A small study by the US EPA investigated healthy young non-smoking male highway troupers measuring effects from speed-changing traffic conditions on heart rate and blood, in relationship to detailed air pollution sampling

inside their patrol car. Effects were seen on heart rate variability, supraventricular atopic beats and the von Willebrand coagulation factor. The strongest correlations were found with fine particulate matter from brake wear and engine emissions while a role of crustal material could not be excluded. The study gave some support for traffic exposure to have the potential for worsening cardiovascular disease but was not able to separate motor engine exhaust effects from those of metal from brake wear [139].

### **3.2.3. Tyre wear**

#### **3.2.3.1. PM composition**

Analysis of the chemical composition from car tyres has shown that it varies a lot between different manufactures. Apart from various mixtures of rubber, tyres from cars and trucks also contain a number of different chemicals. Wear PM from tyres has been shown to contain high concentrations of Zn and a few studies also report considerable amounts of PAH:s. These hydrocarbons have not been specified in any detail though, which limits possibilities to estimate toxic capacity [124, 140].

Mechanically formed wear particles are often larger than the particles produced during the combustion process. However, researchers from the Swedish National Road and Transport Research Institute (VTI) have recently published a study in which they have used a road simulator to investigate the wear particles generated from different tyres and road pavements [16]. They found that not only particles from the coarse particle mode were formed but the interaction between tyres and pavement may also produce ultrafine particles. In a recent study, the characteristics of the ultra fine particles were investigated [26]. The results indicated that the size distribution of the particles varies between different tyres. Since these ultrafine particles mainly consist of carbon reinforcing filler material (soot agglomerates) and softening filler (mineral oils), the mixture of oils used in the tyre manufacturing process influence the characteristics of the wear particles produced. It was also concluded that the total number emission factor was more dependent on vehicle speed and the use of studs in winter tyres, than of tyre brand or pavement that was used.

### **3.2.3.2. Toxicology**

It has been suggested that the toxicity of tyre debris might be due to the presence of organic compounds. Investigations have shown that the organic extract of tyre debris contain high concentrations of isoprene polymers, which is commonly used in the production of rubber. Exposure of human lung cell line A549 to the organic extracts of tyre debris have shown that growth and viability of the cells were affected in a dose-dependent manner, as shown by increased cell mortality and DNA damage [141].

It has also been shown that tyre debris contains relatively high concentrations of zinc (Zn), primarily as zinc oxide, which may contribute to toxic effects in various systems. When the tyre debris deposited on the roads interact with water, for example rain, a number of different chemicals may be released, among them Zn. The toxicity of the eluates seems to be dependent on the size of the exposed surface, pH, the quantity of released Zn and degree of aggregation [142, 143]. It has also been shown that Zn in PM may cause various health effects, including inflammation and airway hyperreactivity [144-147].

The inhalation of metal compounds, especially those with high oxidative capacity like the transition metals, may lead to the production of ROS. As mentioned above, these have the capacity to initiate various signalling pathways, leading to the induction of an inflammatory response, which might cause damage to cells and tissues.

Another explanation to the toxicity of tyre debris, that has not been thoroughly investigated, is the wear from studded tyres that are often used during winter time in the Nordic countries. Common material in studs are carbon steel and carbides of Al, Si, Mn, Mg and Fe [124]. Since the studs are mainly composed of metal it is likely that the PM emitted from studded tyre wear will include traces of metals.

### **3.2.3.3 Epidemiology**

There is no literature available addressing the role of tyre wear specifically from an epidemiological perspective. So far it has not been possible to distinguish influence from tyre wear from other components in road dust. However, it has been shown that allergens also from latex are transported by the combustion particles in ambient air, that may act as carriers and depots of allergens inhaled into the airways, why latex from tyres may have a role in latex allergy and asthma symptoms [148].

### 3.2.4. Summary of wear PM and health risks

*Wear particles are mainly found in the coarse particle fraction 2.5 – 10 µm to which road surface, tyre and break wear particles, sand, gravel and wind blown dust contribute. In the systematic review of Forsberg and Brunekreef from 2005 it was concluded that the coarse fraction is not generally associated with mortality and cardiovascular effects, in contrast to the fine particle fraction (PM <2.5 µm).*

*Coarse PM seems to have stronger associations, than fine particles, with hospital admissions for respiratory conditions. This entity may involve cases of respiratory diseases such as pneumonias, asthma, chronic obstructive pulmonary disease (COPD) and other illnesses. Epidemiological studies have indicated respiratory symptoms, asthma medication and lung functions measurements to relate to the coarse particle fraction more than to the fine fraction. It is possible that the coarse particles are more related to eliciting symptoms of already established asthma, than causing reduced lung growth and inducing asthma, to which motor engine exhaust has been linked in some recent studies. Consequently, it may be of importance to consider what severity and magnitude of health effect that fine and coarse particles may be causing.*

*The literature on more specific components of wear particles is not extensive. There are few studies of road dust or coarse particles where studded tyres were used. The three Finnish panel studies showed somewhat inconsistent respiratory effects that are hard to link primarily to road dust. In the PEACE panel study on asthmatic children PM<sub>10</sub> silicon and iron concentrations, indicators of crustal material, tended to be negatively associated with peak expiratory flow and positively associated with the prevalence of phlegm.*

*Road wear particles contain asphalt remnants like bitumen as well as the mineral used in the road surface. An appearing literature suggests that different minerals may cause very different toxic capacity and that some of these minerals possibly should be avoided. This is an area which has not been much looked into. The studies from VTI in Linköping have been very important in this aspect but far more work of this*

