Traffic related air pollution with emphasis on particle associated polycyclic aromatic hydrocarbons

Tire wear and biodiesel exhaust emissions

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Till min familj
List of publications

This doctoral thesis is based upon the following publications, which are referred to its corresponding Roman numeral throughout the thesis:


* These authors contributed equally to the paper.
The author’s contribution to the above mentioned publications are as follows:

**Paper I:** The author was responsible for the experimental work, majority of the data analysis, some of the statistical analyses, and majority of writing the paper.

**Paper II:** The author was partly responsible for outlining the experimental work and responsible for the majority of the experimental work, data analysis and writing of the paper.

**Paper III:** The author was responsible for conceiving the idea and partly responsible for outlining the experimental work and a minor part of writing the paper.

**Paper IV:** The author was responsible for conceiving the idea and outlining the experimental work and minor part of the experimental work and data analysis, and partly responsible for writing the paper.

**Paper V:** The author was partly responsible for the chemical analysis, and writing a minor part of the paper.
Aim of thesis

With the growing importance of non-exhaust particles relative to vehicular tail-pipe emissions in urban air, it is necessary to investigate the possible contribution of polycyclic aromatic hydrocarbons (PAHs) from the different non-exhaust sources as these inputs are far less characterized than tail-pipe emissions. Tire wear is a contributor to particles in urban air, alongside road dust resuspension and brake wear. Due to its constituents \textit{i.e.}, extender oils and carbon black filler material, tires contain PAHs. Studies regarding the PAH contents in tire rubber are scarce and no study has focused on the highly carcinogenic dibenzopyrenes.

One of the aims of this thesis has been to explore the possibilities of whether tire wear could be a source of PAHs, in particular to the highly carcinogenic dibenzopyrenes in urban air. To be able to quantitatively measure the tire wear contribution to urban air, it is necessary to identify a chemical marker compound or several marker compounds that ideally are unique for that particular source and stable enough to be measured. Benzothiazoles, used as vulcanization accelerators in tire manufacture, were evaluated for their suitability as marker compounds for tire wear particles in urban air.

Diminishing oil reserves and concern for global warming caused by atmospheric inputs of \text{CO}_2 from fossil fuel combustion has led to intensified efforts to find a suitable renewable alternative to petroleum based fuels. The worldwide production volume of biofuels has increased considerably during the last decade. Biodiesel, a biofuel produced from plant and animal fats, has been suggested as a suitable replacement for conventional petroleum based diesel fuels. While the majority of studies have focused on health outcomes from petroleum diesel exhaust exposure, human health effects related to biodiesel exhaust exposure is much less investigated.

The second aim of this thesis has been to investigate how biodiesel fuel influence the emissions of PAHs from diesel engines in comparison to petroleum based diesel. Particles collected on filters where analyzed for >40 PAHs in two separate studies on two different diesel engines, running on neat rapeseed methyl ester (RME), petroleum diesel and a 30 \% RME fuel blend. One of the studies also included determination of four oxygenated PAHs.
List of publications not included in this thesis

The author has also contributed to the following peer-reviewed publications:


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Abbreviations

B30  Biodiesel fuel blend with 30 % biodiesel
B100 100 % biodiesel
CRM Certified Reference Material
Da Daltons
DCM Dichloromethane/methylene chloride
DNA Deoxyribonucleic acid
Dp Particle diameter
EC/OC Elemental carbon/organic carbon ratio
EC/TC Elemental carbon/total carbon ratio
EFSA European Food Safety Agency
EI Electron ionization
EU European Union
FAME Fatty acid methyl ester
FS Full scan
GC Gas chromatography
HA Highly aromatic
HC Hydrocarbons
HPLC High performance liquid chromatography
IARC International Agency for Research on Cancer
IS Internal standard
LVI Large volume injection
m/z Mass-to-charge ratio
MS Mass spectrometry
NIST National Institute of Standards and Technology
Nitro-PAHs Nitro substituted polycyclic aromatic hydrocarbons
Oxy-PAHs Oxygenated polycyclic aromatic hydrocarbons
PACs Polycyclic aromatic compounds
PAHs Polycyclic aromatic hydrocarbons
PFE Pressurized fluid extraction
PLS Partial least squares
PM$_1$ Fine particles with an aerodynamic diameter < 1 µm.
PM$_{2.5}$ Particles with an aerodynamic diameter < 2.5µm.
PM$_{10}$ Particles with an aerodynamic diameter < 10 µm.
PM$_{\text{coarse}}$ Coarse particles, particles with an aerodynamic diameter between 2.5–10 µm.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Term</th>
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<tbody>
<tr>
<td>PN</td>
<td>Particle number</td>
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<tr>
<td>PNC</td>
<td>Particle number concentration</td>
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<tr>
<td>RME</td>
<td>Rapeseed methyl ester</td>
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<tr>
<td>ROS</td>
<td>Reactive oxygen species.</td>
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<tr>
<td>S/SL</td>
<td>Split/splitless</td>
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<tr>
<td>SIM</td>
<td>Selected ion monitoring</td>
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<tr>
<td>SOF</td>
<td>Soluble organic fraction</td>
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<tr>
<td>SRM</td>
<td>Standard Reference Material – Trademarked name for certified reference materials issued by National Institute of Standards and Technology.</td>
</tr>
<tr>
<td>TC</td>
<td>Total carbon</td>
</tr>
<tr>
<td>TEF</td>
<td>Toxic equivalency factor</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>TSP</td>
<td>Total suspended particulates. Airborne particles of all sizes.</td>
</tr>
<tr>
<td>UFP</td>
<td>Ultra-fine particles, particles with an aerodynamic diameter &lt; 0.1 µm.</td>
</tr>
<tr>
<td>USE</td>
<td>Ultrasonic assisted extraction</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume fraction</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>wt-%</td>
<td>Weight percent</td>
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1. Introduction

Air pollution

In the 17\textsuperscript{th} century the English diarist and writer John Evelyn (1620–1706) sent his essay “Fumifugium” to King Charles II to draw his attention to the possible health hazards from breathing smoke originating from the combustion of the cheap sea coal used at the time as fuel for domestic heating in the city of London. He proposed two solutions to this environmental issue by suggesting an exchange to a cleaner fuel, \textit{i.e.} a fuel that produces less smoke when burnt, and a separation of the polluting industry from the general public (Environmental Protection UK, 2011).

The health hazard posed by air pollution were not generally accepted as a problem until after The Great Smog of 1952 in London, where sulfur dioxide (SO\textsubscript{2}) and PM pollution levels where soaring, which – according to contemporary estimates caused at least 4,000 premature deaths (Wilkins, 1954). A more recent estimate suggests that The Great Smog of 1952 caused a staggering 12,000 premature deaths (Bell and Davis, 2001). This smog episode led to one of the first environmental legislation known as the Clean Air Act of 1956, which enforced an exchange of fuel for domestic heating, and a relocation of power stations from cities as a measure to control air pollution and to protect the public health (HMSO, 1956). Although, The Great Smog of London was not the first smog episode with deadly outcomes, it has because of its distinct effect on the public health, and its unmistakable connection between air pollution and adverse health effects become a landmark event in epidemiological studies on air pollution (Anderson, 1999).

Today, the association between increased hospital admissions and daily mortality with air pollution is well established. Increased relative risks of health outcomes such as respiratory diseases (Brunekreef and Holgate, 2002; Bernstein et al., 2004), cardiovascular diseases (Kunzli et al., 2000; Brunekreef and Holgate, 2002; Brook et al., 2010), and lung cancer (Abbey et al., 1999; Raaschou-Nielsen et al., 2013) have all been linked with exposure to air pollution. Air pollution and PM has recently been classified by the IARC as a human carcinogen (Loomis et al., 2013). In a recent report from the WHO it was estimated that air pollution was the cause for 7 million premature deaths worldwide in 2012. This makes air pollution the world’s largest single environmental health risk (WHO, 2014). The number of deaths includes both deaths from ambient air pollution (outdoor) and from
Almost all of the attributed deaths from household air pollution (4.3 million) occurred in low and middle income countries, mainly in the South East Asian and the Western Pacific region. Similarly, the majority (88%) of the attributed deaths from ambient air pollution (3.7 million) occurred in low and middle income countries. Similar to the household air pollution most of these deaths occurred in the South East Asian and the Western Pacific region. In rural areas in developing countries, indoor air pollution occurs at concentrations that are substantially higher than in the developed world due to the use of biomass fuels for heating and cooking (Brunekreef and Holgate, 2002).

PM is the commonly measured air pollutant that has shown a stronger correlation with an increased mortality rate than any other of the major air pollutants such as SO$_2$, nitrogen dioxide (NO$_2$), and ground-level ozone (O$_3$) (Dockery et al., 1993; Schwartz, 1994). The adverse health effects can be observed already at very low concentrations, indicating a no safe threshold for PM (Brunekreef and Holgate, 2002). A large number of epidemiological studies have indicated the important role of PM regarding both short-term and long-term health effects. PM has shown to cause or exacerbate several cardiopulmonary diseases such as, chronic obstructive pulmonary disease, asthma, coronary heart disease and lung cancer (Valavanidis et al., 2008; Brook et al., 2010). The typical susceptible groups are asthmatic children, individuals with pre-existing cardiovascular diseases and elderly people (Ruckerl et al., 2011). Besides being a health hazard, particulate air pollution lowers visibility and contributes to global warming. Because of the sheer chemical complexity and the highly diverse nature of airborne particles, taken together with the gaps of knowledge in the underlying mechanisms for adverse health effects from PM inhalation, the characteristics of PM deserve to be elaborated in a section of its own.
2. Particulate matter

Physicochemical characteristics
Airborne particles include suspended liquid or solid particles in the atmosphere originating from both natural and anthropogenic sources. They are emitted either as primary particles or formed through reactions as secondary particles. In the atmosphere the physicochemical properties of airborne particles can be altered through condensation, evaporation or coagulation processes (Turner and Colbeck, 2009). Airborne particles include a broad range of different particle diameters from a few nm up to approximately 100 µm (Harrison and Yin, 2000). Particles can be characterized by their particle number-, surface area- or particle volume-distributions. (Turner and Colbeck, 2009). Figure 1 shows how these different particle properties are typically distributed across the particle size range in urban air. One of the main characteristics of these distributions is that the ultrafine particles (UFP), with an aerodynamic diameter less than 0.1 µm, constitute the largest fraction of the total number of particles in the atmosphere, while the total surface area is mainly attributed to fine particles with an aerodynamic diameter <1µm (PM$_1$). The particle volume distribution is bimodal i.e., two prominent peaks in its distribution, where the major part of the total volume can be attributed to particles with an aerodynamic diameter up to 0.1 µm. The particle volume distribution and particle mass distribution are similar as long as the particle density does not change with the particle diameter (Turner and Colbeck, 2009).

The smallest particles are the nuclei mode particles with diameters within the range 5–100 nm. They originate from recently emitted hot gases that are formed by gas-to-particle conversion processes. These particles are short-lived and will rapidly be subjected to coagulation and/or condensation to form accumulation mode particles (size range 0.1–2 µm). In this process mass is conserved while the number of particles is not. The concentration by number of nuclei mode particles is usually at its maximum level close to the emission source and declines rapidly with the distance from the source (Turner and Colbeck, 2009). The accumulation mode particles have an atmospheric life time of 1–2 weeks and can be subjected to long range transport and/or atmospheric removal processes such as wet and dry deposition (Turner and Colbeck, 2009). In cities, nuclei mode particles primarily originate from combustion related sources, such as traffic, while
larger particles (>1µm) primarily originate from other sources such as sea spray, wind-blown soils/mineral dust or are mechanically generated from traffic, quarrying, construction and demolition (Thorpe and Harrison, 2008; Turner and Colbeck, 2009; Heal et al., 2012). Naturally occurring particles account for the majority of the particles in the atmosphere, while, in urban areas the characteristics of the airborne particles are highly influenced by anthropogenic activities (Turner and Colbeck, 2009).

Figure 1. Particle number- (upper left), particle surface area- (upper right) and particle volume- (bottom) distributions across different particle diameters in a typical urban aerosol. Adopted from Heal et al. (2012) with permission of The Royal Society of Chemistry.

Currently, the convention is to monitor the two particle metrics PM\textsubscript{10} and PM\textsubscript{2.5} (particles with an aerodynamic diameter of ≤10 µm and ≤2.5 µm, respectively) that both are described by their mass concentrations, for instance in µg/m\textsuperscript{3}. Since PM\textsubscript{2.5} is a subset of PM\textsubscript{10} they comprise of the same nuclei mode and accumulation mode particles. To distinguish between these two particle metrics, another metric could be used. PM\textsubscript{coarse} includes particles in the size range 2.5–10 µm. The sum of all the airborne particles of different sizes is referred to as total suspended particles (TSP). To distinguish the combustion originating components of the airborne particles, measurements of the carbonaceous constituents, rather than particle size is used. These include measurements of metrics such as black smoke, black carbon, and elemental-/organic- carbon (EC/OC). The two former metrics is determined through optical methods, while EC/OC is a thermal-optical measurement of the carbonaceous content of the particles (Heal et al., 2012).

The bulk chemical composition of ambient PM is highly diverse and largely dependent on both the source of primary particles and the processes
involved in the formation of the secondary particles (Turner and Colbeck, 2009), thus the PM composition shows a high spatiotemporal variation, and also varies with particle size. Common major components, that together account for a considerable mass fraction of ambient PM are sulfates, nitrates, ammonium salts/ions, chlorides, carbonaceous material, crustal material derived from e.g., soils, biological material such as spores, pollen, bacteria, viruses, as well as fragments of plant materials (Harrison and Yin, 2000; Turner and Colbeck, 2009). In addition, PM consists of trace metals and trace organic compounds comprising the differentiated compounds of the organic carbon fraction (Turner and Colbeck, 2009). Typically in urban air, UFP are composed to a high extent of carbonaceous materials, both organic and elemental carbon, whereas PM$_{2.5}$ is largely composed of nitrates, sulfates and organic carbon. PM$_{coarse}$ is to a large extent composed of crustal elements (Kennedy, 2007).

Particle toxicity

Inhaled particles are deposited in different regions of the respiratory system depending on particle properties such as size, density and shape (Kocbach Bølling et al., 2009). Adverse health effects from inhaled particles are thought to depend on different factors, such as deposition site and their clearance rates (Kocbach Bølling et al., 2009). In general, larger particles are deposited in the upper lung regions, while UFP have a high probability of deposition in the alveolar region (Hinds, 1999; Ruckerl et al., 2011). Figure 2, shows particle size distribution for deposition in different lung regions, as well as the total deposition in the respiratory system.

It is not known what physicochemical properties of airborne particles are responsible for adverse health effects and neither are the underlying biological mechanisms fully understood (Harrison and Yin, 2000). There is little evidence that any single bulk constituent or trace components of PM could be responsible for the adverse health outcomes shown in epidemiological studies. Furthermore, there is no hitherto known single chemical substance potent enough to cause the observed health effects from PM exposure, given the mass concentrations of the different components in ambient PM (Harrison and Yin, 2000). The lack of a chemical-based toxicological explanation for the epidemiological observations could according to Valberg (2004) be due to:

(1) Synergism, i.e., the chemical mixture is much more toxic than its individual chemical components.
(2) A small portion of the population is especially sensitive to certain ambient PM chemicals.
The adverse health outcomes are unrelated to the chemical constituents of PM.

The apparent toxicity of PM is caused by a previously unmeasured co-pollutant, or depends on some other confounding factor.

Figure 2.  Predicted total and regional deposition fraction as a function of particle diameter according to International Commission on Radiological Protections model during light exercise and nose breathing. Reproduced from Kuempel et al. (2015), published by Taylor & Francis under Open Access.