*type is needed. The same applies for rubber asphalt which has been used in the US for many years where the literature essentially only reflexes the exposure in asphalt workers and not the emission components from rubber asphalt.*

*Tyre wear particles include organic components which have in some studies been indicated to have a toxic capacity. There are also studies indicating the generation of ultrafine particles from tyre wear, but no health related research is yet available on this particle fraction. A suggested difference between old and newer, or fresh and worn, tyres regarding particle production has not been resolved.*

*Brake wear particles may be of importance due to their metal content and the ability of metals do cause oxidative stress and affect different enzymes. There is a good and strong literature on toxicity from metals. The EU project HEPMEAP coordinated from Sweden demonstrated metals to be main components in driving toxicity from particles in traffic situations, together with organic component which may appear from motor engines and other sources. When considering metals it should be noted that the rubber from tyres also contains substantial amounts of zinc which adds to the metal amounts in road dust. The use of studied tyres increases road wear substantially and also contributes to the metal amounts in road dust. In addition, the recent findings that the interaction between pavement and tyres also may produce ultrafine particles, mainly of carbonaceous material, are also very interesting for further consideration regarding potential health effects.*

*In summary, keeping in mind that the epidemiological studies of road dust are very few, studies of coarse PM in general adds support for the view that dust particles, largely crustal, do not have the same influence on mortality and cardiovascular effects that has been widely demonstrated for fine combustion particles. Coarse particles seem to have the stronger effect on respiratory endpoints such as symptoms and emergency visits and hospitalisation for asthma and other lung diseases. Some wear components are likely much more toxic than others, and role of different sources is important to clarify.*

## HÄLSOEFFEKTER AV VÄGDAMM

Slitage partiklar från däck, vägbana och bromsar förekommer i huvudsak i den grova partikelfractionen (2,5-10 µm). Dessa komponenter återfinns i vägdamm som även kan innehålla sand och grus och samt grövre dammpartiklar som transporterats med vinden. Jord, växt och bakteriekomponenter, bland annat endotoxin förekommer också. En uppmärksam källa, som diskuterats mycket på senare tid, är väg och dubbslitage som kommer av dubbdäcksanvändning.

En systematisk översikt av epidemiologiska studier, där effekter av både grova (2,5-10 µm) och fina partiklar (<2,5 µm) undersökts, har visat att de grova partiklarna som innehåller vägdamm är kopplade till symptom och försämring av astma och andra lungsjukdomar. Däremot finns inte bevis för att dessa partiklar skulle ha avgörande samband med ökad dödlighet, vilket istället kopplats starkt till de fina partiklarna som i huvudsak genereras av förbränningsprocesser.

Endast ett fåtal epidemiologiska studier har försökt analysera specifika källors bidrag till de grova partiklarna och hälsoeffekter. Tre finska panelstudier har försökt titta på effekter av vägdamm där dubbdäck användes utan att ge entydigt svar på om dammet gav lungpåverkan. Den internationella PEACE studien rapporterade att silica och järn som indikatorer på mineralursprung var kopplat till lättare försämring av PEF som mått på lungfunktion och slembesvär. Däremot inte tydligt mot mer definitiva mått på försämrad astma. Den amerikanska "Childrens Health study" som visade på försämrad tillväxt av barns lungor såg inte något starkt samband med de grova partiklarna, utan istället till fina partiklar, kvävedioxid och elementärt kol, vilket tolkades som motoravgaser framför allt från dieselfordon.

Vissa slitagekomponenter som återfinns i den grova fraktionen har troligen en högre toxicitet än andra partiklar. Tester från bland annat VTI och Oslo har indikerat att vägbanor med olika uppbyggnad, stenstorlek och mineraltyper kan ge högst olika bidrag till cellreaktioner, studerat i laboriemiljö.

Olika typer av däck tycks kunna ge olika effekter där dubbdäck ger överlägset mest partiklar från vägbana och däck, och även bidrar med mycket små nanopartiklar. Effekterna av dessa komponenter är ännu föga klarlagda liksom betydelsen av bromsslitagepartiklar.

Sammantaget förefaller inte vägdam och grova partiklar vara kopplade till ökning av dödsfall som är fallet för fina förbränningspartiklar. Däremot är de kopplade till indikatorer på försämring av lungsjukdomar såsom tex astma.

### **3.3. SECONDARY PARTICLES**

#### **3.3.1. Sulphates**

##### ***Epidemiology***

Both gasoline and diesel fuels in Sweden have very low sulphur contents. According to the Swedish Petroleum Institute (SPI) the sulphur content in Swedish gasoline (Environmental class 1) is  $\leq 50$  ppm and in diesel fuel (Environmental class 1) is  $\leq 10$  ppm [149], respectively. However, in the lubricating oil used for combustion engines, additives containing sulphur are present. Li and co-workers [150] investigated the regeneration mode of a diesel particulate trap emissions from a diesel particulate trap and it was found that calcium sulphate (gypsum) was emitted. The problem of calcium sulphate formation can be limited by using lubricating oils with lower sulphur contents. According to Per-Martin Roos [151] the trend is to develop engine lubricating oils with lower sulphur contents, which will prolong the lifetime for diesel particulate traps. These new lubricating oils are called low SAPS (Sulphate Ash, Phosphorus, Sulphur) lubricating oils. Generally speaking fully synthetic engine lubricating oils have lower sulphur contents (below 200-300 ppm) compared to mineral based engine lubricating oils which are in the range of 0.4 to 0.9% [151]. Due to these facts the sulphate contents in ambient particles emanating from vehicles in Sweden are expected to be decreasing i.e. other sources in the environment will be the main contributor to ambient air particle sulphate contents.

According to a recent review on sulphate, despite low spatial variability it is generally less associated with health endpoints than  $PM_{2.5}$  [152]. There are however not too many studies, and their results are quite inconsistent.

In a study of daily mortality, size-fractionated particulate mass and gaseous pollutants were obtained in eight of Canada's largest cities from 1986 to 1996, sulphate, iron, nickel, and zinc from the fine fraction were most strongly associated with mortality [153].

The secondary aerosol factor was significantly associated with cardiovascular mortality but not total mortality in a time-series study from Phoenix [106]. This was confirmed in a later analysis of the apportioned anthropogenic PM<sub>2.5</sub> source categories, where secondary sulphate, traffic, and copper smelter-derived particles were most consistently associated with cardiovascular mortality [98]. Again the associations were weaker for total mortality.

In the Harvard Six Cities study the sulphate-related factor was significant in the combined analysis [104] and close to significant in the Health Effects Institute coordinated reanalysis [154].

When daily mortality in three New Jersey cities was investigated after a factor-analysis, statistically significant associations were found between mortality and several sources including sulphate [105].

An examination of reported associations between daily mortality and source investigators' estimated source-apportioned PM<sub>2.5</sub> for Washington, DC for 1988-1997 found that patterns were similar across investigators/methods, with the largest and most significant percent excess deaths per 5-95(th) percentile increment for secondary sulphate [107]. When results from seven research institutions that studied effects of source-apportioned PM<sub>2.5</sub> in Washington, DC, and Phoenix were evaluated, the sulphate-related PM<sub>2.5</sub> component was most consistently significant across analyses in these cities [155].

In the long-term studies the Harvard Six Cities cohort (Dockery et al, 2003) and the American Cancer Society (ACS) II cohort study [156, 157] there were significant associations between both PM<sub>2.5</sub> and sulphate and mortality. In The Six Cities Study the correlation between PM<sub>2.5</sub> and sulphate was so high ( $r=0.98$ ) that they reflect the same exposure contrast. In a separate study ACS data were analyzed at the county scale instead of using larger metropolitan areas [158]. At the county scale, long-term exposure to sulphates was more strongly associated with increased risk of all-cause and cardiopulmonary mortality than previously indicated. The new spatial analytic methods applied to the ACS data resulted in various levels of spatial autocorrelations

and demonstrated a significant association between sulphur dioxide and mortality [159]. This suggest that mortality may be attributable to more than one component of the complex mixture of ambient air pollutants for U.S. urban areas, but SO<sub>2</sub> is likely not a causal component but a good indicator for the relevant mixture.

### **Toxicology**

Studies from the 1970's and 1980's explored health effects from sulphur compounds including sulphur dioxide and sulphur acid particles. Bronchoconstrictive and hyperreactive airway responses were seen together with some inflammatory responses. Overall there were high concentrations demanded to elicit effects which are far beyond ambient levels today. In-vitro experiments with sulphur compounds indicate low toxicity. It has recently been highlighted that sulphate levels measured from ambient air may be markers for other sources and metals which may carry the toxic effects. One such example is as mentioned above distance transport of sulphate and metal containing particles from a nickel smelter in Ontario, Canada, causing adverse health effects at vast distances in the US, to which air packages had been tracked in detail [160].

### **3.3.2. Nitrates**

Zhao and Hopke [161] investigated particle source apportionment from San Gorgonio wilderness and concluded that nitrates present on PM<sub>2.5</sub> atmospheric particles are secondary formed nitrates. The relative contribution of nitrates on the particulate material investigated was about 38%. Secondary nitrates are formed in the atmosphere by oxidation of NO<sub>x</sub> (partly emitted from mobile sources).

There are almost no epidemiological studies using nitrate levels. One published study of daily mortality found no significant association between nitrate and mortality [162]. In a time-series of six California counties, ambient concentrations of several

constituents of fine particles, including nitrate, demonstrated stronger effects on daily mortality than did PM<sub>2.5</sub> mass [163]. One cohort study included nitrate, the Veterans Administration Cohort, and found a statistically significant effect on mortality [164]. However, traffic density was a more robust predictor of mortality than was sulphate or PM<sub>2.5</sub> [165]. From a toxicological perspective nitrate particles appear to have low toxic capacity.

### **3.3.3. Secondary organics**

There has been shown that the atmospheric PM mass may increase significantly (up to 50%) due to formation of secondary organic aerosols (SOA) [166]. Emitted gaseous and semi-volatile organic compounds from combustion sources, e. g. traffic, may in this aspect be of great importance and it is important to consider the transformation of such primary emissions [166]. According to Cass et al. [167] there is limited knowledge available about the chemical contents of atmospheric particles originating from cities. According to them, ultrafine particles comprise on average 50% organic compounds, 14% trace metal oxides, 8.7% elemental carbon, 8.2% sulphate, 6.8% nitrate, 3.7% ammonium, 0.6% sodium and 0.5% chloride. This implies a strong research need to identify and quantify organic compounds present in ambient air particles both from an academic and a health point of view.

Certain organic components can be associated with a strong toxic capacity. For effects of both primary and secondary organics in traffic, see Chapter 4.2 for more details.