Nevertheless, in vitro and in vivo studies have highlighted the importance of inflammation and oxidative stress as key mechanisms in PM-induced adverse health effects (Donaldson and Stone, 2003; Lodovici and Bigagli, 2011; Kelly and Fussell, 2015). Oxidative stress occurs from an imbalance between the anti-oxidant capacity of the cell and the production of reactive oxygen species (ROS). The resulting depletion of cellular anti-oxidants may cause damage to cellular components such as lipids, proteins and DNA. Oxidative stress is believed to play an important role in the inflammatory process which is linked to several pulmonary and cardiovascular diseases (Donaldson et al., 2003). There are several trace components associated with PM that have been ascribed a high importance for overall particle toxicity because of their capability to induce the formation of ROS. Trace components of PM such as metals, in particular transition metals such as Fe, Cu, Ni, Cr, Co, V, has been associated with ROS formation and oxidative stress in airway epithelial cells (Valavanidis et al., 2008). It has been suggested that trace metals of PM do not necessarily have to be water-soluble to exert toxicity, although the potency appears to be higher for the
water-soluble fraction (Ghio et al., 1999). Also, organic constituents such as polycyclic aromatic compounds (PACs) and in particular quinones (Oxy-PAH) can induce ROS production though redox cycling (Ayres et al., 2008). It is expected that the effect of these organic constituents of PM is dependent on their bioavailability (Ayres et al., 2008; Møller et al., 2010), but also that the particles themselves, independent of their chemical composition, can cause oxidative stress. UFP made of low-toxicity material exhibit pro-inflammatory effects \textit{in vivo} and \textit{in vitro}, suggesting that the surface area is an important metric for particle toxicity (Brown et al., 2001). There are more indications in the literature that for the toxicity of small particles, both the chemical composition and the surface area are of importance, while for larger particles the chemical composition is more important (Møller et al., 2010). Moreover, the endotoxin content of particles, which predominantly is associated with PM$_{\text{coarse}}$, has been suggested to be a major factor for PM-induced adverse health effects (Schwarze et al., 2006).

The carcinogenic effect of PM is primarily linked to its genotoxic potential, which includes two major types of DNA damage, oxidative damage from ROS and the formation of DNA adducts of organic compounds (Schins and Knaapen, 2007). PAHs which constitute a large group of organic compounds, commonly associated with urban PM, can after metabolic transformation covalently bind to DNA to form bulky DNA adducts (Boström et al., 2002).

Traffic-related particles

Particle emissions from road vehicles, from both exhaust and wear, contribute with a substantial part of the total PM mass in urban environments. At curbside locations in several European cities, vehicular traffic has been estimated to account for 35–55 and 40–60 % by mass of PM$_{10}$ and PM$_{2.5}$, respectively (Querol et al., 2004). It has also been shown that the majority of the particle number concentration (PNC) of UFP in the urban atmosphere can be attributed to road traffic emissions (Pey et al., 2009).

Since traffic PM emissions are significant contributors to urban PM, both PM$_{10}$ and PM$_{2.5}$, it is likely that the observed health effects from PM exposure are substantially driven by traffic related particles. In fact, it has been suggested that almost 50 % of all-cause mortality is due to traffic related PM emissions in some European cities (Kunzli et al., 2000). Several other studies have also highlighted the importance of vehicular PM emissions to adverse health effects (Laden et al., 2000; Sarnat et al., 2008; Ostro et al., 2011).

Vehicular emissions can be separated into exhaust emissions or non-exhaust emissions. Exhaust emissions, which originate from the combustion
process of the engine and is emitted through the tail-pipe, is also referred to as tail-pipe emissions. Non-exhaust emissions on the other hand are produced primarily from mechanical processes such as abrasion of tire treads and road surfaces. Non-exhaust and tail-pipe emissions differentiate substantially in their physical and chemical characteristics. Hence, it is reasonable to suspect that they have different biological effects which might result in different health outcomes upon exposure.

Exhaust emissions

In the urban atmosphere, emissions from mobile sources have a significant impact on the chemical composition and the size distribution of the particles. Mobile sources typically include diesel and gasoline powered road vehicles. Tail-pipe emissions are what typically constitute the majority of the UFP found in the urban atmosphere (Pey et al., 2009). Given the small particle diameters of tail-pipe emissions they have a higher relative contribution of mass to PM$_{2.5}$ than to PM$_{10}$. Also, tail-pipe emissions are typically rich in EC and OC, where diesel particles typically have a higher EC/OC ratio than gasoline related particles (Schauer, 2003). Several epidemiological studies have shown a stronger correlation of PM$_{2.5}$ than PM$_{10}$ to adverse health outcomes (Pope and Dockery, 2006). Furthermore, an association between black smoke and increased mortality has been demonstrated (Pope and Dockery, 2006). Also, the large specific surface area (surface area per mass unit) of smaller particles, is a cause for concern. The reported health effects of small particles, together with the associated biologically active chemical compounds, have made tail-pipe emissions a subject for intense scrutiny.

Diesel-powered vehicular emissions have been ascribed the major role as source of fine particulates in the urban atmosphere, even more so than gasoline-fueled vehicles, despite the prevalence of the latter. Less than two decades ago, the particle mass emissions from diesel engines were typically 10–100 times that of gasoline engines (Kittelson, 1998). However, during the past decades there have been significant advances of the engine- and exhaust after-treatment technologies to meet increasingly stringent emission standards, which ultimately have led to a reduction of diesel particle emissions. The current emission standard in Europe is Euro 6 that was implemented in September 2014 and is valid for passenger cars, as well as for light trucks. The same emission limits are set for both diesel and gasoline engines, i.e. 0.005 g/km for PM and 6.0×10$^{11}$ particles/km, respectively. For heavy-duty diesel engines, the emissions are measured using an entirely different test procedure and the current emission standard for heavy-duty vehicles, Euro VI implemented in January 2013, is 0.01 g/kWh, and 8.0×10$^{11}$ particles/kWh, respectively (DieselNet, 2016). However, the entire vehicle fleet changes more slowly than the implementation of a new emission standard, thus diesel exhaust emissions could still have an appreciable
contribution of particles to urban atmosphere. For instance, Lawrence and co-workers attributed 21 % of the PM$_{10}$ in a road tunnel to diesel exhaust emissions, while gasoline accounted for 12 % (Lawrence et al., 2013). Yin and co-workers used chemical mass balance models and attributed 11.6, 1.4, and 9.7 % of PM$_{2.5}$ (by mass) at an urban background site to diesel engines, gasoline engines, and smoking engines, respectively (Yin et al., 2010). Smoking engines are small engines with atypical high emission of lubrication oil e.g., lawn movers and other small off-road engines. Another scenario is when light-duty diesel engines constitute a large part of the vehicle fleet, consequently resulting in a high contribution from diesel engine emission (El Haddad et al., 2009). However, it should be pointed out that the source apportionment of vehicular exhaust emissions is not without conflicting results regarding the relative contribution of diesel emissions contra gasoline emissions (Gertler, 2005). What appears to be important is the contribution of exhaust particles from the heavy-duty vehicle fleet. A higher particle emission factor of UFP for heavy-duty vehicles in comparison to light-duty vehicles has been demonstrated (Ban-Weiss et al., 2010; Nickel et al., 2013).

An idealized particle size distribution from a diesel engine is shown in Figure 3. The typical features of the particle distribution are that nuclei mode particles (D$_p$ <0.050 µm) make up for the majority of PNC (>90 %) but a minor part of the mass (1–20 %), while the accumulation mode (D$_p$ 0.050–1 µm) particles typically contribute with most of the emitted particle mass. There is also a mode, consisting of coarse particles that have been detached and re-suspended after being deposited on surfaces in the exhaust system which, contributes to the total mass with between 5–20 % (Kittelson, 1998). The nuclei mode particles originate primarily from the condensation of volatile organic compounds that nucleate upon dilution and cooling of the exhaust gas. Nucleation also occurs from the reaction between SO$_3$ and water which leads to the formation of sulfuric acid droplets and sulfates (Kittelson, 1998; Maricq, 2007). The accumulation mode particles typically consist of solid chain aggregates of graphite spherules with adsorbed hydrocarbons, sulfates, and traces of metallic ash (Maricq, 2007). The particle size distribution of diesel particle emissions which is shown in Figure 3, implicates that diesel particles mostly deposit in the two lower airway regions, i.e., tracheobronchial- and alveolar regions (Figure 2).
Unburned fuel and lubrication oil, and partially combusted byproducts thereof, provide hydrocarbons that form nucleation mode particles or condense on the larger accumulation mode particles. Hence, the chemical composition of fuel and/or lubrication oil can be reflected on the chemical composition of the particles. These adsorbed hydrocarbons comprises a vast array of different organic constituents such as PACs (Rogge et al., 1993b; Schauer et al., 1999, 2002; Maricq, 2007; Cheung et al., 2010). The metallic ash content of the diesel particles originates primarily from the lubrication oil (Maricq, 2007). It has been suggested that unburned lubrication oil primarily make up for ~95 % of the volatile hydrocarbons adsorbed on the particles and almost all of the nucleation mode particles (Sakurai et al., 2003). The levels of adsorbed hydrocarbons sometimes referred to as the soluble organic fraction (SOF), can vary between 10 to 90 % by mass depending on operational conditions and engine designs (Kittelson, 1998). It has also been reported that biodiesel-fuel combustion generates particles that contain more SOF in comparison to petroleum-based diesel fuels (Swanson et al., 2007). An oxidation catalyst, removing gaseous hydrocarbons, lowers the SOF content of the diesel particulate material in more modern diesel powered vehicles (Maricq, 2007).

To be able to meet new and more stringent emission standards, with levels well below what is achievable with engine design alone, further development of exhaust after-treatment technology is necessary. There are
different after-treatment systems to control different parts of the emission such as diesel particulate filters for removal of solid particles mainly the accumulation mode, as well as oxidation catalysts for removal of hydrocarbons, and reduction of nucleation mode particles. NO\textsubscript{x} emissions can be controlled with for instance exhaust gas recirculation. The different technical details on these emission reduction strategies and the possible consequences for the emission characteristics are thoroughly reviewed by Maricq (2007).

Since it became known several decades ago that diesel particulates contain mutagenic organic compounds, much of the research has focused on the potential risk of cancer from diesel particle exposure (Lewtas, 2007). Bioassay-directed chemical analysis has made it possible to identify several PAHs and related compounds, such as Nitro-PAHs, as causative agents for the high mutagenic activity of diesel-particle extracts (Lewtas, 2007). Today, diesel-engine exhaust is classified as a human carcinogen by IARC, where PAHs and Nitro-PAHs are the chemical groups of highest concern (IARC, 2014). In addition to cancer, human exposure studies on diesel engine exhaust have shown effects on the pulmonary and cardiovascular system, likely initiated by oxidative stress (Ghio et al., 2012). Diesel particles and organic extracts thereof (obtained by Soxhlet extraction in dichloromethane) have shown to cause oxidative stress in vitro. It was suggested that the SOF may play a major role for oxidative stress, and it was postulated that the ROS was partly originating from the metabolism of PAHs as well as from quinone redox cycling (Baulig et al., 2003).

Non-exhaust emissions

Non-exhaust emissions include particles generated from interaction of road surfaces and tire treads, particles generated from wear of brake linings, and resuspension of road dust (Thorpe and Harrison, 2008). In general non-exhaust particles have larger aerodynamic diameters than tail-pipe emission particles (APEG, 1999) and consist predominantly of coarse mode particles (Pant and Harrison, 2013). Since non-exhaust emissions are not affected by the improvements in engine and after-treatment technologies, they have become of increasing relative importance as tail-pipe emissions have decreased. It should also be pointed out that a vehicle without tail-pipe emissions, e.g., an electric car would still have a non-exhaust emission component. It has in fact been suggested that electric vehicles will have a limited reduction of traffic-related particle emissions (Timmers and Achten, 2016). Using emission factor modelling, Ketzel and co-workers suggested that 50–85 % of the total traffic emissions of PM\textsubscript{10} could be attributed to non-exhaust emissions sources (Ketzel et al., 2007), implying that the latter have a significant impact on urban air quality. Several studies have highlighted the relative importance of vehicular-derived non-exhaust
particles, in particular those resuspended from road dust (Harrison et al., 2001; Lenschow et al., 2001; Abu-Allaban et al., 2003; Bukowiecki et al., 2010; Lawrence et al., 2013; Lawrence et al., 2016). Although, road dust resuspension is considered to be the major non-exhaust source to particles, road abrasion could constitute up to 90 % of the locally emitted PM$_{10}$ due to the extensive use of studded tires and traction sand during winter (Johansson et al., 2007). It has been predicted that by 2020, 80–90 % of the traffic-derived PM$_{10}$ will be of non-exhaust origin in central Europe, given that the non-exhaust emissions remain unchanged (Rexeis and Hausberger, 2009).

**Brake wear particles**

Brake wear particles typically have their mass mean diameters within the PM$_{\text{coarse}}$ size range (Sanders et al., 2003; Iijima et al., 2007), while the PNC is composed of around 90 % of PM$_{2.5}$ particles (Iijima et al., 2007). It has been estimated that 50–70 % of the wear particles escapes the vehicle to become airborne (Sanders et al., 2003). Brake wear particles are typically composed of the metals Fe, Cu, Ba and Sb as major constituents, while K, Ti, Mg and Sn are constituents at lower levels (Thorpe and Harrison, 2008). Important factors for the wear rates of the brake linings and for the particle size distribution for the abraded material are vehicle speed, vehicle weight, severity of the braking and the materials used in the braking system (Sanders et al., 2003; Boulter, 2005).

**Road surface wear particles**

Paved road surfaces could be made of either asphalt or concrete. Asphalt road surfaces are to a large extent (~95 %) composed of mineral aggregates of different sizes along with materials such as bitumen, fillers and adhesives. Concrete road surfaces are made out of mineral aggregates, sand and cement (Thorpe and Harrison, 2008). Road surface wear is particularly pronounced when studded tires or traction sanding are used extensively (Kupiainen et al., 2005; Gustafsson et al., 2009). Gustafsson and co-workers used a road simulator and found that pavement wear is strongly influenced by both vehicle speed and the type of aggregates used in the pavement (Gustafsson et al., 2009). Furthermore, the authors concluded that in comparison to studded tires, the emission of PM$_{10}$ from the non-studded winter tires (frictional tires) was low. Furthermore, summer tires caused a negligible wear of the road surface in comparison. Kupiainen and co-workers observed an increased particle concentration when using traction sand in a road simulator, in which they tested both frictional tires and studded tires. The generated particles were shown to originate from both the pavement and the traction sand, and were mostly composed of minerals and occurred mainly within the PM$_{\text{coarse}}$ range (Kupiainen et al., 2005).
**Tire wear particles**

Tire wear particles are the particles generated from the interaction between the road surface and the tire tread. A significant portion consists of particles larger than PM$_{10}$ (Thorpe and Harrison, 2008) and the majority of the generated particles are deposited directly on or in the vicinity of the road surface (Wik and Dave, 2009). Tire rubber is mainly composed of OC and EC (Hildemann et al., 1991), and usually contains a substantial amount of Zn, due to the use of ZnO in the tire manufacturing process (Thorpe and Harrison, 2008). Rogge and co-workers analyzed tire dust for several different organic compound classes. Out of the identified and chromatographically resolved organic constituents (< 20 % of extracted mass) they found that $n$-alkanes, $n$-alkanoic acids, and natural resins were the most abundant compound classes (Rogge et al., 1993a).

The physicochemical properties of the tire wear particles are dependent on several factors, such as tire, and road-surface characteristics, as well as speed and operation of the vehicle (Thorpe and Harrison, 2008). It has been estimated that merely 5 % of the tire wear particles actually becomes airborne (Cadle and Williams, 1978). Another and more recent estimate suggests that less than 10 % of the tire wear particles is emitted as PM$_{10}$ under typical driving conditions (Boulter, 2005). Nevertheless, sub-micrometer particles have shown to be generated at the road/tire interface using a road simulator (Dahl et al., 2006). The mean particle number diameters measured in that study was similar to those originating from light-duty vehicle tail-pipe emissions. However, the estimated real-world emission factors were only 0.1–1 % of the corresponding light-duty vehicle tail-pipe emission at similar speeds. The authors suggested that sub-micrometer particles mainly originate from mineral oils and carbon black filler material used in tire manufacturing. A typical feature of the larger tire wear particles are their elongated shapes (Cadle and Williams, 1978; Adachi and Tainosho, 2004; Kreider et al., 2010; Gunawardana et al., 2012) with incorporated road surface materials (Adachi and Tainosho, 2004; Kreider et al., 2010). These intermixed particles of tire rubber with incorporated road surface material could account for as much as 95 % of the total tire wear emission of PM$_{2.5}$. Hence, using chemical marker compounds for quantitation of the tire rubber component alone might underestimate the actual contribution of tire wear particles in the atmosphere (Dall'Osto et al., 2014).