### 3.3.4. Summary of secondary PM and health risks

*Sulphate and nitrates have sometimes been associated with adverse health effects in epidemiological studies, but may to some extent be markers for other components which may have caused health effects. Sulphates may for instance be related to metals from chemical and combustion processes. There is no immediate concern to direct special attention to these components. Significant formation of secondary organic aerosols in the atmosphere have been shown, but the relative importance of traffic exhaust emissions and the potential change in toxicological potential during such "ageing" has not been studied.*

#### **HÄLSOEFFEKTER OCH SEKUNDÄRA PARTIKLAR**

Sekundära partiklar såsom sulfater och nitrater har ibland associerats med hälsoeffekter i befolkningsstudier. Särskilt tydligt har sambandet varit mellan sulfathalt och dödlighet i amerikanska långstidsstudier. Enligt toxikologiska studier förefaller dessa partiklar att ha en relativ låg toxicitet och mätdata från studier kan i vissa fall vara indikatorer för andra komponenter i partiklarna (t.ex. kolväten och metaller) som kan ha orsakat effekter. Bildning av organiska sekundära partiklar i atmosfären kan vara betydande, men den specifika betydelsen av trafikavgaser för detta samt hur potentiell toxicitet av avgaspartiklar förändras vid åldring i atmosfären har inte studerats ännu.

## **4. SPECIFIC FINE PARTICLE CHEMISTRY AND TOXICOLOGICAL ISSUES**

### **4.1. METALS**

Metal particles are derived from fuel and lubricant additives, fuel contamination, engine wear, catalysts, brakes and ambient PM that have passed through the engine system. They may also be produced during tyre and road wear.

#### **4.1.1 Toxicology**

Metal content in traffic related PM often originates from exhaust emission, wearing from brakes and studded tyres and the use of lubricating oils. A number of studies have found correlations between metal content and various biological effects, for example cytokine production, cellular stress, oxidative damage to DNA and radical generating capacity, which might lead to negative health impacts [21, 168-173]. For biological effects issues like water solubility and valence states of metals may be crucial for their ability to interact with biological systems. These aspects are often lacking when metal measurements have been done, which may fog the interpretation of certain studies and may lead both to overestimation and underestimation of toxic capacity.

The oxidative capacity of particular matter sampled from traffic situations has been shown to be strongly associated with the presence of metals. Metals, especially transition metals, have a strong oxidative activity and a capacity to produce reactive oxygen species (ROS). These have the potential to damage membrane lipids, proteins and DNA, which may eventually result in cell death [22, 28, 34, 174, 175]. ROS can be derived either directly from PM or endogenously produced by chemical components of PM, such as metals, PAH and quinones [176-179]. Additionally, ROS are likely involved in the initiation of redox-sensitive signalling pathways including

activation various transcription factors (e.g. NF- $\kappa$ B) , and thereby induction of cytokines and chemotactic factors (e.g. IL-6, IL-8, ICAM-1), which may result in pulmonary inflammation, impairment of lung development, or lung diseases [22, 180-183]

In the epithelial lining fluid (ELF), antioxidants like ascorbate, urate and glutathione are present and may act as a first line of defence, but when they fail to disarm the ROS an oxidative stress might appear. It is defined as a disturbance in the pro-oxidant and antioxidant balance and may be quantified as a decrease in the glutathione/oxidized glutathione ratio in humans [180, 184-186]. Researchers have hypothesized that an undermined antioxidant defence may increase the susceptibility of PM-induced airway inflammation, decreased resistance to infection and worsening of airway symptoms [65, 76, 187-189]. This might explain why some people are more sensitive and more liable to experience adverse health effects after exposure to air pollution.

Apart from the oxidative capacity of metals present in PM, Ghio and co-workers propose a common mechanism behind the biological effects reported following exposure to various particles. Iron is essential in many aspects for the cells to function normally and Ghio et al suggest that the inhaled PM interferes with the iron homeostasis in the lung's cells and tissues. This disruption results in an oxidative stress which is associated with the initiation and activation of a range of signalling pathways and transcription factor involved in the inflammatory response. This proposed mechanism would be common to all particles, including those that do not contain metals when inhaled [190].

### **4.1.2 Epidemiology**

When elements were studied simultaneously in the meta-analysis of daily mortality in "The Harvard Six Cities" during 1979-1988 the metals significantly associated with mortality were nickel and lead [104]. At that time point lead could still be seen as a marker of traffic.

As discussed earlier, the Canadian study of daily mortality showed strong associations between mortality and ambient metal (Fe, Ni and Zn) exposure [153].

Utah Valley has provided a unique opportunity to evaluate the health effects of particulate pollution due to the intermittent operation of a local integrated steel mill. A significant positive association between daily mortality and PM<sub>10</sub> pollution has been observed [191]. Other health effects of elevated PM<sub>10</sub> pollution observed in Utah Valley include decreased lung function; increased incidence of respiratory symptoms; increased school absenteeism and increased respiratory hospital admissions [192].

Relative to the natural experiment studies of Utah Valley, a nationwide 8.5-month strike period involving copper smelters in New Mexico, Arizona, Utah, and Nevada resulted in smaller reductions in fine particulate pollution that occurred over a much larger geographic [193]. Over the strike period, a regional improvement in visibility accompanied an approximately 60% decrease in concentrations of suspended sulphate particles. The strike-related estimated percent decrease in mortality was 2.5%. Detailed data on changes in levels of transition metals are not available, but it is likely that there were important reductions in transition metals associated with the temporary closure of the copper smelters.

In a time-series analysis of PM and daily mortality in California, ambient concentrations of EC and many of the other species including Zn demonstrated higher excess risks than PM<sub>2.5</sub> mass [163].

In multi-centre PEACE panel study on asthmatic children there were few associations between metals in PM<sub>10</sub> and respiratory outcomes [194]. Silicon and iron concentrations tended to be negatively associated with peak expiratory flow and positively associated with the prevalence of phlegm. The effects of silicon and iron could not be separated, which indicates that the crustal coarse PM<sub>10</sub> fraction was involved.

A small panel study of COPD patients in Rome found short-term effects on lung function from PM<sub>2.5</sub>, NO<sub>2</sub>, zinc and iron [195].

## **4.2. ORGANICS**

As mentioned earlier the complex chemistry of exhaust emission is only partly understood presently. Of those over 20 000 individual compounds, mainly organics, only approximately 500 have been identified [56]. There is an emerging literature on the biomedical effects of different organic components from engine exhaust, however, little has been presented on gasoline exhaust. The focus in this report has therefore been directed to diesel exhaust related organic compounds.

PM in diesel exhaust contains a high degree of organic components and studies have shown inflammatory responses both in human and animals after exposure. However, when it comes to assigning different effects to specific components, the knowledge is still scarce. This is an important research area, especially since motor engine exhaust has been indicated to bring substantial adverse health effects. A detailed understanding of what chemicals are causing what toxic and health effects is important to establish.

Brooks and colleagues exposed 24 healthy subjects to concentrated ambient particles using a particle concentration and also added ozone to the exposure. The study demonstrated decrease in brachial artery diameter indicating a vasoconstrictive response to the combination of urban particles and ozone [118]. In a follow-up analysis the authors investigated correlations between different chemical constituents during exposure and the acute vascular response. A linear regression analysis indicated both organic and elemental carbon to contribute to the adverse effect on blood vessels. These correlations were considerably stronger than those of PM<sub>2.5</sub> mass [196].

### **4.2.1. Aliphatics**

Both gasoline and diesel fuels as well as engine lubricating oils comprises of a complex chemical mixture. A group of compounds present is aliphatic hydrocarbons

(both normal (n) and branched) which ranges from a carbon chain length of C<sub>4</sub> (butane, gasoline) to more than C<sub>40</sub> (tetradecane, engine lubricating oil).

The aliphatic hydrocarbon emission from vehicles comprises of unburned aliphatic hydrocarbons that originates from the fuel and the engine lubricating oil. Furthermore, in the combustion process in the engine and in the exhaust after treatment system, aliphatic hydrocarbon chain lengths are reduced as the aliphatic hydrocarbons are partly burned in the combustion process. This means for example that an aliphatic hydrocarbon with initially a carbon chain length of C<sub>30</sub> can theoretically in the combustion process produce aliphatic hydrocarbons with carbon chain lengths from C<sub>1</sub> to C<sub>29</sub>. In a study by Carlsson and co-workers [197], aliphatic hydrocarbons in diesel fuels and exhaust emissions from two heavy duty diesel vehicles were investigated. The analysis indicated that the diesel fuels contain aliphatic hydrocarbons ranging from C<sub>12</sub> to C<sub>40</sub>. In addition, a correlation was found between increased final boiling point of the diesel fuel correlates and a high content of larger molecular weight aliphatic hydrocarbons. Both n-alkanes as well as branched alkanes were detected in the diesel fuels investigated. Chemical analyses of the particulate emissions revealed aliphatic (n-alkanes and branched alkanes) chain lengths of mainly C<sub>16</sub> to C<sub>24</sub> with a major fraction of C<sub>21</sub>. Corresponding aliphatic emissions (n-alkanes and branched alkanes) in the semivolatile phase was mainly C<sub>14</sub> to C<sub>21</sub>. Kalaitzoglou et al. (2004) investigated particle phase aliphatic concentrations in urban air in Greece and concluded that n-alkanes (C<sub>21</sub> to C<sub>28</sub>) determined originate from fossil fuel combustion [198].

In summary, this implies that both fuel (gasoline and diesel) and lubricating oil aliphatics have an impact on the aliphatic exhaust emissions. Furthermore, aliphatic exhaust emissions reported from alternative fuels are missing in the scientific literature.

#### **4.2.2. PAH and quinones**

Polycyclic Aromatic Compounds (PAC) is a group of compounds which comprises of mutagenic carcinogenic compounds. Polycyclic Aromatic Hydrocarbons (PAH) area

subgroup of the PAC compounds, which also comprises of mutagenic carcinogenic hydrocarbons [84, 85]. The PAHs is a relative large group of hydrocarbons consisting of two or more condensed aromatic rings. In Sweden a large number of the PAHs (listed below) are recommended for air monitoring programs by the Swedish Environmental Protection Agency (SEPA).

benzo(a)pyrene	fluoranthene
benzo(b)fluoranthene	indeno(1,2,3-cd)pyrene
benzo(ghi)perylene	methyl anthracenes/phenanthrenes
benzo(k)fluoranthene	phenanthrene
dibenz(a,h)anthracene	pyrene
dibenzo(a,l)pyrene	retene

In Sweden, recommended guideline value concentrations in ambient air are set to 0.1 ng/m<sup>3</sup> for benzo(a)pyrene (B(a)P) and for fluoranthene 2 ng/m<sup>3</sup> [199]. So far B(a)P is the only single PAH that is currently regulated within EU. The target value is 1 ng/m<sup>3</sup> as an annual average sampled in the PM<sub>10</sub> fraction [200].