**Road dust resuspension**

Road dust could be resuspended by naturally occurring winds, or by the turbulence arising from road traffic. The levels of resuspended particles depend on several factors such as dust load on the road surface, vehicle speed, road maintenance, road wetness and meteorological parameters (Pant and Harrison, 2013). In addition, resuspension has shown to correlate well
with the number of passing heavy-duty vehicles (Thorpe et al., 2007). Road dust is composed of materials from a wide variety of sources, for instance exhaust particles, de-icing salts, soils, plant fragments and other materials of biological origin, as well as particles from other non-exhaust sources (Rogge et al., 1993a; Thorpe and Harrison, 2008). Road dust typically contains crustal elements such as Al, Si, Ca, Fe (Pant and Harrison, 2013). Wear particles from tire treads, road surfaces, and brake linings that are deposited on the road surface that become airborne through resuspension are not easily distinguishable from the particles directly emitted to the atmosphere (Thorpe and Harrison, 2008). Nevertheless, by combining the use of chemical markers and particle size distributions, Harrison and co-workers estimated the individual source contribution of non-exhaust components to 38.1 ± 9.7, 55.3 ± 7.0 and 10.7 ± 2.3 % of particle mass in the size range: 0.9–11.5 μm for resuspension of dust, brake dust and tire dust, respectively (Harrison et al., 2012). Furthermore, the authors concluded that, although site specific, those sources accounted for all road traffic particles with diameters >0.9 μm.

Health effects from non-exhaust particles

Despite the large contribution of coarse particles from non-exhaust sources in urban environments, studies on their potential health effects from exposure are limited. However, PM_{coarse} has been associated with respiratory morbidity in short-term exposure studies (Brunekreef and Forsberg, 2005), increased cardiovascular, cerebrovascular (Perez et al., 2009) and respiratory mortality (Castillejos et al., 2000). Furthermore, in vitro studies have shown that brake wear particles can induce oxidative stress and increase pro-inflammatory responses (Gasser et al., 2009), road wear particles generated between the interaction of pavement and studded tires are at least as inflammatory as diesel exhaust particles (Gustafsson et al., 2008) and organic extracts of tire debris are cytotoxic, have the ability to generate ROS, and cause DNA damage (Gualtieri et al., 2005; Gualtieri et al., 2008).
Polycyclic aromatic hydrocarbons (PAHs) constitute a large group of organic compounds composed of hydrogens and carbons structurally arranged as honeycomb-like ring systems with two or more fused aromatic rings. PAHs can be classified as alternant or non-alternant. Alternant PAH compounds are solely composed of six-membered rings, whereas non-alternant PAH compounds contain at least one four- or five-membered ring (typically the latter) in their chemical structure. Non-alternant PAHs are less stable than the alternant PAHs because of their lower resonance stability (Fetzer, 2000). PAHs with rings that are joined together by only one shared face are called ortho-fused or cata-condensed. Peri-fused or peri-condensed PAHs are those with a ring joined by sharing more than one face. Peri-condensed PAHs have internal $sp^2$-hybridized carbon atoms bonding to three other carbon atoms. Since those carbon atoms are not in the periphery of the structure, they lack covalent bonds to hydrogen atoms (Fetzer, 2000). Some examples of commonly determined PAHs are shown in Figure 4.

Since PAHs constitute such a large and diverse class of compounds, members could have widely different properties. For instance the two-ring alternant naphthalene has a boiling point of approximately 218 °C, while the seven-ring coronene has a boiling point of 525 °C at atmospheric pressure (Achten and Andersson, 2015). Furthermore, a different arrangement of the rings entails different physicochemical properties and biological effects. Thus, two different PAH isomers can have highly different biological effects in a biological system such as the carcinogenic potential (Boström et al., 2002). Some general features of PAHs are their lipophilicities and low water solubilities. The water solubility of PAHs typically decrease with increasing molecular weight (Lee et al., 1981).

PAHs are ubiquitous and persistent environmental pollutants that usually occur in the environment as complex mixtures. PAHs primarily originate from incomplete combustion of organic materials. The mechanisms of PAH formation from incomplete combustion is not fully understood. However, it has been suggested to involve both pyrolysis and pyrosynthesis. During combustion at high temperatures and low oxygen concentrations the organic material is fragmented to smaller species, mostly free radicals (pyrolysis), which subsequently recombine to form simpler PAHs through pyrosynthesis. Once formed, these simpler PAHs can undergo further pyrosynthesis to form larger ring systems (Lee et al., 1981). Other formation processes, un-related
to combustion, involve biosynthesis and degradation of biological materials. PAHs can be of natural or anthropogenic origin of which the latter is the major source of PAHs in the atmospheric environment (Zhang and Tao, 2009). Examples of some natural sources of PAHs are wild fires, volcanic eruptions and crude oils. Anthropogenic sources include residential heating or cooking, waste incinerators, power generation using fossil fuel or biomass, and exhaust emissions from the transport sector. In addition to their abundancies in the geosphere PAHs are also ubiquitous in the interstellar medium of galaxies (Tielens, 2008).

![Diagram of PAHs](attachment:image.png)

**Figure 4.** Examples of some commonly measured PAHs and their structural assignment.

The relative composition of the PAH mixture varies between different sources primarily depending on the formation process. A distinction has been made between long-time produced petrogenic PAHs formed at low temperatures (100–150 °C) and high pressures, short-time produced pyrogenic PAHs formed at high temperatures (>400 °C) and biogenic PAHs formed relatively rapid by biological/chemical processes (Stout et al., 2015). What is typical for petrogenic PAHs is that the mixture is rich in alkyl-substituted PAHs such as methylated- and ethylated- phenanthrenes. Pyrogenic PAH sources display a lower relative abundance of alkylated PAHs in their PAH profile than do petrogenic sources along with a higher ratio of unsubstituted PAHs with higher molecular weights (Andersson and
Achten, 2015). Biogenic PAH source profiles are usually simple and could consist of a single PAH compound, such as retene or perylene (Stout et al., 2015). It has been suggested that some specific PAHs could be used as markers for source attribution. For instance, dominance of chrysene and benzo[k]fluoranthene would indicate an origin from coal combustion, while high levels of benzo[ghi]perylene, coronene and phenanthrene would suggest motor vehicles as major source (Ravindra et al., 2008). Diagnostic ratios between unsubstituted and/or alkylated PAHs have been used as tools to identify different origins of PAHs in the environment, for instance diesel or gasoline exhaust, wood combustion, fossil fuel contamination etc. However, a limitation to the use of diagnostic ratios is that they may change from the source to the recipient either through heterogeneous degradation in the atmosphere, or by gas phase and particle partitioning, together leading to wrong conclusions (Tobiszewski and Namieśnik, 2012). Because of the intrinsic relation between the formation process and the composition of the PAH mixture, PAH profiles have been utilized in source apportionment modelling. However, to be able to more efficiently differentiate between sources the number of PAHs and other PACs in the profile should be substantially increased. Inclusion of other organic and inorganic marker compounds is to be preferred as well (Larsen and Baker, 2003; Stout et al., 2015).

In 1775 the English surgeon sir Percival Pott (1714–1788) found an association between exposure to soot and scrotal cancer of chimney sweeps. His account was the first description of an occupational cancer disease (Brown and Thornton, 1957). A similar observation was made by Volkmann and Bell who described scrotal skin tumors in German and Scottish paraffin workers (Luch, 2005). The carcinogenic properties of pitch, tar and tarry compounds were well known in the early 20th century, but it was not until the 1930’s that Cook and co-workers identified benzo[a]pyrene, a five-ring PAH, as a cancer-causing constituent in coal tar (Cook et al., 1932). Because of their chemical stability PAHs require metabolic activation by enzymes before they can exert any mutagenic and carcinogenic effects. The enzymatic activation of PAHs results in a wide variety of different reactive intermediates such as epoxides, diolepoxides and quinones (Oxy-PAH) which can react and covalently bind to DNA. However, the diol-epoxide metabolite is regarded to play a central role in adduct formation of PAH-induced mutagenesis and carcinogenesis (Luch, 2005).
Exposure situations and monitoring

Because of the wide distribution of PAHs in our environment, there are several sources of exposure to these compounds. The major source of PAH exposure for a non-smoker, neither exposed through occupation, is the diet. Elevated levels of PAHs can be produced during cooking of food, in particular when grilled over open flames or smoked (Phillips, 1999). PAHs in food could also originate from atmospheric deposition of particles, industrial processing methods or from PAH contaminated water and soil (Bansal and Kim, 2015). Important major contributors to dietary intake of PAHs include cereals, oils, fats and vegetables (Phillips, 1999). Typical occupations with high exposure levels of PAHs include aluminum production workers, coke-oven workers, chimney sweeps, and workers exposed to coal-tar products. These exposures occur primarily through inhalation or dermal contact (IARC, 2010).

Since humans spend the majority (>80 %) of their lives in indoor environments, the indoor PAH levels may constitute a major portion of the total exposure, but the relative contributions of those exposure situations are poorly understood (Choi and Spengler, 2014). Sources of indoor PAHs include emissions from cooking and heating, cigarette smoking, candle and incense burning, but also infiltration from outdoor sources (Maertens et al., 2004). It has been suggested that infiltration of PAH may be a major source of PAHs in indoor environments (Choi and Spengler, 2014). However, in the rural areas of the developing countries indoor PAHs are more likely to originate from biomass-burning for heating and cooking.

Currently benzo[a]pyrene is the only PAH classified as a human carcinogen (Group 1) by the IARC, while several are classified either in Group 2A (probably carcinogenic to humans) or Group 2B (possibly carcinogenic to humans). Both the United States Environmental Protection Agency (US EPA) and the European Union (EU) have setup priority lists of PAHs. EPA have suggested 16 PAH for environmental monitoring and EU have its 15+1 priority list of PAHs for food safety monitoring, both include PAHs that are of particular concern for the environment or for human health due to their wide distribution and toxicity (Table 1).

The EPA 16 PAH, which was introduced 4 decades ago, has received some criticism for not including three important groups of the PAC family: known highly toxic PAHs e.g., dibenzopyrenes and benzo[c]fluorene, alkylated PAHs, and PACs containing heteroatoms (Andersson and Achten, 2015). The EU 15+1 priority list contains the highly toxic PAHs benzo[c]fluorene and four dibenzopyrenes, but the number of alkylated PAHs is limited to only 5-methylchrysene and no heteroatom PAC is included.
Table 1. Individual PAH compounds included in EPA 16 PAH and EU 15+1 priority lists and their classification according to IARC.

<table>
<thead>
<tr>
<th>PAH compound</th>
<th>EPA 16 PAH</th>
<th>EU 15+1</th>
<th>IARC Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Fluorene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Anthracene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Pyrene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Benzo[c]fluorene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>X X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Chrysene</td>
<td>X X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Cyclopenta[cd]pyrene</td>
<td>X</td>
<td></td>
<td>Group 2A</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>X X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>X X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Benzo[j]fluoranthene</td>
<td>X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>X X</td>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>X X</td>
<td></td>
<td>Group 2A</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>X X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Dibenzo[a,e]pyrene</td>
<td>X</td>
<td></td>
<td>Group 3</td>
</tr>
<tr>
<td>Dibenzo[a,l]pyrene</td>
<td>X</td>
<td></td>
<td>Group 2A</td>
</tr>
<tr>
<td>Dibenzo[a,j]pyrene</td>
<td>X</td>
<td></td>
<td>Group 2B</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>X</td>
<td></td>
<td>Group 2B</td>
</tr>
</tbody>
</table>

Group 1: Carcinogenic to humans, Group 2A: Probably carcinogenic to humans, Group 2B: Possibly carcinogenic to humans, Group 3: Not classifiable as to its carcinogenicity to humans.

Risk assessment

Currently, there are two common methods for quantitative cancer risk assessment of PAH mixtures in air. One uses the concentration of benzo[a]pyrene as a surrogate marker for the entire mixture of PAHs. A lifetime exposure to a concentration of 0.1 ng/m\(^3\) benzo[a]pyrene would correspond to one additional case of cancer in 100,000 (Boström et al., 2002). The current EU limit for benzo[a]pyrene in ambient air is set to 1 ng/m\(^3\) in PM\(_{10}\) (The European Parliament and the Council of the European Union, 2004). The major weakness using this approach is that it was derived...
from lung cancer risk assessments based on exposure to coke-oven emissions, which may not be representative for other types of PAH exposures (Boström et al., 2002). In reality, the relative composition of PAH mixtures varies substantially with source. However, there are studies that support the use of benzo[a]pyrene as a surrogate marker for the occurrence of PAH in ambient air (Brown and Brown, 2012) and marker for carcinogenic potential of indoor and outdoor air (Delgado-Saborit et al., 2011). The surrogate marker approach has been adopted by WHO in their air quality guidelines and by the European Food Safety Agency (EFSA) (WHO, 2000; EFSA, 2008). Because of the poor predictability of the PAH content in food by using solely the benzo[a]pyrene concentration, EFSA has suggested the sum of the four PAHs benzo[a]pyrene, chrysene, benz[a]anthracene, and benzo[b]fluoranthene (PAH4) as a suitable markers for the occurrence of PAHs in food (EFSA, 2008).

The second approach to quantitative risk assessment is the use of toxic equivalency factors. Different PAHs have widely different potencies of causing cancer, thus being assigned different relative potency factors or TEF-values in relation to benzo[a]pyrene. The quantitative cancer risk of the mixture is calculated as the sum of each components assigned TEF-value multiplied by its concentration. The sum is expressed as benzo[a]pyrene equivalents (Boström et al., 2002). There are several limitations attached to this approach, one being the lack of TEF-values for several commonly occurring PAH compounds. Furthermore, there is a high variability in reported TEF-values. Moreover, there is an assumption of additivity for each component, while it is in many cases reasonable to assume synergistic or antagonistic effects when occurring as mixtures (Jarvis et al., 2014).

The wide spread in TEF-values among different PAH compounds, has in one theoretical model been attributed to the geometrical structure of the molecules, i.e. the fjord and bay theory. A PAH molecule that has a fjord or a bay region (examples shown in Figure 5) is expected to exhibit a high activity. Fjord-region PAHs are in general more active than bay-region PAHs, and one of the most potent PAH, dibenzo[a,l]pyrene, is a fjord-region PAH with an assigned TEF-value of 1–100 (Boström et al., 2002). This implies that dibenzo[a,l]pyrene could have a significant contribution to the overall effect albeit being present at low concentrations. Exceptions to this structure-activity relationship exist and the structural requirements for non-alternant PAHs differ from alternant PAHs (Boström et al., 2002).
Sources of PAH compounds in the atmosphere

In ambient air PAHs and other PACs are present as gas phase species and/or associated with PM. The distribution between the gas phase and PM depends on the compound specific vapor pressure, where a lower vapor pressure results in a distribution shifted towards the PM. Other important factors for distribution between the gas phase and PM are ambient temperature and the affinity for the particle matrix (Keyte et al., 2013). PAH compounds with five rings or more are almost entirely associated with PM in ambient air, while PAHs with fewer than three rings, such as anthracene or phenanthrene (178 Da) are mostly gaseous under such conditions. The relative mass of the gaseous PAHs exceeds that of particle-associated PAHs (Landlová et al., 2014) and the majority of the latter is typically occurring on smaller airborne particles, such as PM$_1$ (Layshock et al., 2010; Landlová et al., 2014). The reason may be either that PAH-rich emission sources are typically combustion related and produce smaller particles, or that the higher specific surface area of smaller particles leads to a higher adsorption capacity for PAHs (de Kok et al., 2006).