Combustion of fossil fuels in car and truck engines contributes to the production of polycyclic aromatic hydrocarbons (PAH), which are known to correlate with a high toxic potency of combustion derived particulate matter [13, 196, 201-204]. In general, smaller particles have a higher content of PAH bound to the carbonaceous core, which might be due to a relatively high surface area for PAH adsorption [202, 203, 205-207]. The emissions of PAH have been shown to vary between gasoline engines and diesel engines. Gasoline engines emit higher amounts of high molecular weight PAH, while diesel engines are primarily producing low molecular weight PAH [13]. Since motor exhaust contains substantial amount of PAHs, a detailed toxicological understanding is important. Amore detailed effects have been demonstrated in subjects exposed in a PAH-rich work situations.

Asphalt with its content of bitumen may also result in exposure to organic components. A multinational investigation of more than 12 000 asphalt workers indicated benzo-(a) pyrene to be positively associated with mortality from ischemic heart disease. In addition, animal studies, as well as epidemiological studies in

humans, have indicated that PAHs adversely affect blood pressure and heart rate and also have the ability to promote the development of atherosclerosis.

DNA damage is another negative effect connected with exposure to urban PM and both the organic and inorganic components of PM have been shown to contribute to the induction of DNA damage. Investigations have shown that the majority is connected to the organic extract of PM [208]. In addition, they have indicated that the DNA damaging capacity is correlated to the particle size and that the smaller fractions of PM, PM<sub>2.5</sub> or smaller, seem to conduct the highest DNA-reactivity [206, 208-211]. It has been found that DNA reactivity correlate positively with total PAH, carcinogenic PAH, metals and the interaction between PAH and metals. A positive relationship has also been found between radical formation (superoxides, hydroxyperoxide, hydroxyl radicals, semiquinone radicals) and the induction of DNA breakages [13].

### ***Special considerations of quinones***

Quinones have recently been pointed out as a particularly reactive PAH component, which can cause adverse biological events. Quinones are a group of organic compounds that consists of diketones (carbonyls) which are present in diesel exhaust particles [212] and ambient air [213]. Aromatic quinones have previously been identified and measured in gasoline particulate exhaust extracts [214]. The origin of the aromatic quinones is however not fully understood. It is not clear whether they are originating from the gasoline fuel, as uncombusted quinones (initially in the fuel), or whether they are formed in the combustion process. It is well known that fuel properties will affect the chemical composition of the exhaust emitted from engines. By adding ethanol to gasoline there are increased emissions of aldehydes (carbonyls). This might also be true to quinones. It is possible that the addition of ethanol to gasoline fuel increases the emission of quinones. However, there are presently no studies published on this topic.

Quinoid substances found on the surface of vehicle PM emission are mainly generated during the combustion process [35, 215]. As previous mentioned it is known that combustion generated particles have the ability to generate ROS, which is correlated to pulmonary and vascular effects through the induction of different pro-

inflammatory mediators. Redox cycling of quinoid compounds may partially explain the toxicity of PM since they have the ability to reduce oxygen to produce ROS [35]. A number of different quinones have been shown to be present in DEP and the toxicity of these has been evaluated in various experimental studies.

In a recent publication, Xia and colleagues wanted to investigate if the adverse health effects connected to oxidative stress were mainly mediated by the diesel particles themselves, or the chemical components bound to the particle surface [216]. RAW 264.7 cells were used to study the toxic effects of diesel exhaust particles and chemicals, focusing on mitochondrial function. The results showed that a quinone enriched polar fraction from a diesel particulate extract was more potent inducing toxic effects in RAW 264.7 cells than PAH, as reflected in oxygen radical formation, decreased membrane potential, loss of mitochondrial membrane mass and induction of apoptosis.

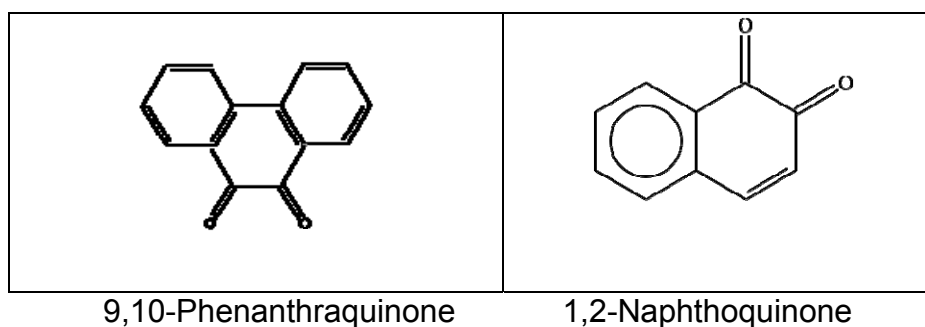


Figure 4.

*Phenanthraquinone* has been identified as a component of diesel exhaust. Investigations have suggested phenanthraquinone to be involved in DEP-induced oxidative stress. This might be due to its interference with oxidative stress-mediated signal transduction and capability to interact with NADPH-cytochrome P450 reductase, leading to an overproduction of ROS [217]. Studies in mice have also shown that intratracheal administration of phenanthraquinone may induce the recruitment of inflammatory cells, such as neutrophils and eosinophils, to the lung, together with increased expression of pro-inflammatory cytokines such as IL-5 and eotaxin. An aggravation of antigen-related airway inflammation together with enhanced production of antigen specific immunoglobulins (IgE and IgG1) has also

been shown [218, 219]. Exposure to *naphthoquinone*, another quinoid substance present in DE, has been shown to exacerbate allergic airway inflammation, which might be due to an increased expression of IL-4, IL-5, eotaxin, macrophage chemoattractant protein-1 and keratinocyte chemoattractant in lung tissue [220].