On a global scale it has been estimated that in 2007 the total emissions of PAHs (EPA 16 PAH) to the atmosphere amounted up to 5.04 × 10$^8$ kg, where residential and commercial biomass burning accounts for 60.5 %, agricultural waste burning, deforestation, and wildfires for 13.6 %, and on-road vehicular tail-pipe emissions for 12.8 % (Shen et al., 2013). The sources of PAHs differ substantially between different countries. As an example, 32.9 % of the PAH emission in Russia in 2007 was attributed to motor vehicles, while this source the same year was accounting for merely 3.2 % in Angola, in which PAH-emissions mainly originated from deforestation and wildfires (77.5 %) (Shen et al., 2013). Atmospheric PAH concentrations in urban environments are generally higher than those in rural and remote locations. There is also a seasonal variation, where the concentrations in the winter are higher than in the summer (Prevedouros et al., 2004; Ravindra et al., 2006). Higher PAH concentrations in winter time
have been suggested to be caused by meteorological conditions as well as increased emissions from heat-generation such as domestic wood burning (Ravindra et al., 2008). A declining trend of the PAH emissions on a global scale has been observed over the last decade, which has been attributed to emission mitigation technologies for motor vehicles in the developed countries, centralized heating, and replacement of coal with natural gas for cooking in developing countries (Shen et al., 2013).

After-treatment systems such as catalytic converters for both gasoline and diesel powered engines have had a significant reduction on the automotive PAH emissions (Rogge et al., 1993b; Jalava et al., 2010). Before the catalytic converter is fully functional it needs to reach its operational temperature. Thus, the PAH emissions from a cold engine in comparison to a warm engine can differ substantially. This phenomenon is more pronounced for gasoline engines (10 times higher for Euro 3 engines) than for light-duty diesel engines (2 times higher for Euro 3 engines). Cold-start PAH emissions from gasoline engines could surpass those from diesel engines (Devos et al., 2006) and have indeed been suggested as an important contributor of PAHs (Boström et al., 2002). Diesel particulate filters used for particle removal also removes PAHs associated with the particles from the exhaust downstream the diesel particulate filter.

Automotive exhaust emissions from both diesel and gasoline powered vehicles are significant contributors to PAHs in urban environments (Ravindra et al., 2008). Source apportionment of the sum of the total PAH including both gas phase- and particle-associated PAHs, at urban sites in the UK by Jang and co-workers, suggests that 50.5 % of the PAHs originated from unburned petroleum, 21.4 % was from diesel emissions, while 15.0 % and 13.1 % were from coal and wood combustion, respectively (Jang et al., 2013). In a study by Westerholm and co-workers, the estimated source contribution of benzo[a]pyrene and the four dibenzopyrene isomers dibenzo[a,e]pyrene, dibenzo[a,I]pyrene, dibenzo[a,i]pyrene, and dibenzo[a,h]pyrene in PM$_{10}$ measured at Hornsgatan in Stockholm, was 22 % from diesel emissions, 13 % from gasoline emissions, 43 % from small scale wood burning emissions, and 22 % from long-range transport (Westerholm et al., 2012). By weighting the concentrations of these components with their worst case scenario TEF-values, the corresponding benzo[a]pyrene equivalent contributions were 37 % for diesel emissions, 7 % for gasoline emissions, 24 % for small scale wood burning, and 32 % for long-range transport (Westerholm et al., 2012). Moeinaddini and co-workers attributed the relative contribution to PAHs (EPA 16 PAH) in PM$_4$ in Teheran to 56.5 % from diesel emissions, 15.5 %, from gasoline emissions, 13 %, from wood combustion and incineration, 9.2 % for industrial emissions, and 6.0 % from road soil particle emissions (Moeinaddini et al., 2014).
Non-exhaust emissions as sources to airborne PAHs are by far less characterized than exhaust emissions. All different non-exhaust particle sources have the potential to contribute with PAHs to the atmosphere. Tires contain PAHs as a consequence of the use of extender oils and carbon black filler material in tire manufacturing (Ahlbom and Duus, 1994). Road surfaces, particularly pavements with bituminous binder (asphalt) contain PAHs as well (Gadd and Kennedy, 2003). PAHs have also been detected in small amounts in brake lining particles (Rogge et al., 1993a), and road dust resuspension, has already been identified as an important contributor to atmospheric PAHs (Harrison et al., 1996).

PAH derivatives

The National Toxicology Program has estimated that the PAC family consist of more than 1500 different compounds (NTP, 2012). Among these are several PAH derivatives, such as methylated and other alkylated PAHs, nitro-substituted PAH (Nitro-PAH), Oxy-PAHs, and heterocyclic arenes with at least one sulfur-, oxygen- or nitrogen atom in one of the aromatic rings. Structures of some representative compounds from the different classes are depicted in Figure 6.

The importance of the alkylated PAHs arises from their high prevalence and that several compounds of this class are more toxic than their parent compound. As an example, 7,12-dimethylbenz[a]anthracene has been suggested to be 20 times as potent carcinogen than benz[a]anthracene and twice that of benzo[a]pyrene (Andersson and Achten, 2015). Another potent methyl derivative is 5-methylchrysene with a suggested TEF-value of 7, thus being at least 7 times more potent than chrysene itself (TEF-value = 0.001–0.89) (Boström et al., 2002). It should however be emphasized that surprisingly little is known about the toxicity of alkylated PAHs, despite their high abundancy in petroleum (Stout et al., 2015) and on diesel exhaust particles (Williams et al., 1989; Poster et al., 2003a). In diesel fuel, the content of heterocyclic PACs, consists primarily of carbazole and dibenzothiophene and their alkyl derivatives, which is reflected in the exhaust emission (Williams et al., 1986). Similarly to the alkyl substituted PAHs, the toxicity of heterocyclic PACs is far less characterized than PAHs (Andersson and Achten, 2015). The carcinogenicity of some heterocyclic aromatic compounds has been evaluated by IARC, who classified dibenz[a,j]acridine as belonging to Group 2A (probably carcinogenic to humans), dibenz[a,h]acridine, dibenz[c,h]acridine, carbazole, and 7H-dibenzo[c,g]carbazole to Group 2B (possibly carcinogenic to humans), and benz[a]acridine, benzen[c]acridine, dibenzothiophene, and benzo[b]naphtho[2,1-d]thiophene to Group 3 (not classifiable as to its carcinogenicity to humans) (IARC, 2013).
A substantial amount of bioassay-directed studies on the mutagenic activity of particulate matter extracts has shown that the moderately polar and/or polar fraction is usually responsible for the majority of the total mutagenicity exerted by the sample. This fraction contain compounds such as Nitro-PAHs, aromatic amines, and aromatic ketones (Claxton et al., 2004). Several studies have also highlighted the importance of the quinone enriched organic fraction from different particulate matrices due to their capability of causing oxidative stress in vitro (Li et al., 2002; Xia et al., 2004; Kubatova et al., 2006). Besides being products of incomplete combustion, both Oxy-PAH and Nitro-PAHs can be formed in different atmospheric reactions between PAHs and ozone or atmospheric radicals (Tsapakis and Stephanou, 2007). The mutagenicity of ambient PM has shown to correlate with the content of PAHs and Nitro-PAHs on the particles (de Kok et al., 2006). An important source of Nitro-PAH derivatives in the urban atmosphere is diesel exhaust emissions, which are suggested to be the main contributor of 1-nitropyrene, 2-nitrofluoranthene, and 6-nitrochrysene (Albinet et al., 2007). The two compounds 6-nitrochrysene and 1,6-dinitropyrene are highly potent carcinogens with suggested TEF-values of 10 (Collins et al., 1998). Also some Oxy-PAHs derivatives, such as 9,10-anthraquinone, can be present in the urban atmosphere at concentrations comparable with the more abundant PAHs (Albinet et al., 2007; Ahmed et al., 2015a; Alam et al., 2015).
Figure 6. Some members of the PAC family, depicting the heterocyclic compounds quinoline, carbazole, dibenzofuran, and benzo[b]naphtho[1,2-d]thiophene. Also shown are Nitro-PAHs, the Oxy-PAH 9,10-anthraquinone, and the alkylated 7,12-dimethylbenz[a]anthracene.
4. Analytical methodology for PAHs

There is a wide variety of different approaches for the determination of PAHs associated with particles. Those different approaches include an appropriate sampling strategy, choice of a suitable extraction technique and solvent, selection of methods for cleanup and isolation of the analytes, selection of a chromatographic separation method that provides sufficient resolution between compounds and a suitable detector with sufficient sensitivity and selectivity. Given the high diversity of the entire PAC family, there is a wide variety of possibilities of analytical methods that can be used. However, all possible approaches for chemical analysis of the entire PAC family will not be covered within this thesis. Focus will be on the techniques and procedures that were used in the studies underlying this thesis.

Particle sampling

Testing of regulated emissions and sampling of exhaust particles are typically performed in engine dynamometers for heavy-duty vehicles or chassis dynamometers for light-duty vehicles. The engine/vehicle is operated under preset conditions, which consist of simulated speeds for urban or extra-urban driving (highway or rural) conditions, known as transient testing. Emission testing of heavy-duty vehicles can also involve steady-state testing where the engine is operated at different modes with preset engine load and speeds (DieselNet, 2016). The raw exhaust gas is diluted with filtered ambient air in a dilution tunnel to simulate a real world dilution of exhaust gases. Flow rate of the exhaust and filtered air are maintained constant during the sampling and the regulations stipulates that the temperature during sampling of PM on filters should be kept between 42–52 °C (IRSG, 2012).

Dynamometer testing typically accounts for tail-pipe emissions. This does not reflect the total vehicle fleet and, furthermore particle aging is not accounted for (Pant and Harrison, 2013). Other approaches for assessing vehicle contribution of particles include, roadway/tunnel measurements, twin-site studies and road simulator studies (Pant and Harrison, 2013).

The collection of PM on filters for PAH determination is afflicted by systematic errors, that are caused by the sampling procedure itself. One common sampling artifact is the volatilization of semi-volatile organic
compounds adsorbed to the PM. Likewise, gas phase components can adsorb on both the particles and sampling filters (Turpin et al., 2000). The positive or negative sampling bias resulting from the gas-phase/particle equilibrium can be reduced by equipping the sampling train with a denuder that absorbs gas phase compounds. To include the off-gassed material from the particles in the measurement, a polyurethane foam plug capturing the semi-volatiles downstream the collection filter could be used (Maricq, 2007). Sampling artifacts as a result of the formation of oxidation products of PAHs similar to those that occur in the atmosphere may lead to an underestimation of the PAH concentration on the particles (Tsapakis and Stephanou, 2007; Ravindra et al., 2008). This error is of especially high importance when sampling diesel exhaust particles where Nitro-PAHs could be formed as a result of reaction between PAHs and the NOx rich exhaust gases (IRSG, 2012).

**Sample extraction**

A quantitative determination of the individual components of the organic fraction adsorbed to suspended particles, usually present at trace levels, most often involves extraction using organic solvents. A requirement for any extraction technique is that the procedure leads to stable and high recoveries of the analytes. Low recoveries could lead to large errors in the quantitative results (Colmsjö, 1998) and may also cause that the isolated amount of materials are insufficient for detection.

The basic principle of solvent extraction from particles is the breaking of particle-analyte interaction and to solvate the analytes. The solubility depends to a large extent on the polarity of the solvent and solute. Although PAHs are readily extracted with many different organic solvents, special consideration on the solvent selection is necessary if the analysis includes other PACs, since the extraction yield could differ widely between PAHs and for instance Oxy-PAHs using solvents with different polarities (Stanley et al., 1967). This is of high importance if there is an intention to use the extract in any kind of biological testing (Marvin and Hewitt, 2007). However, when determining the bio-accessible concentration of an analyte, the objective is not necessarily to exhaustively extract the sample but rather to mimic the conditions in the human body, providing a more accurate risk assessment (Mukhtar and Limbeck, 2013). The solvents used for this approach are aqueous, thus low recoveries for non-polar compounds like PAHs are expected. Since some organic solvents such as benzene and dichloromethane (DCM), constitute occupational hazards and produce hazardous wastes their use is limited despite being efficient extraction solvents for PAHs (Colmsjö, 1998).
There are several different extraction techniques and solvent combinations used for extraction of PAHs from different PM matrices, which include techniques such as simple solvent extraction, Soxhlet extraction, ultrasound-assisted solvent extraction (USE), supercritical fluid extraction (SFE), and pressurized fluid extraction (PFE). They all have advantages and disadvantages, which have been reviewed by Colmsjö (1998). Ideally, any extraction technique should give high extraction yields, be quick, cheap, reproducible, be as safe as possible for the operator and require small amounts of solvent. The three extraction techniques USE, Soxhlet and PFE will be described more thoroughly due to their common use in the determination of PM-associated PACs.

To compensate for the inevitable losses of analytes during sample preparation, an internal standard (IS) is added to the sample prior to extraction. The IS should be chemically similar to the analyte, to which it is calibrated against, and should be distinguishable at detection and not occur in the sample. Isotope labelled analogs of the analytes serve as good IS, such as perdeuterated benzo[a]pyrene, in which all hydrogens have been replaced with deuterium ($^2$H). However, the added IS is usually not as deeply incorporated into the sample and is therefore more readily extracted than the analytes. To evaluate the accuracy and precision of an analytical protocol a certified reference material (CRM) could be used (Ulberth, 2006). There are several commercially available particulate matter CRMs, with certified mass fractions for PAHs, such as those issued by the National Institute of Standards and Technology (NIST). Those are trademarked as standard reference material (SRM) e.g., SRM 1650b Diesel Particulate Matter. Extraction yields of PAHs and related compounds using different techniques or solvents have been evaluated using SRMs in numerous of studies, mentioned in the text below. To ensure that the analytes are not introduced into the samples in the laboratory, the samples are accompanied by a blank, which is treated in the same manner as the samples but should not contain the analytes. Possible contaminations of samples will be detected as the presence of analytes in the blank.

**Soxhlet extraction**

Soxhlet extraction was proposed by Franz Ritter von Soxhlet in 1879, who developed his technique for the determination of milk fat (Jensen, 2007). Today Soxhlet extraction is somewhat regarded as a benchmark technique for solvent extraction of solid samples. The equipment is an all glass lab ware consisting of an extraction chamber, a flask and a water-cooled reflux condenser (Figure 7). The sample is put inside a thimble, typically made of cellulose, and placed in the extraction chamber. The flask containing the extraction solvent is boiled and the solvent vapors pass through the bypass arm and are condensed above the sample, slowly filling the extraction...
chamber with solvent. When the solvent has reached up to the level of the siphon arm, the solvent with the extracted components from the sample are drawn back down into the flask, completing one extraction cycle. The extraction cycles are typically repeated for approximately 20 h (Colmsjö, 1998), which usually makes Soxhlet extraction a time-consuming technique. However, the dynamic extraction usually results in high recoveries for PAHs.

Figure 7. Schematic overview of a Soxhlet apparatus depicting: (A) Condenser (B) Cooling water outlet (C) Cooling water inlet (D) Bypass arm (E) Extraction chamber (F) Siphon arm (G) Thimble (H) Solvent flask.
Ultrasound-assisted solvent extraction

Ultra-sonication or ultrasound-assisted solvent extraction (USE) utilizes acoustic waves with frequencies above 20 kHz. The ultrasonic waves cause a rapid alternating compression and decompression of the liquid creating bubbles or cavities which when they collapse produces very high temperatures and pressures, and the hot spots can reach 5000 °C and 1000 atm (Luque-García and Luque de Castro, 2003). Close to a solid surface, the cavity collapse is asymmetric resulting in a high-speed jet of liquid (Luque-García and Luque de Castro, 2003), which accelerates the transfer of the analytes into the solvent.

One of the major advantages with USE is that it is fast and several samples can be extracted simultaneously leading to a high sample throughput. However, extraction parameters are difficult to control, and the lack of uniformity in the distribution of the ultrasonic wave could have a negative impact on the repeatability and reproducibility of the method (Luque-García and Luque de Castro, 2003). The extraction yields of PAHs from PM with a high content of EC have been suggested to be inversely proportional to the number of aromatic rings. (Griest et al., 1980).

Pressurized fluid extraction

PFE also known as pressurized liquid extraction or accelerated solvent extraction was introduced in 1996. It utilizes elevated pressures and temperatures (Richter et al., 1996). An elevated extraction temperature facilitates the disruption of the analyte-particle surface interaction and increases the solubility. It also lowers both the viscosity and surface tension of the extraction solvent, thereby allowing a better penetration of the solvent into the sample. An elevated pressure is primarily kept to maintain the solvent in its liquid state, but has been suggested to also force the solvent into pores, and thus increase the recoveries (Richter et al., 1996). In many applications PFE has been regarded as exhaustive, as is Soxhlet, but with the additional advantages of shorter extraction times and lower solvent consumption (Schantz, 2006).

A schematic overview of a PFE instrument is shown in Figure 8. In practice, samples are inserted into extraction cells which in turn are placed into the cell holder tray of the instrument. From a software the extraction parameters such as temperatures, pressures, solvent composition, duration and number of static extraction cycles, can be controlled by the operator. The extraction sequence can be initiated as soon as the set-point temperature has been reached. A pump delivers solvent to fill the cell, now situated in the oven, and the cell is pre-heated. Temperature and pressure are maintained during a defined amount of time (static cycle time), after which the extract is collected into vials completing one static cycle. As an optional procedure the
extraction cell can be filled with different sorbents, either to retain co-extracted interferents or to achieve in-cell fractionation of for instance PAHs and Oxy-PAHs (Lundstedt et al., 2006).

**Figure 8.** A schematic representation of the PFE instrumentation. Courtesy of Christoffer Bergvall.

There have been several reports suggesting that the extraction of some PAHs (and Nitro-PAHs) from diesel PM would be more difficult compared to ambient air PM, which would results in unsatisfactory recoveries (Pineiro-Iglesias et al., 2002). This phenomenon has been attributed to strong analyte–particle interactions (Paschke et al., 1992b). The low recoveries of PAHs, in particular those of high molecular weights, appear to be influenced by the amount of SOF on the diesel particles, i.e., more SOF, leads to higher recoveries (Turrio-Baldassarri et al., 2003). Nguyen and Ball suggested a dual-sorption model, in which PAHs absorbed into the SOF are more readily desorbed by solvent than those adsorbed on the diesel particle surface (Nguyen and Ball, 2006).

Studies have indicated that higher molecular weight PAHs are more readily extracted from diesel particles using PFE than Soxhlet (Schantz et al., 1997; Turrio-Baldassarri et al., 2003) or USE (Bergvall and Westerholm, 2008). Masala and co-workers investigated different extraction solvents and solvent compositions of toluene and methanol and concluded that the most effective solvent composition for extracting PAHs from diesel PM was toluene/methanol 9:1 (Masala et al., 2011). The temperature at PFE has been suggested to be the most important parameter, rather than the choice of solvent or pressure, for high extraction recoveries of several PAHs and Nitro-PAHs in diesel PM (Schantz et al., 2012). Ahmed and co-workers evaluated the extraction efficiency of PAHs and Oxy-PAHs from diesel PM.
and concluded that none of the tested extraction methods were optimal for extracting both compound classes simultaneously (Ahmed et al., 2015b).

The use of PFE has been the natural choice in the studies of PM-associated PAHs underlying this thesis, due to the high extraction recoveries reported for this technique. However, in Paper III USE was selected instead to avoid the risk of decomposition of the thermolabile benzothiazoles at elevated temperatures.

Sample cleanup/isolation

A particle extract is highly complex and contains a large number of different constituents. Hence, isolation of the analytes is often necessary before the final instrumental analysis. By reducing the complexity of the sample, interferents can be removed, thus improving the method detection limit, and maintenance of the instrumentation can be kept to minimum.

Solid phase extraction

Solid phase extraction (SPE) is a common technique for sample pretreatment and isolation of the analytes. SPE cleanup is typically performed with commercially available plastic disposable cartridges, with a wide variety in size of the reservoir and amount of sorbent, as well as sorbents commercially available for different applications (Majors, 2011).

A typical SPE cleanup procedure starts by conditioning of the sorbent, followed by loading of the liquid sample, e.g., PM extract, on top of the sorbent. After the liquid sample has penetrated the sorbent, the sorbent is washed with an appropriate amount of solvent to remove potentially interfering compounds, after which the analytes retained on the sorbent can be eluted using an appropriate eluent. The type of sorbent, eluent (type and amount) and washing solution (type and amount) used for the cleanup is highly dependent on the specific application and on the characteristics of both analytes and matrix. Examples of SPE stationary phases used for cleanup of PAHs are e.g., dual-layer sorbent consisting of Florisil, octadecyl (C18)-bonded and zirconium-coated silica used for separation from edible oils (Stenerson et al., 2015), cyanopropyl silica sorbent for fractionation of PAHs, Nitro-PAHs, Oxy-PAHs, and OH-PAHs (Cochran et al., 2012), while an aminopropyl silica sorbent is used by NIST for their cleanup of PAHs extracted from diesel particulate materials (Poster et al., 2003b).

In the work underlying this thesis, SPE with unmodified silica has been applied as the first step of sample pre-treatment. The cleanup procedure did not include a washing step, and the analytes were directly eluted while the more polar constituents of the sample were retained in the sorbent. In Paper I, Paper II, Paper IV, hexane was used as eluent to obtain a non-polar...
fraction consisting of PAHs and other non-polar hydrocarbons e.g., alkanes. In Paper V, the eluent was changed to toluene to elute the more polar Oxy-PAHs from the cartridges.

HPLC fractionation techniques

Samples, such as diesel PM extracts, may still be exceedingly complex after SPE and therefore most often require further cleanup. HPLC applied in preparative mode gives the possibility to isolate and purify complex sample extracts with a higher degree of selectivity. There are two major modes of HPLC, normal phase HPLC (NP-HPLC) and reversed phase HPLC (RP-HPLC), where the former is an established method for group separations and sample cleanup of PACs, including PAHs, dioxins and organochlorine pesticides (Ballschmiter and Wößner, 1998). In NP-HPLC the stationary phase is usually more polar than the mobile phase (HILIC separations excluded) and the retention of the solutes commonly is governed by their adsorption to the stationary phase (Snyder et al., 2010), for instance by polar forces or π-π interactions. Wise and co-workers have demonstrated that PAHs elute mainly according to their number of aromatic rings on aminopropyl silica in NP mode (Wise et al., 1977), which makes it possible to collect discrete isomer ring-fractions or even a single-compound fraction for determination with a procedure known as heart-cut fractionation. HPLC heart-cut fractionation is commonly used in the certification process of SRMs and is considered to produce more reliable quantitative data for PAHs than the whole PAH fraction (Poster et al., 1998). Since the retention in NP-HPLC with stationary phases such as silica usually is governed by polar interactions, it is possible to separate PACs as groups of compounds according to their polarities. An example is shown in Figure 9. Group separation in NP-HPLC has indeed shown to be an invaluable tool aiding in the isolation and identification of biological active compounds in complex extracts. By using HPLC for bioassay-directed fractionations, it has been possible to pin-point possible causative agents to specific biological responses, as in the case of the important contribution of PAHs and Nitro-PAHs to the mutagenic effects of diesel particle extracts (Lewtas, 2007).
Figure 9. Elution sequence of PAHs, Nitro-PAHs, carbazoles, Oxy-PAHs and aza-arenes from a silica stationary phase using a mobile phase gradient of increasing polarity. Reprinted from Stray et al. (1984), Copyright Wiley-VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

Back-flush as a mean of isolating a PAC fraction from a complex sample was first described by Östman and Colmsjö, almost three decades ago (Östman and Colmsjö, 1987). In the back-flush technique the first eluting compound of interest is chosen to serve as a retention time marker for flow reversal. Fractionation of a sample is then done by reversing the flow just before the elution of the marker. All compounds with shorter retention times have at that time already exited the column with the effluent, while the desired back-flush fraction still within the HPLC column begin to migrate backwards to the injector end of the column. Ideally, as long as there are no unwanted adsorption effects, all compounds with a higher capacity factor ($k'$) than the marker will be eluted in a single symmetrical (Gaussian) peak. By using back-flush fractionation it is possible to faster elute compounds, such as high molecular weight PAHs or more polar compounds that otherwise would require very long run times. Hence, it is possible to obtain a fraction containing a wide span of different PACs. Östman and Colmsjö applied this
procedure to isolate a PAC fraction from used crankcase oil, using an aminopropyl stationary phase with hexane as mobile phase. Alkanes, olefins, mono- and di- cyclic aromatic hydrocarbons, which otherwise would interfere with detection of the PACs, were in this way removed from the sample. The differences between a crank case oil sample subjected to only silica SPE cleanup and a sample from additional back-flush fractionation are shown Figure 10. This cleanup procedure has been successfully employed for different compound classes and several different stationary phases, such as PAHs in several different PM matrices (Christensen et al., 2005; Bergvall and Westerholm, 2006), chlorinated-PAHs in urban PM (Östman and Nilsson, 1992), non-ortho substituted PCBs in Aroclor 1254 (Grimvall and Östman, 1994), and polyaromatic nitrogen heterocycles in air samples collected at an aluminum reduction plant (Tollbäck et al., 2000). In Paper IV it is described how back-flush can be used to extract PAHs from lipid rich matrices.

Figure 10. GC/MS full scan (m/z 50–350) chromatograms of (A) used crank-case oil sample only subjected to SPE-cleanup. (B) The same sample with an additional cleanup using back-flush HPLC. Both chromatograms have the same scaling of the y-axis. Courtesy of Conny Östman.
An interesting development of back-flush fractionation is described by Ahmed and co-workers, who used two sequential back-flush fractionation procedures, one to remove polar compounds, while retaining the non-polar fraction including PAHs, followed by isolating a >3-ring fraction of PAHs. This procedure almost entirely replaces manual handling in the sample cleanup (Ahmed et al., 2013).

Instrumental analysis

Both gas chromatography (GC) and RP-HPLC are suitable chromatographic separation techniques for the determination of PAHs. When the analytes occur as complex mixtures, capillary GC is the method of choice, due to its high peak capacity, separating far more components than is usually possible with HPLC (Poster et al., 2006). GC also offers a variety of injection techniques that may decrease the method detection limits. Among these is the large-volume injection (LVI) technique, which is described below.

GC separation with MS detection was used for the separation and detection in all studies underlying this thesis with the exception of Paper III, in which RP-HPLC with tandem MS was used for benzothiazoles.

Large volume injection

There are several different approaches to perform LVI, including different types of injector interfaces in combination with injection techniques. This has been reviewed elsewhere (Hoh and Mastovska, 2008). What they all have in common is that a much larger volume of the sample can be introduced into the GC system than with conventional techniques, thus lowering the method detection limit. This is of high importance when determining PAHs occurring at very low levels, such as dibenzo[a,l]pyrenes. In the work underlying this thesis a programmable temperature vaporizer (PTV) has been used to perform LVI. It is possible to operate the PTV injector as a conventional split/splitless-injector (S/SL-injector), while it has the additional features of rapid heating and cooling.

LVI is conveniently performed by operating the PTV in so called solvent vent mode. In this mode the temperature is kept roughly equal to the boiling point of the solvent. The solvent vapors generated by the injected solvent are eliminated through the split exit of the injector with the aid of an increased carrier gas flow. By eliminating the solvent it is possible to inject a substantially larger volume than the typical 1 µL at S/SL injection, without the risk of flashback from the expanding solvent vapor in a limited injector liner volume. The sample can be introduced into the PTV injector either as one large injection or multiple injections at fixed intervals. By using solvent vent mode it is possible to introduce a HPLC effluent directly into the PTV.
injector. The HPLC mobile phase is introduced at a rate at which the solvent vapors are concurrently eliminated from the injector. The introduction flow rate requires optimization to avoid flooding of the injector liner to ensure keeping a liquid film retaining the otherwise lost analytes in the liner (Tollback et al., 2003). Evaporative losses of analytes can be reduced by using a co-solvent with a higher boiling point (Mol et al., 1996). In most of the studies underlying this thesis n-dodecane was added to the hexane mobile phase as a co-solvent.

After the solvent has been eliminated from the liner, the split valve is closed and the PTV temperature is rapidly increased to desorb and transfer the analytes to the GC using splitless injection.

Gas chromatographic separations

GC is a common separation technique for volatile, thermally stable compounds with limited polarity. In GC the mobile phase is a carrier gas (typically helium in GC/MS analysis), while the stationary phase consist of an immobilized thin film coated on the inner walls of a long and thin capillary column. The solutes partition between the stationary phase and the gas phase and is transported by the carrier gas further down the carrier gas direction to its final destination, the detector. The separation of the solutes is primarily according to their vapor pressure, i.e., volatile solutes elutes earlier than those with a lower vapor pressure. The partition into the gas phase is favored at higher temperatures, thus it is common to increase the GC oven temperature to elute the solutes more quickly. Typical operation temperature ranges between roughly 30 °C to slightly above 300 °C. The chosen operational temperature interval is highly dependent on the type of column stationary phase, boiling points of the solvent and characteristics of the analytes.

One of the most common and non-polar stationary phases is the 100 % methyl substituted silicone. Other common stationary phases are phenyl-substituted silicone and cyanopropyl-phenyl silicone, trifluoroethyl silicone and polyethylene glycol (Sparkman et al., 2011). The multipurpose GC stationary phase, DB-5 consists of 5 % phenyl- and 95 % of methyl-substituted silicone and is one of the most common stationary phases in environmental analysis. In the work underlying this thesis the chromatographic separations have been carried out on a DB-17 column (60 m × 0.25 mm inner diameter with 0.15 µm film thickness). This stationary phase consists of 50 % phenyl- and 50 % methyl-substituted silicone and was selected as the best choice as it provides sufficient resolution of some important PAH compounds, among others benzo[a]fluoranthene / benzo[j]fluoranthene, and dibenz[a,c]anthracene / dibenz[a,h]anthracene (Poster et al., 2006). DB-17 has also shown to be the most suitable stationary
phase for resolving 302-Da PAH isomers such as dibenzopyrenes (Schubert et al., 2003).

GC separation prior to detection is of high importance for the detection of PAHs, especially for isomers such as benzo[a]pyrene and benzo[c]pyrene, which cannot be distinguished by their mass spectra. Co-eluting or poorly separated solutes with common ions may impair the accuracy and precision of the quantitative analysis. There are several PAH compounds that are not fully baseline-resolved and some that completely co-elutes on a DB-17 column. A well-known example is chrysene and triphenylene with identical MS spectra. However, these compounds can be chromatographically separated on a dimethyl 50% liquid crystal column (Poster et al., 2006). In the work presented in Paper I, Paper II, and Paper V several not fully baseline resolved PAH compounds were observed. In most of the cases poor resolution was found between the isomer in the following pairs phenanthrene / anthracene, 2-methylphenanthrene / 2-methylantracene, 9-methylphenanthrene / 1-methylphenanthrene, 3-methylfluoranthene / 1-methylfluoranthene (full co-elution), 1-methylfluoranthene / benzo[a]fluorene, 4-methylpyrene / 1-methylpyrene, chrysene/triphenylene (full co-elution), 6-methylchrysene / 5-methylchrysene and benzo[b]fluoranthene / benzo[k]fluoranthene. Examples of some un-resolved methyl substituted three-ring PAHs are shown in Figure 11. A consequence of poor peak resolution is that when the relative peak heights are very different, the smaller peak could be entirely obscured by the larger, as in the case of 2-methylphenanthrene and 2-methylantracene shown for SRM 1650b in Figure 11B. Those compounds appear more resolved in another diesel particle sample, as shown in Figure 11C. Proper integration is severely hampered by co-elution in the former example. Other problematic examples are co-eluting monomethyl- and dimethyl- phenanthrenes and anthracenes, for which a common m/z 192 ion causes cross-sensitivity. The compound 9-methylantracene (not shown) is an example of this.
Figure 11. Reconstructed ion chromatograms showing GC/MS traces of \( m/z \) 192 (black trace) and \( m/z \) 206 (red trace) from (A) PAH standard (B) SRM 1650b (C) Diesel particulate sample (from Paper V). Peak numbering: (1) 3-methylphenanthrene, (2) 2-methylphenanthrene, (3) 2-methylanthracene, (4) 9-methylphenanthrene, (5) 1-methylphenanthrene, and (6) 1,7-dimethylphenanthrene.