Exposure to diesel exhaust does not only affect the respiratory system but effects have also been reported in the vascular system. Phenanthraquinone has the capability to reduce endothelial nitric oxide synthase activity, resulting in a suppressed NO-mediated vasorelaxation and increased blood pressure. This indicates that quinoid substances may be involved in the harmful mechanisms of particle exposure connected to the vasculature, and might partially explain the impairment of vasodilatation seen after diesel exhaust exposure [221].

Together, these results strengthen the hypothesis of quinones being involved in the negative health effects associated with diesel exhaust exposure.

### ***Special considerations of nitro-PAHs***

The diesel combustion process occurs at relative higher combustion temperatures, compared to gasoline engines, which will increase formation of NO<sub>x</sub> (NO<sub>x</sub> = in principal NO + NO<sub>2</sub>) from nitrogen and oxygen from the intake air. As PAH are present in the diesel exhaust, nitro derivatives of PAH are formed by the nitration of the mother PAH into corresponding Nitro-PAH during the combustion process and in the tail pipe. Lately, the use of diesel particulate traps as exhaust after treatment technology on diesel engines has increased. The diesel particulate traps contains catalytic material with the purpose to lower ignition temperatures of diesel particulate material collected inside the particulate trap to reduce particulate emissions from diesel engines. By the use of catalytic material in the diesel particulate trap an increased formation of NO<sub>2</sub> may occur and an increased risk for formation of Nitro-PAH can be the case. As nitro derivatives of PAH are highly mutagenic and are present in diesel exhaust [85] increased emissions of nitro-PAH using particulate traps should be evaluated and avoided.

Nitro-PAHs (mono and dinitro PAH) appear to have a very strong toxic potential but so far this has been little studied and come out as an important research area, especially for oxidative diesel particulate traps..

Nitro-PAHs, which are also formed during combustion processes, is one the most potent directly acting bacterial mutagens. Studies have shown that diesel engines emit at least ten times more nitro-PAH as compared with gasoline engines concentration [222-224].

### **4.3. SOOT AND CARBON CORE**

From an epidemiological perspective, studies of short-term effects suggest that acute exposure to elemental carbon (EC) and organic carbon (OC) components of PM have been associated with stronger effects than that associated with total fine particle mass for cardiovascular hospital admissions as well as for mortality [103, 106, 163]. A meta-analysis combined results and found a similar relative risks for PM<sub>10</sub> and black smoke for daily cardiovascular mortality (per unit increase), but for cardiovascular admissions the effect of black smoke was greater than the effect of PM<sub>10</sub> [225]. This difference was seen also in APHEA2 [226].

#### ***Toxicological mechanisms***

Nanometer sized carbon particles (nanoparticles) have been indicated to be extremely toxic due to their small size, which means that they may be able to directly interact with molecules and penetrate cell membranes. Importantly, they have been demonstrated to exert toxicological effects in relationship to their combined surface area. Thus, the total surface area generated by high numbers of small sized particles is often larger than the surface area produced by a few bigger particles. This has been investigated in a number of studies using (carbon black) and comparing it with metal particles, diesel exhaust particles with organics on the surface as well as particles from other sources. Particles may be ingested by alveolar macrophages after inhalation and macrophages may then trigger the airway epithelium through secretion by factors such as TNF- $\alpha$ . The particles may also by themselves induce an oxidative stress in the epithelial lining fluid, which may trigger inflammatory events. The particles may also penetrate epithelial cells and through signalling pathways, including epidermal growth factor receptor (EGFR) and mitogen activator protein kinases (MAPK), active transcription factors such as NF- $\kappa$ B and AP1, leading to inflammation [227]. The cellular pathways involved have been investigated in both human, animal and in-vitro systems. Diesel exhaust particles have generally not been shown to be among the most reactive as regards oxidative stress. Metal rich particles such as coal fly ash seem to have stronger effects.

As mentioned, the nanosized particles appear to be able to penetrate into the vascular system, following inhalation, as has been shown in some animal studies. The particles may thereby influence the functions in the vascular system including the heart. This hypothesis has found some support [228] while two other studies in human subjects have failed to confirm a particle penetration [229, 230]. If particles are able to penetrate it has been calculated that the concentration may be less than 1 % of the total inhaled amount. This may still represent a substantial combined surface area of a large number of nanoparticles and could consequently be related to toxicological events.

Overall, the toxicological literature indicates that nanometre sized PM appear to be very toxic due to size aspects. In addition, this toxicological capacity may also be further enhanced by chemical constituents (e g in vehicle exhaust components) adsorbed to surfaces of such nanoparticles.

#### **4.4. SUMMARY OF SPECIFIC FINE PM CHEMISTRY AND TOXICOLOGICAL ISSUES**

*It is clear that the very small particles often referred to as nanoparticles appear to be very toxic and that this may be associated to size aspects and the circumstance that very large number of particles, even though they may have a small mass due to their small size, may still have an extremely large total surface area. This surface area is what cells in the body has contact with and it has been indicated by Donaldson and colleagues that the surface area of particles may be even more important than sheer particle mass. An additional important issue is that chemical components on the particle surface and properties of the particle itself (e.g. solubility) are extremely important when modulating the toxic effects.*

*As regards chemical components and toxicology it is clear that particle bound transition metals have the ability to cause oxidative stress and that this may relate to adverse health effects in populations. Organic components appear very important for toxicological events and adverse health effects. A detailed understanding of the chemistry associated with lubrication oils, fuels, engine combustion, catalysts and exhaust after treatment devices is very important to study. Some very important studies from Nel and co-workers have demonstrated aliphatics, aromatics and polar components such as quinones to give toxic effects, but in totally different directions. Polar organic components and quinones are together with nitro-PAH (mono/di-nitro PAH) chemicals which now are critical to explore.*

## **DEN FINA PARTIKELFRAKTIONENS SPECIELLA KEMI OCH FARLIGHET**

Ett flertal studier har visat att nanopartiklar (mindre än 0,1  $\mu\text{m}$ ) har en hög toxisk kapacitet. Detta kan sammanhånga med att de pga. sin ringa storlek kan penetrera cellmembran lättare, samt att de ofta förekommer i enormt stort antal, vilket medför att den totala ytan hos partiklarna blir stor, trots att massan är låg. När vi andas in partiklar är det i första hand ytan på dessa partiklar som interagerar med celler i kroppen. Experimentella studier har även indikerat att partikelytan kan vara av större betydelse än partikelmassan när det gäller toxisk effekt.

En annan viktig parameter när det gäller partiklarnas toxiska kapacitet är kemiska komponenter på partiklarna som kan orsaka oönskade biologiska reaktioner. Löslighet och kemisk form (tex valens) kan spela stor roll för om komponenter är inerta och inte orsakar effekter, eller om de är speciellt reaktiva.

Övergångsmetaller på partiklar kan inducera oxidativ stress vilket kan leda till negativa hälsoeffekter. Olika kolvätekomponenter kan också vara av stor betydelse för toxiciteten. Studier har visat toxiska effekter av alifatiska och aromatiska kolväten samt kinoner men effekterna verkar skilja mellan de olika föreningarna. Polära föreningar som kinoner kan t.ex. orsaka oxidativ stress och är mycket reaktiva.

Det är av stor betydelse att försöka förstå vilka kemiska komponenter som leder till vilka effekter och detta är viktigt att studera mer i detalj.

## **5. CONCLUSIONS AND RESEARCH NEEDS**

There is today over a hundred epidemiological studies worldwide that have demonstrated adverse respiratory and cardiovascular health effects, including increased mortality associated with particulate air pollution. Effects have been seen both in adults and children which has attracted growing concern.

Epidemiological and experimental studies have now clearly demonstrated that not all particles are equally toxic, but give different risks for health effects. This is not covered in the current air quality guidelines and limit values. There is a considerable interest to determine the contribution of different particle sources to health effects, and likewise to determine the role of particle size fractions and chemical composition.

The current understanding indicates the fine particle fraction ( $< 2.5 \mu\text{m}$ ), which mainly has its origin from combustion, to be strongly associated with increased death rates from lung- and cardiovascular conditions as well as worsening of diseases. Fine particles have also been indicated to be involved in the observed impairment of lung growth in children living close to main roads. Diesel exhaust has been implied as a major component in the effects on children's lungs and asthma development, as well as acute effects on the cardiovascular system and heart, which is linked to heart attacks after exposure to traffic related pollution.

Coarse particles such as road dust and crustal material has been more strongly associated with worsening of respiratory symptoms including asthma than the fine fraction, but has not been strongly linked to cardiovascular effects and mortality.

Areas where knowledge is especially important and lacking in relationship to health effects:

- The role of different diesel and gasoline engines
- Different fuels – present and future standard and alternative fuels
- Vehicle cold start emissions (at low ambient temperatures) from present and future standard and alternative fuels

- Specific particle components and properties
- Atmospherically transformed (aged) vehicle exhaust
- Lung deposition behaviour in relation to particle properties
- Efficacy of exhaust after-treatment devices
- Efficacy of vehicle cabin filters
- Road dust and health effects
- Road wear components – tyres, studded tyres, road minerals, sanding, brake wear
- Long term health effects of traffic related pollution in children and adults
- Early and later effects of exposure during foetal life

There is also a strong research need to identify and quantify unknown organic and inorganic compounds present in vehicle particulate emissions and in ambient air particles both from an academic and health effects related point of view. Compounds of interest from a health point of view to identify and to be determined in particles in general are: PAH, mono/di nitro PAH and quinones as well as metals and their chemical speciation.

## **FORSKNINGSBEHOV**

Dessa kan baseras på svårighetsgrad och omfattning av hälsoeffekter av partiklar i trafikmiljön. I Sverige avlider närmare 2000 personer varje år pga luftföroreningar vilket vida överskrider trafikolyckornas dödsstatistik. Det är därför rimligt att överväga en nollvision för att "ingen skall behöva dö på grund av de luftföroreningar som trafiken medför". Det är vidare rimligt att trafikrelaterade luftföroreningar inte skall leda till att barns lungor får kroniska skador som leder till långtidseffekter. Dessutom är det betydelsefullt att trafiken inte leder till akuta besvär och symptom från lungor och hjärt-kärlsystemet.

Följande prioriterade forskningsbehov har identifierats:

### **Övergripande frågeställning:**

Trafikrelaterade partiklars ursprung, storlek och kemiska egenskapers betydelse för hälsoeffekter.

### **Detaljfrågeställningar:**

- Hälsoeffekter av avgaser från olika typer av diesel- och bensinmotorer med och utan partikelfilter, körda med nuvarande, framtida samt alternativa bränslen under olika driftsförhållanden.
- Betydelsen av vägdamm och olika slitagekomponenter för hälsoeffekter.
- Långtidseffekter på barns och vuxnas hälsa av trafikrelaterade luftföroreningar.
- Ökade kunskaper om trafikrelaterade partiklars kemiska karakteristika med betydelse för hälsoeffekter, såsom PAH, nitro PAH, kinoner och metaller.
- Olika trafikrelaterade partiklars deponering i lungorna och relation till hälsoeffekter.

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