There are cases where non-isomeric PAH compounds can cause cross-sensitivity. In Paper II, cyclopenta\([cd]\)pyrene and chrysene, but not triphenylene, were included in the calibration standard. The molecular ion of chrysene is \( m/z \) 228 and \( m/z \) 226 for cyclopenta\([cd]\)pyrene, but the MS spectrum of chrysene also contain \( m/z \) 226. When those PAHs are not well separated on the column the peak shape of cyclopenta\([cd]\)pyrene becomes distorted, Figure 12A. When analyzing real samples, which also includes triphenylene (molecular ion is \( m/z \) 228) as a native constituent, the peak shape of cyclopenta\([cd]\)pyrene becomes even more distorted (Figure 12B). A similar scenario, but less severe, occurs for the pair benzo\([c]\)phenanthrene / benzo\([ghi]\)fluoranthene, with the targeted ions \( m/z \) 228 and \( m/z \) 226, respectively, where a peak distortion occurs on the latter. Cyclopenta\([cd]\)pyrene, although an important PAH compound was not quantified because of the cross-sensitivity from the co-elution with chrysene and triphenylene. Another example of cross-sensitivity is the unresolved pair...
indeno[1,2,3-cd]pyrene (targeted ion: m/z 276) and dibenz[a,h]anthracene (targeted ion: m/z 278), where the peak of the former is distorted by the m/z 276 ion from dibenz[a,h]anthracene. However, the peak resolution of indeno[1,2,3-cd]pyrene and dibenz[a,h]anthracene has been reported to increase as the GC-column gets worn (Bergvall and Westerholm, 2008).

Figure 12. Reconstructed ion chromatograms showing GC/MS traces of m/z 226 (red trace) and m/z 228 (black trace): (A) in a PAH standard containing chrysene and cyclopenta[cd]pyrene and (B) in a diesel particulate sample.

Besides a poor resolution between targeted compounds, unknown matrix constituents as well as other PAHs may co-elute with the targeted compounds. In an especially troublesome case the unresolved peak is an IS. Figure 13 shows how the deuterated IS, 1-methylpyrene-d₉ co-elutes with matrix constituents in the different particulate samples. Since IS is used for quantitative purposes, the selection of IS is critical. Table 2, shows quantitative results obtained for 1-methylpyrene and 4-methylpyrene, using either pyrene-d₁₀ or 1-methylpyrene-d₉ as IS. The calculated mass fractions using the different IS for the traffic related PM₁₀ (sampled in a road tunnel) or neat biodiesel (B100) emission particles (Paper V) were not significantly different at 5 % level using a student t-test, while the mass fractions of NIST SRM 1650b (diesel particulate material) were significantly different at this level. The mass fractions obtained using pyrene-d₁₀ as IS were in line with the reference values for this diesel particulate material. The wood smoke, and biomass-burning related particle samples (TSP from a cashew nut roasting site in Brazil) consisted of only one replicate, but the chromatogram in Figure 13 shows that 1-methylpyrene-d₉ appears isolated from other nearby peaks. However, it should be pointed out that these interfering matrix constituent may be specific for this particular cleanup procedure (Si SPE with 2 mL toluene and back-flush fractionation using a pentabromobenzyl column, described in Paper V).
Figure 13. A reconstructed ion chromatogram of m/z 225 showing interferences with the IS 1-methylpyrene-d₉ in the different particulate matrices traffic related PM₁₀, NIST SRM1650b, B100 combustion particles, wood smoke particles (generated from a wood stove under oxygen rich environment), and biomass-burning related particles (TSP).
Table 2. Quantitative data for 1-methylpyrene and 4-methylpyrene using either 1-methylpyrene-d₉ or pyrene-d₁₀ as IS in different types of particulate matrices.

<table>
<thead>
<tr>
<th></th>
<th>1-methylpyrene mean ± SD (ng/mg)</th>
<th>4-methylpyrene mean ± SD (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1-methylpyrene-d₉</td>
<td>Pyrene-d₁₀</td>
</tr>
<tr>
<td>Traffic related PM₁₀, n = 3</td>
<td>2.97 ± 0.34</td>
<td>2.65 ± 0.16</td>
</tr>
<tr>
<td>NIST SRM 1650b, n = 3</td>
<td>0.734 ± 0.049</td>
<td>1.59 ± 0.05</td>
</tr>
<tr>
<td>B100 combustion particles, n = 2</td>
<td>1.59 ± 0.28</td>
<td>1.50 ± 0.57</td>
</tr>
<tr>
<td>Wood Smoke particles, n = 1</td>
<td>0.800</td>
<td>0.724</td>
</tr>
<tr>
<td>Biomass-burning*, n = 1</td>
<td>1.23</td>
<td>1.38</td>
</tr>
</tbody>
</table>

* = Data reported as ng/m³. SD = One standard deviation of mean.
Even for a limited number of alkylated, mostly mono-methylated PAHs, it is obvious that a full separation of these compounds puts a high demand on the gas chromatographic method. The number of possible isomers for alkylated PAHs is very large, for instance the number of possible isomers of mono-methylated PAHs with the elemental composition C_{20}H_{12} (252 Da PAHs) is 192 (Andersson and Achten, 2015). It should be noted that there is no stationary phase capable of resolving all priority PAH compounds in a single gas chromatographic run and the number of separation issues is likely to increase as the number of compounds expands beyond the priority lists. Even multidimensional chromatographic techniques such as GC × GC is unable to resolve the vast number of methylated PAHs that could be present in a sample (Skoczynska et al., 2013). Nevertheless, heart-cut techniques in GC have shown promising results for separating otherwise unresolved unsubstituted PAHs (Ahmed et al., 2013).

Mass spectrometry of PAHs

There is a large number of different techniques that encompasses MS. What they all have in common is that the MS instrumentation consists of a pumping system, an ion source, a mass analyzer and a detector, and what is being measured is the mass-to-charge ratio (m/z) as well as the current from the produced ions. In GC separations of PAHs it is common to use electron ionization (EI) as an ionization technique. Other MS ionization techniques and analyzers for PAHs have been reviewed elsewhere (Poster et al., 2006).

In EI, the ionization of analytes is achieved by subjecting them to electrons emitted from a hot filament. These electrons are usually accelerated to a kinetic energy of 70 eV. In the interaction between the electron beam and a molecule an electron is expelled from the molecule, thereby producing a radical cation (molecular ion, M²⁺). Typically the energy transferred to the molecule widely exceeds the energy required for ionization of organic molecules, hence this excess of energy leads to fragmentation of the molecule (de Hoffmann and Stroobant, 2007). The produced fragmentation pattern at 70 eV and at the low pressure in the ion source is very reproducible, hence it is possible to compare the recorded mass spectrum of the molecule, like a fingerprint, with MS spectral libraries to aid in its identification.

Once the molecules have been ionized they are transferred to the mass analyzer. In Paper I, Paper II, Paper IV, and Paper V the mass analyzer consisted of a single quadrupole mass filter. A schematic of the ion source and the quadrupole mass filter is shown in Figure 14.
A single quadrupole mass filter can be operated in two modes, full scan (FS) or selected ion monitoring (SIM), by applying combination of radio frequency and direct current voltage to the rods. In FS a pre-set range of m/z are transmitted through the quadrupole to the detector, which makes up the spectrum. SIM on the other hand limits the transmission of ions to a few, typically one or two are chosen per compound. SIM typically exhibits a higher signal-to-noise ratio and thus a lower limit of detection than FS and is the customary operation mode in quantitation of PACs.

Mass spectra of PAHs are relatively simple in that sense that they often consist of only a few m/z signals. The low tendency of fragmentation of PAHs is a result of their ability to delocalize the excessive electron energies. Typically, the mass spectra of PAHs exhibits a relative high peak intensity for the molecular ion, M⁺, most often corresponding to the base peak (most intense signal), with smaller peaks from losses of one to four hydrogens i.e., [M-1]⁺, [M-2]⁺, [M-3]⁺, and [M-4]⁺. Also, an [M+1]⁺ ion is usually observable in the spectrum originating from the occurrence of the ¹³C isotope, naturally present in the molecules. The intensity is dependent on the number of carbons in the analyte. For larger ring systems, the doubly charged molecular ion (M²⁺) is common. A fragment corresponding to the neutral loss of C₂H₂ via a retro-Diels-Alder mechanism (loss of 26 Da) is frequently observable, but typically at lower abundance (Lee et al., 1981). Mass spectra of methyl-substituted PAHs typically show the molecular ion as base peak. Another peak corresponding to [M-27]⁺, is a result of the loss of one hydrogen, followed by rearrangement to a benzotropylium ion and then loss of a C₂H₂ fragment (Lee et al., 1981). As examples, EI-MS spectra of chrysene and cyclopenta[cd]pyrene are shown in Figure 15A to further illustrate the cross-sensitivity that was previously discussed and shown in Figure 12. PAHs with longer alkyl chains typically show mass spectra with

---

**Figure 14.** Simplified schematics of the EI source and the quadrupole mass filter of the MS system used for PAH determination in the studies underlying this thesis. The sample entry is outside of the plane.
signals corresponding to alkyl losses \( e.g., [M-15]^+ \) and \([M-29]^+ \) of an ethyl-substituted PAH. The mass spectra of dimethylated PAHs usually exhibit the molecular ion as base peak and also an intense \([M-15]^+ \) fragment ion from the loss of one methyl group. Mass spectra of both monomethylated and dimethylated phenanthrene are shown in Figure 15B.

The mass spectra of PACs containing hetero-atoms within their ring system are commonly very similar to PAHs with the base peak typically being the molecular ion. The mass spectra of the Oxy-PAHs studied in Paper V show the molecular ion along with an intense \([M-28]^+ \) ion, the latter corresponding to the loss of CO. If the molecule contains two carbonyl moieties another intense signal, corresponding to the fragment ion \([M-56]^+ \) will be present (Pierce and Katz, 1976). The \([M-28]^+ \) ion signal is the base peak for some Oxy-PAHs, such as for both 9,10-anthraquinone and its perdeuterated analog 9,10-anthraquinone-d₈ (Ahmed et al., 2015b).
Figure 15. Mass spectra of (A) cyclopenta[cd]pyrene (left), and chrysene (right). The [M-2]$^+$ ion corresponding to m/z 226 is highlighted in the mass spectrum of chrysene. (B) Mass spectra of 3-methylphenanthrene (left) and 3,6-dimethylphenanthrene (right).
Coupled HPLC/GC/MS for PAH determination

Online coupled HPLC and GC separation is a powerful technique for measurements of trace level compounds in complex matrices, by combining the sample cleanup possibilities of HPLC with the high peak capacity of GC. As the sample preparation is taking place in a closed system the risk of contamination and sample loss is reduced due to less manual handling. Also the automation possibility of a coupled system significantly increases the sample throughput. There are several different designs and applications of coupled HPLC/GC systems, which are reviewed elsewhere (Hyotylainen and Riekkola, 2003).

The HPLC/GC/MS system used in Paper I, Paper II, Paper IV and Paper V consists of an auto-sampler, a HPLC pump, an UV-detector, three switching valves, and a GC/MS system. Both HPLC and UV detector are programmed from their front panel keypads while the auto-sampler, GC/MS system, valves and acquisition of UV data is programmed and operated by the PC software CMA (CMA Microdialysis AB), ChemStation (Agilent Technologies), and ELDS (Chromatography Data System AB, Svartsjö, Sweden), respectively. The HPLC is interfaced with the GC by a fused-silica capillary connected to the PTV injector of the GC.

The different valve positions and mobile phase flow paths are shown in Figure 16. When a sample is introduced into the HPLC system all the valves are configured according to Figure 16A. With these valve positions the HPLC effluent is diverted to the waste. Just before elution of the retention time marker, valve 1 switches to reverse the flow and the solutes migrates backwards through the HPLC column (Figure 16B). Before a real analytical run the system is calibrated by recording both the time for flow reversal and the elution time of the back-flush peak, in order to successfully collect the back-flush fraction in the loop situated at valve 3. The collection of the back-flush fraction is done by switching valve 2 (Figure 16C). The width of the back-flush peak, together with mobile phase flow, determines the loop size. Once the entire back-flush peak has been eluted from the column, the valve configuration is immediately changed back to the configuration shown in Figure 16B preventing it from being diverted to the waste. The back-flush fraction collected in the loop can now be introduced into the PTV injector of the GC system by switching valve 3, Figure 16D, by using a reduced mobile phase flow, in this case 0.3 mL/min. When the injection of the back-flush fraction is complete, the system returns to the valve configuration shown in Figure 16B to flush mobile phase through the loop, column and capillaries.
Figure 16. Schematic overview of the valve positions of the HPLC compartment of the hyphenated chromatographic system. Arrows denote flow path. ALS = auto-sampler, EPC = Electronic pressure control. The carrier gas is split between the valve and PTV injector. (A) Straight flush: the default position on sample injection. (B) Back-flush: flow is reversed by switching valve 1. (C) Loop connected on valve 3 is filled with the back-flush fraction, by switching valve 2. (D) The sample is introduced into the PTV injector of the GC/MS instrument by switching valve 3.
5. Results

Paper I

Some of the major constituents used in tire rubber are known to contain PAHs, such as mineral oils used as softener and carbon black filler material. Before regulating the PAH content of the mineral oils used in tire manufacturing or re-treading industry, the PAH content could amount to 10–30 % (Ahlbom and Duus, 2003). Since January 2010, the highly aromatic (HA) oils have been replaced in EU with alternatives which according to legislation cannot contain more than 1 mg/kg (1 ppm) of benzo[a]pyrene, or more than 10 mg/kg (10 ppm) of the sum of benzo[a]pyrene, benzo[e]pyrene, benz[a]anthracene, chrysene, benzo[h]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, and dibenzo[a,h]anthracene (The European Parliament and the Council of the European Union, 2005).

Tire wear contributes with a substantial mass loadings of tire debris to the environment (references within Paper I), but few studies have measured the content of PAHs with higher molecular weights in tire rubber. The literature data on benzo[a]pyrene in tire rubber is scarce and even absent for the dibenzopyrene isomers. The aim of Paper I was to evaluate whether tire wear could contribute with highly carcinogenic dibenzopyrenes to the environment by using a previously developed method for determination of 15 PAHs of molecular weights ≥252 Da in diesel exhaust particles.

Tire treads from eight makes of tires from four different manufacturers were abraded using a coarse stainless steel file. The investigated tires included summer, winter (non-studded), and studded winter tires. All of the analyzed tires were manufactured between the years 2004 and 2005. Two different tires of same make were analyzed in triplicate. The abraded rubber material was extracted using PFE and analyzed using online back-flush fractionation and GC/MS analysis.

The extraction recoveries were evaluated by consecutively extracting a tire sample three times using PFE. The first round of extraction recovered 88.9–98.8 % of the PAHs from the tire rubber.

The difference in measured PAH mass fractions between the tires was found to be large, with a ratio of almost 23 between the lowest and the highest measured PAH level. No significant general difference in PAH content was observed between summer and winter tires. However, there was a significant difference from the same manufacturer, with a higher PAH
content in the winter tires compared summer tires. The relative PAH composition of the investigated tires were quite similar and the majority (>92 %) of the studied compounds could be attributed to the five PAHs benzo[ghi]perylene, coronene, indeno[1,2,3-cd]pyrene, benzo[e]pyrene, and benzo[a]pyrene. A linear correlation was observed between the benzo[a]pyrene content and the content of the four dibenzopyrene isomers, suggesting that the content of the dibenzopyrenes in tires can be estimated from the content of benzo[a]pyrene. The annual emission in Sweden of the dibenzopyrenes from tire wear was estimated to 3.6 kg/yr (median). Similarly, the benzo[a]pyrene emission was estimated to 50 kg/yr (median) surpassing the contribution from the estimated diesel particulate emissions in Sweden in 1994 (Boström et al., 2002).

Paper II

Biodiesel, is an alternative fuel composed of fatty acid alkyl esters, usually the methyl ester (FAME), produced from transesterification of fatty acids from plant and animal fats (Gunstone et al., 2007). For the majority of the commercially available biodiesel fuels the carbon chain length of the fatty acid ranges between 16 to 18, with between 0 and 3 desaturations (Gunstone et al., 2007). The relative composition of the different FAMEs varies between fuels produced from different feedstocks (Hoekman et al., 2012). Biodiesel and ethanol together constitute the vast majority of biofuels produced for the global transport sector (REN21, 2015).

Biodiesel fuels are biodegradable, considered non-toxic, and typically produce lower emissions of the regulated pollutants PM, HC, CO, but increased emissions of NOx compared to petroleum based diesel fuel (Laipuerta et al., 2008). Biodiesel can be used as fuel using the current diesel engine technology without any engine modifications (Swanson et al., 2007). The current European fuel standard (EN590:2009) for on-road diesel fuel allows a FAME content up to 7 % (v/v). The aim of EU directive 2009/28/EC implemented to promote the use of renewable energy sources, is that renewable energy sources should account for 10 % of the total energy used in the transport sector by 2020 (The European Parliament and the Council of the European Union, 2009).

In this study, the range of PAHs was expanded from Paper I to include PAHs in the molecular weight range 178–302 Da. A previously developed analytical methodology was applied to determine PAHs on particles collected on filters from a portable power generator. Three different diesel fuels were used, neat petroleum diesel, a 30/70 wt-% blend rapeseed methyl ester (RME) and petroleum diesel (B30), and a neat RME biodiesel (B100).

The generator, although not representative for real world emissions, allows studying biodiesel emissions with a reduced set of variables and is a
cheap and fast way of generating exhaust emission particles. In addition to PAH emissions, online measurements of NO$_x$, CO, HC, PM and PNC and particle size distribution were conducted.

The emissions from the biodiesel fuels were lower in PAHs, CO, HC, and PN, while NO$_x$ emissions were higher for the biodiesel fuels as expected. Furthermore, the median particle diameter was smallest for B100.

The major PAH constituents determined on the particles were phenanthrene, alkylated phenanthrenes, fluoranthene and pyrene. Although the total PAH emission for B100 fuel was lower, the PAH profiles were different between the B100 particles and the particles from the combustion of the two other fuels. The B100 particles contained a higher percentage of PAHs with a molecular weight $\geq$202, and the emission factors of some PAHs of higher molecular weights i.e., indeno[1,2,3-cd]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, picene and coronene, were higher when combusting B100 fuel. The emission factors of the sum of the four dibenzopyrenes were on the other hand 20 times lower when using B100 as fuel.

Contradictory results were obtained when comparing filter measurements and online measurement of the PM emission from the B100 fuel. Filter measurement is the method stipulated by legislations regarding particle mass emission. This method showed an increased emission of PM mass when using the B100 fuel. The online measurements on the other hand showed a decreased PM emission for this fuel. A possible explanation could be that unburned fuel components from the biodiesel fuel to a larger extent condense either on the filter substrate per se or on the collected particles, thus resulting in a sampling artifact with positive sampling bias.

Thermogravimetric analysis (TGA) of the petroleum diesel and B100 exhaust particles showed that the latter contained considerable more volatile components, which was suggested to originate from unburned or partially combusted fuel components. This supports the suggestion of bias for sampling of B100 fuel emissions. Some issues in the GC/MS analysis of the PAH content were observed when analyzing B100 particle extracts. A FS GC/MS analysis of B100 particle extract revealed the presence of FAMEs. This led to the development of a HPLC cleanup method for the removal of FAMEs from the PAHs prior to the GC/MS analysis in Paper IV.
Benzothiazoles is a group of nitrogen and sulphur containing heterocyclic aromatic compounds with a substituent on the 2 position (Table 3). Within this class of compounds there are several high production volume chemicals commonly used as vulcanization accelerators in tire manufacturing (Kloepfer et al., 2004). These compounds that can constitute up to 2 % of the rubber mix (Ahlbom and Duus, 1994). Benzothiazole has previously been used as a marker compound for tire wear debris (Rogge et al., 1993a; Cass, 1998). Other marker compounds used for tire debris in the environment include e.g., styrene butadiene rubber (Cadle and Williams, 1978; Lee et al., 1989), organic zinc compounds (Fauser et al., 2002), hydrogenated resin acids (Kumat et al., 2011), the benzothiazole amines 2-(4-morpholinyl)benzothiazole and \( N \)-cyclohexyl-2-benzothiazoleamine (Kumat et al., 2002).

Table 3. Molecular structure and abbreviation of the studied benzothiazoles

<table>
<thead>
<tr>
<th>Base structure</th>
<th>( R = )</th>
<th>Compound</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>–H</td>
<td>Benzothiazole</td>
<td>BT</td>
</tr>
<tr>
<td></td>
<td>–SH</td>
<td>2-mercaptobenzothiazole</td>
<td>MBT</td>
</tr>
<tr>
<td></td>
<td>–SCH(_3)</td>
<td>2-methylthiobenzothiazole</td>
<td>MTBT</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( N )-cyclohexyl-2-benzothiazole sulphenamide</td>
<td>CBS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2,2′-dithiobisbenzothiazole</td>
<td>MBTS</td>
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</table>

A number of tires, an asphalt sample collected at a busy street in urban Stockholm, and TSP and PM\(_{10}\) samples collected at the same street were all analyzed for five different benzothiazoles (Table 3). The samples were extracted using USE and analyzed using RP-HPLC with tandem MS.

Initially, this study aimed at exploring the possibility of using several benzothiazole derivatives as marker compounds for tire wear particles in urban air. However, only BT and MBT were found above the method quantification limit in both the air particulate samples. Similarly to the PAH content the benzothiazoles, in particular MBT displayed a large variability in mass fractions for the different tire samples, suggesting a limited use for this compound as a quantitative marker. However, MBT was not detected in the asphalt sample, thus narrowing down the possible non-exhaust sources of this compound in urban air.
The airborne concentration of BT, and MBT in PM$_{10}$ was on average 17 pg/m$^3$ and 64 pg/m$^3$, respectively. The corresponding concentration in TSP was 199 pg/m$^3$ and 591 p/m$^3$ for BT and MBT, respectively. Using benzothiazole as marker compound the estimated rubber content of the airborne particles was calculated to be 0.7 % in PM$_{10}$ and 5.5 % in TSP. When using MBT as marker the calculated tire rubber content was 0.2 % and 1.4 % in PM$_{10}$ and TSP. It should however be pointed out that the PM$_{10}$ samples were quite old (10 yrs), thus degradation during storage is not unlikely, which might underestimate the actual concentrations. The storage stability of these benzothiazoles in air PM is however not known.

The relative high content of MBT in tires makes it possible that tire wear can contribute with this compound to urban air. This should be subject to further studies since MBT is considered as being very toxic with allergenic properties (Geier et al., 2002), have documented toxicity to microorganisms (De Wever and Verachtert, 1997), and has recently been classified as a Group 2A compound (probably carcinogenic to humans) by IARC (Grosse et al., 2016).

Paper IV

As a consequence of the chromatographic interference in the analysis of biodiesel exhaust particles in Paper II, the retention behaviors were studied for some major biodiesel fuel constituents and some representative PAHs in five different NP-HPLC systems. The goal was to find a suitable stationary phase and mobile phase composition (hexane/MTBE ratios) to be used for sample cleanup using back-flush HPLC fractionation of biodiesel particulate extracts and in extension edible (vegetable) oil matrices.

Capacity factors of the selected acylglycerols, FAMEs and PAHs, were determined from isocratic runs for five HPLC stationary phases: cyanopropyl, phenylpropyl, pentabromobenzylxypropyl (PBB), nitrophenylpropyl, and aminopropyl modified silica. A stepwise increment of the MTBE content of the mobile phase was made and a simple structure retention relationship model based on partial least squares (PLS) regression was established to find a rationale for different retention behaviours across the tested columns.

The independent variables used in the PLS model were calculated from the relative mass contribution of characteristic moieties for each compound, e.g., mass contribution of aromatic carbons to the total mass of the molecule. The dependent variable, \( f \), were obtained from transforming the experimentally determined capacity factors \( (k') \) using the function:

\[
f = \frac{1}{k+1} \quad (\text{Eq. 1})
\]
The condition with 0 % MTBE was omitted from the model, due to the indication of non-linear effects on the retention between 0 and 5 % MTBE content in the mobile phase. Compounds that did not elute within 30 min were assigned the value of 0, while a compound eluting with the void volume will have a value of 1.

The retention characteristics for the compounds across the tested column can be interpreted from the regression coefficients for the independent variables in the PLS model shown in Figure 17. A negative value of the regression coefficient (y-axis) can be interpreted as a contribution from that particular moiety (x-axis) to the retention for each column. For example, the retention is increased due to the presence of hydroxyl oxygens in the molecule for all the columns, where the strongest effects can be seen for the cyano column and the weakest for the PBB-column. The strongest interaction between moieties typical for PAHs i.e., aromatic hydrogens and aromatic carbons can be observed for the PBB column. The difference between the PBB column and the other tested columns is illustrated in a loadings-plot shown in Figure 2 in Paper IV. This suggests that the PBB column differs from the other tested columns in terms of retention behavior.

A likely retention mechanism for PAHs on the PBB column is the formation of a donor-acceptor complex. The bromine atoms are withdrawing the electrons from the benzene ring of the benzyl group creating a deficiency of electrons in the ring, thus making it an electron acceptor. The aromatic rings systems of PAHs are electron rich, hence they are electron donors. The interaction between the electron rich PAHs and electron poor benzene ring of the stationary phase is what governs the retention.

In order to isolate a back-flush fraction of PAHs from the unwanted lipid constituents, the latter must have a weaker retention than the PAH analytes. In this particular case, that means that the selectivity factor compared to the backflush retention time marker (in this case anthracene) for flow reversal of the compounds must be below one. Figure 18 shows how the selectivity of the investigated compounds changes with increasing MTBE composition for the five columns. All compounds with a selectivity more than 1, i.e., above the dotted line in the diagrams elutes after anthracene at that particular mobile phase composition and would thus be isolated in a back-flush fraction using anthracene as retention time marker for flow reversal.
Figure 17. Regression coefficients for the independent variables in the PLS model across the tested HPLC columns. The independent variables are the relative mass contributions for each moiety to the overall molecular weight, except Molecular weight and MTBE %.
Figure 18. Selectivity compared to anthracene for the investigated acylglycerols, FAMEs and PAHs across the different columns and with different mixtures of MTBE in hexane as mobile phases.
From Figure 18 it is clear that PBB has the satisfying retention characteristics for the intended application since the lipid compounds elute before the PAHs. A biodiesel particle extract, was analyzed with HPLC/GC/MS in FS mode with the PBB column installed in the HPLC. The extract was also analyzed using the same parameters as used in Paper II (Nitrophenylpropyl column, using 100 % hexane as the mobile phase) to compare the cleanup efficiency of the two columns. The chromatograms from the two analytical runs are shown in Figure 19. The differences in selectivity and cleanup capability of the two columns determining PAHs in a lipid rich matrix are clearly shown. The accuracy of the method was tested by analyzing a vegetable oil CRM spiked with PAHs (BCR-458 issued by Institute for Reference Materials and Measurements, Geel, Belgium). Using Student’s t-test no significant differences were found at a 5 % level, between the measured mass fractions and the certified mass fractions for pyrene and benzo[ghi]perylene. Chrysene, benzo[k]fluoranthene and benzo[a]pyrene were overestimated by 16–27 %, while, the measured mass fraction of indeno[1,2,3-cd]pyrene was underestimated by 30 %.

Figure 19. Overlaid GC/MS chromatograms of a biodiesel particle extract using different back-flush fractionation parameters, PBB column and 20 % MTBE in hexane as mobile phase (black trace), nitrophenylpropyl column with 100 % hexane as mobile phase (red trace). Refer to Figure 3 in Paper IV for tentatively identified peaks.
Paper V

In this paper the exhaust emission from biodiesel combustion from another type of diesel engine was investigated. The test engine consisted of a 4 cylinder non-road engine. Particles for offline measurement were collected during the urban part of the European Transient Cycle, while online measurements of particle properties, i.e., PN and particle size distribution, were performed during both the urban part and the idling mode using the three different fuels petroleum diesel, B30, and B100.

A total number of 46 PAHs were included in the analysis of the particle exhaust emissions. In this study the rarely determined but potent carcinogenic PAHs 7,12-dimethylbenz[a]anthracene, 5-methylchrysene and benz[j]aceanthrylene were included as well as four Oxy-PAHs. The emission particles originating from the different fuels were also characterized according to carbon fractioning in OC and EC, and the ratio of EC to total carbon (TC) could be determined.

We could show that when using neat biodiesel, the exhaust emissions of total PM and CO were reduced compared to petroleum diesel, but showed a small increase in NOx emissions. Biodiesel combustion showed a lower number concentration and a shift to smaller particles than the petroleum diesel. However, PN and particle size were less influenced by the different fuels used during the idling operation mode. The EC/TC ratio of the particles indicate that B100 particles contain a higher fraction of organic carbon than the other two particle types, which were similar.

The particles originating from B100 combustion contained less PAH per mass unit of particles (136 µg/g) than particles from the other two fuels (251 µg/g and 312 µg/g for B30 and petroleum diesel, respectively). Also, the emission factor (PAH per kWh) was lower for almost all of the PAHs, except coronene when combusting B100. The most abundant unsubstituted PAHs were phenanthrene, fluoranthene and pyrene regardless of fuel. The methyl substituted three-ringed PAHs, accounted for 29–34 % of the total PAH content of the particles from the fuels. Similar to the previous study in Paper II did the B100 particles contain a higher percentage of PAHs with a higher molecular weight. The mass fractions of several high molecular weight PAHs and of one Oxy-PAH were also higher for the B100 particles. The highest mass fractions of benz[j]aceanthrylene were observed for biodiesel particles. However, the lower PM emission from combusting B100 results in a lower emission factor of this highly potent PAH. The potent carcinogens 7,12-dimethylbenz[a]anthracene and 5-methylchrysene included in this study was not detected in any of the analyzed particle samples.
6. Discussions

Tire wear

It has been shown that the mass contributions of tire wear particles to PM\(_{2.5}\) and PM\(_{10}\) is usually around a few percent, however for PM\(_{10}\) up to 10 % has been reported (Panko et al., 2013). In Paper III, it was estimated that tire rubber contributes with 0.7 % of the mass of PM\(_{10}\) and 5.5 % of TSP. However, as pointed out previously, measurements on the rubber components alone might underestimate the actual contribution of the particles produced in the interface between the road and tire (Kreider et al., 2010; Dall'Osto et al., 2014). Another consequence of this is that the toxic constituents present in the tire rubber are different to particles generated at a road/tire interface because of the inclusion of road material as well as the possible change in chemical composition due to frictional heating (Kreider et al., 2010). Many of the reported source contributions of tire wear particles are based solely on rubber composition. However, quantifying intermixed wear particles, i.e., particles generated from the interaction between tire rubber and road surface, the contribution to PM\(_{10}\) is still relatively low. Panko and co-workers reported a mass contribution ranging between 0.18–2.80 % of these intermixed particles to PM\(_{10}\). These measurements were conducted in several cities in France, USA, and Japan and included both urban and rural sites (Panko et al., 2013).

Several studies have suggested that a large part of the abraded tire rubber ends up on the road surface or close to the road without becoming airborne. Given the mass fractions of the dibenzopyrenes in the tires analyzed in Paper I, in comparison to diesel or gasoline exhaust particles, taken together with the relative low contribution of particle mass to e.g., PM\(_{10}\) and even less for PM\(_{2.5}\), that suggests that tire wear is a minor traffic-related contributor to airborne dibenzopyrenes. Kumata and co-workers estimated that tire wear debris contribute with 0.37 ± 0.18 % to the total benzo[a]pyrene content in PM\(_{10}\) samples collected at road side (Kumata et al., 2011). Taken together with the indication of a linear correlation between the content of benzo[a]pyrene and the dibenzopyrenes in tires found in Paper I, it can be concluded that tire wear particles likely have a limited contribution of these highly carcinogenic compounds to the atmosphere. It has been estimated that by replacing HA-oils with low PAH containing alternatives the PAH emissions from tire wear will decrease by 98 % (Null, 1999). This estimate
is based solely on the contribution of the extender oil to the PAH content in the tire, but carbon blacks typically contain merely traces of PAHs (Ahlbom and Duus, 1994). The suggested reduction of PAHs needs to be confirmed by analyses of tire rubber or preferably tire wear particles generated from a road/tire interface. As the contribution of PAHs from tail-pipe emissions diminishes tire wear particles may become an important traffic related contributor to atmospheric inputs of PAHs in the future unless the tire formulations experiences another major change influencing the PAH content like the European ban of HA-oil ban in tires. There are still gaps in the knowledge regarding the total emission and size distribution of tire wear particles, as well as in the knowledge of emission factors from traffic. The high variability in content of known hazardous compounds, along with the lack of knowledge of the tire composition within the vehicle fleet brings an uncertainty of both emission rates and contribution of PAHs from tire wear.

In the evaluation of several benzothiazoles as marker compounds for tire wear, it became evident that their use has some limitations. One limitation is that almost all of the compounds were present at low concentrations in the tires, MBT being the only exception. This implies that large amount of samples needs to be collected to obtain sufficient material for detection, which entails elevated risk of sampling artifacts due to longer sampling times. Another limitation is the decomposition and/or volatilization resulting in unstable concentrations during storage of samples. The high variability in MBT mass fractions between different tires, as well as and within the same tire limits its use as a marker compound.

Although, benzothiazoles might have limited use as marker compounds for tire wear particles in the atmosphere, the compounds themselves could have an environmental as well as human health impact. In particular MBT, recently classified as probably carcinogenic to humans by IARC (Grosse et al., 2016), was the most abundant of the determined benzothiazoles in tire rubber. Its presence in urban air implies that a large population might be exposed to this compound. It should be noted that the measured concentration of MBT (64 pg/m$^3$) in Paper III is low in comparison to e.g., benzo[a]pyrene at the same site, which had an annual mean of 240 pg/m$^3$ during 2010 (Westerholm et al., 2012). However, MBT has a predicted vapor pressure slightly higher than that of phenanthrene (8.44 × 10$^{-4}$ Torr vs 2.06 × 10$^{-4}$ Torr at 25 °C) (ACD/Labs), which is mostly partitioned to the gaseous phase in ambient air (Landlová et al., 2014). This suggests that MBT might be present mainly in the gas phase of the urban atmosphere. Contrary to the PAHs, MBT is intentionally added to the tire as an important part of the tire rubber formulation, thus it is unlikely that measures to reduce the content of MBT in tires will be taken in the near future.

Although, the majority of the worn tire rubber does not become airborne, tire debris can be transported with urban runoff and/or wind erosion to other environmental compartments, such as road side soils, sewage sludge, waters
recipients and sediments (Wik and Dave, 2009). With the substantial amount of tire rubber worn each year, tire wear may have a significant impact at these environmental compartments. The chemical composition of tires are very complex and one of the most thorough characterizations of the organic constituents of tire dust was made more than two decades ago on one tire sample, where the majority (by mass) of the constituents were not chromatographically resolved (Rogge et al., 1993a), implying that the knowledge of what environmental risk tire wear might pose is rather limited. This is of particular concern when assessing the possible risks of e.g., cancer from playing on artificial turfs made with recycled crumb rubber infill. However, risk assessments based on targeted chemical measurements suggests that these chemicals constitute a small risk (not elevated) for cancer because of the low exposure levels (from e.g., PAHs) or low carcinogenic potency of the more abundant compound (e.g., benzothiazole) (Ginsberg and Toal, 2010). Nevertheless, there are most certainly a lot more trace chemical compounds in tire rubber than we are currently aware of and for several of the identified compounds in tire rubber there is a lack of data on carcinogenicity.

**Biodiesel exhaust**

Biodiesel combustion in comparison to petroleum diesel combustion is not an exception. Biodiesel exhaust emissions differ with regard to particle properties such as PN emission and particle size, as well as chemical characteristics such as relative amount and composition of SOF, relative amounts of PAC compounds, and emission of gaseous components.

In recognition of the link between the sulfur content of the diesel fuel and its consequence on the emissions, mainly with regards to SO₂, a standard was imposed in 1994 limiting the sulfur content of the diesel fuel to 0.2 wt-% (EN590:1993). Other characteristics of the fuel have other effects on the emissions, such as the levels of the particle associated PAHs correlating with the PAH content of the fuel (Westerholm and Li, 1994). The PAHs in the fuel could act as building blocks in the pyrosynthesis of larger species or survive the combustion process. The survival of PAHs is typically below 1 % and varies widely for different PAHs and engine operation conditions (Tancell et al., 1995; Rhead and Hardy, 2003). A lower PAH content also results in a lower emission of particles (Westerholm et al., 2001). The current European fuel standard (EN590:2009) stipulates an upper limit of 0.001 wt-% (10 ppm) of sulfur and a maximum PAH content of 11 wt-% (di-aromatics and larger) in on-road diesel fuel (Comité Européen de Normalisation, 2009). However, the difference in PAH emissions using EN590 or a diesel fuel with an even lower PAH content is no longer statistically significant when a diesel particle filter is used (Lindgren et al.,
Biodiesel which is essentially free of PAHs has in a numerous of studies been shown to emit less PAHs than petroleum diesel when combusted (Borrás et al., 2009; Kooter et al., 2011; Vojtisek-Lom et al., 2012). However, some studies have reported an increased emission of some individual PAHs with neat biodiesel fuel or fuel blends including biodiesel (Cheung et al., 2010; Karavalakis et al., 2011; Topinka et al., 2012). Moreover, an increased emission of some Nitro- and Oxy- PAHs from combustion of neat biodiesel or biodiesel fuel blends has been reported (Karavalakis et al., 2010; Karavalakis et al., 2011). These results are however difficult to generalize as they depend on engine, driving cycle, and the feedstock used to produce the biodiesel fuel (Karavalakis et al., 2011; Topinka et al., 2012).

One consequence of the different emissions of biodiesel combustion is that the risk of sampling artifacts is elevated when using techniques originally developed for petroleum diesel emissions. The HC emission is an example of this, where the sampling line to the FTIR, which measures the HC emission, was heated to 185 °C (Paper II). The boiling points of the fuels are within 317–346 °C and 185–334 °C for B100 (RME) and petroleum diesel, respectively. The lower HC emission obtained in the online measurements that was observed in Paper II when using B100 as fuel may be ascribed to condensation of fuel components in the sampling line before reaching the detection system. As was also observed in Paper II, an increase in the particle mass emission of B100 might be a result of positive sampling bias due to condensing unburned fuel components on the filters and/or collected particles rather than an actual increase in mass emissions. The TGA of the particles showed that biodiesel particles (or the sampler filters) contain substantially more volatile components than petroleum diesel. No discrepancies between measurement methods for particle mass emissions were observed in Paper V. A positive sampling bias could severely impair the interpretation of the emission of particle associated compounds e.g., PAHs.

The SOF of particles is of importance for the particle toxicity, as it among other material contains absorbed toxic PAC compounds. Moreover, the amount of SOF has a positive impact on the extractability of e.g., high molecular weight PAH from particles. This may have implications to the bioavailability of absorbed compounds in the SOF once deposited in the respiratory system. Since, biodiesel particles typically contain more SOF than petroleum diesel particles it could be that absorbed compounds are more readily extracted from these particles to a biological system. As indicated in Paper II, unburned or partially combusted biodiesel fuel components were present on the particles, which might have further implication to bioavailability as the fuel itself is mostly composed of lipids, like the cell membranes. This is currently highly speculative and has to be further investigated.
In these studies presented here both the biodiesel combustion emissions in general and the biodiesel fuel blend showed a lower PN emission as well as a shift in particle size distributions toward smaller particles compared to petroleum diesel emissions, both for the generator set and the larger engine used in the investigations. However, the neat biodiesel combustion showed an emission with the lowest PN and the smallest particle diameters. For the larger engine, the PN and particle diameter did not differ substantially between the fuels when operated in idling mode. The particles in idling mode consisted primarily (by number) of nucleation mode particles, while the majority of PN consisted of accumulation mode particles during the modified European Transient Cycle for the larger engine, as well as during the operation of the generator set. Higher emission of NO\textsubscript{x} and lower emission of CO was also observed for B100 combustion in comparison to petroleum diesel in both studies.

The emission of PAHs was shown to be substantially lower for biodiesel than petroleum diesel in both studies, with a general trend of decreasing PAH emission with higher amount of biodiesel (RME) in the fuel. Phenanthrene, alkylated phenanthrenes, fluoranthene and pyrene are all of high abundance regardless of fuel or engine. There was also a higher relative amount of PAHs with more than four rings in the emission from both engines using biodiesel as fuel. The emission factors of the individual PAH compounds were in general lower when combusting B100, with some exceptions: indeno[1,2,3-cd]fluoranthene, indeno[1,2,3-cd]pyrene, dibenz[a,h]anthracene, picene, and coronene in Paper II, and only coronene in Paper V. The lower PAH emission of biodiesel combustion could primarily be attributed to the large decrease in emission of the most abundant PAHs e.g., phenanthrene and alkylated phenanthrenes. The biodiesel combustion emission factor (per kWh) of phenanthrene and benzo[a]pyrene emissions in Paper II was 74 % and 48 % lower in comparison to petroleum diesel. The corresponding decrease in emission factors in Paper V was 87 % and 67 % for phenanthrene and benzo[a]pyrene.

The Oxy-PAHs content of the particles measured in Paper V, as well as the emission factors of those were lower for B100 combustion in comparison to both B30 and petroleum diesel, where petroleum diesel emissions had the highest content and emission factor. The difference for Oxy-PAHs is somewhat lower than for PAHs and thus the relative composition of Oxy-PAHs differs between B100 and the other fuels. This might be due to the higher oxygen content of the B100 fuel, leading to a more extensive formation of Oxy-PAHs. Another reasonable explanation is that the particles originating from the combustion of the two other fuels are more enriched in PAHs than B100 particles.

Since, neat biodiesel fuel is virtually free of PAHs, there is no contribution from PAHs surviving the combustion as in the case of
petroleum diesel or the biodiesel fuel blend. There is also a lack of PAHs to serve as precursors for the formation of larger ring systems, thus the PAHs on the particles originating from neat biodiesel combustion is mostly a result of pyrolysis and pyrosynthesis of the fuel or originate from the lubricating oil. The substantial amount of diesel-fuel related PAH e.g., phenanthrene and methyl-substituted phenanthrenes (Stout et al., 2015) observed in Paper II and Paper IV, suggests that they could originate from another petrogenic source, such as lubrication oil. Lubrication oil has shown to contain phenanthrene and methyl-substituted phenanthrenes at relatively low concentrations (Paschke et al., 1992a). But since biodiesel particles contain relatively small amounts of PAHs it implicates that the contribution from the lubrication oil may become significant. Lubrication oil is typically an important source to the metallic content of particles (Maricq, 2007). Thus, the lubrication oil may play an important role for the overall particle toxicity of biodiesel combustion exhaust particles. In fact, Cheung and co-workers suggested the lubrication oil to be an important factor for oxidative stress induced by gasoline, petroleum diesel, and biodiesel particles (Cheung et al., 2010).

A vast majority of the studies on health related effects from diesel particle exposure have focused on petroleum diesel combustion, while the human health effects from biodiesel exhaust emission exposure are less characterized (Swanson et al., 2007). Biodiesel particles have been reported to exhibit lower oxidative potential (per kWh), but higher relative cytotoxicity compared to petroleum diesel particles (Kooter et al., 2011). On the contrary, increased oxidative potential, similar cytotoxicity and lower genotoxicity have also been reported for biodiesel particles based on mass of PM (Jalava et al., 2012). Another study showed similar genotoxicity of biodiesel and petroleum diesel combustion particles, but attributed the difference to engine technology rather than to the fuel (Topinka et al., 2012). Cheung and co-workers reported a higher oxidative potential for biodiesel particles on a mass basis, but when considering PM emission and the driven distance the petroleum diesel emission particles exhibited a higher oxidative potential (Cheung et al., 2009). A recent human exposure study comparing the acute cardiovascular effects of RME biodiesel combustion with petroleum diesel fuel combustion demonstrated similar effects from both fuels despite the differences in mass concentration (50 % lower for RME) and different physicochemical particle properties of the exhaust (Unosson, 2014).
The analytical challenge

Although, epidemiology has consistently shown a relationship between health effects and air concentrations of particles, \( i.e., \, PM_{10} \) and \( PM_{2.5} \), there are some significant limitations to use these mass based metrics for regulatory purposes. One limitation is that the characterization of the particles is too broad and generalized, treating particles as a single pollutant, implying that their toxicities are independent of the source (Heal et al., 2012). However, the distinction between \( PM_{2.5} \) and \( PM_{10} \) to some extent separates combustion-related particles from larger mechanically generated particles by their relative contribution to mass. Evidence suggests that smaller particles are those who contribute most to adverse health effects at inhalation. Those particles deposit in the most sensitive lung regions, such as the alveoli, and originate from different combustion-related sources, of which traffic is typically associated with PACs and metals, both exhibiting adverse cellular effects, such as cytotoxicity, mutagenicity, pro-inflammatory effects and potential to cause DNA damage (de Kok et al., 2006). Those particles are small and thus do not substantially contribute to the total mass of the airborne particles, implying that regulating mass could have a limited effect on the overall toxicity of the air. However, the current understanding of PM-elicited toxicity does not allow an accurate quantification or ranking of health effects from different particle sources (Kelly and Fussell, 2012) and adverse health effects from \( PM_{coarse} \) should not be disregarded (Schwarze et al., 2006). Thus, it is necessary to identify the underlying mechanisms for PM-induced adverse health effect to be able to identify sources of highly toxic particles and to perform accurate health risk assessments. Ultimately, this could lead to the development of more cost-effective abatement strategies to reduce the health impact from particle exposure.

As a part of this multidisciplinary issue, increased chemical characterization and speciation of particle properties are of great importance. As mentioned earlier in this thesis there are several problems vitiated with the determination of particle-associated PAHs. Every step of the analytical chain inevitably introduces errors, but they are all necessary in order to reach a final result, for instance the sampling which can introduce either positive or negative sampling bias and can alter the original chemical composition of the particles. The effects of the extraction procedure, where tradeoffs might be necessary, such in the case of PFE of Oxy-PAHs and PAHs from diesel particles (Ahmed et al., 2015b). This aspect in the chemical analysis is even more pronounced when joining chemical characterizations with toxicological testing (Marvin and Hewitt, 2007). An isolation procedure that works well for one particulate matrix might not work that well for another, as described in Paper II but solved in Paper IV. With regards to bioassay-directed analysis there is no standardized protocol for group type separations, which
makes comparisons between different methods difficult. Quantification of a larger set of PAHs, but still narrow with regards to the actual number of PAHs, is hampered with co-elutions using conventional one dimensional GC separation, regardless of the column stationary phase chosen. This will certainly become an increasing problem with an expanding set of PAHs and other PAC compounds. When quantifying low-abundant but highly potent compounds such as dibenzo[a,l]pyrene, it also requires that the analytical methodology has a sufficiently low detection limit, which is also important when establishing PAH profiles for e.g., source attribution (Stout et al., 2015). With the rapid improvements in engine and after-treatment technologies which lower the emissions, the method detection limits will certainly become increasingly important. In summary, there is still room for improvements in the determination of PAHs and related compounds.
7. Conclusions and future perspectives

Tire wear particles are likely a minor source of traffic related dibenzopyrenes in urban air. However, the substantial amount of tire rubber worn annually make tire wear an important contributor to hazardous compounds, among others these PAHs, deposited to road side soils and other receiving environmental compartments. However, the contribution of PAHs from tire wear to the environment is expected to decline where a ban on HA-oil has been implemented.

Benzothiazoles appear to have a limited use as a reliable quantitative marker for tire wear in air particles because of their substantial variability in concentration in tire rubber, limited stability, high volatility, and limited occurrence on air particles. Nevertheless, tire wear particles may be an important contributor to the suspected carcinogen MBT in urban PM. Further investigations are needed to identify other possible sources, as well as to determine the gas phase and particle distribution in the ambient atmosphere. The emission of MBT, in contrast to PAHs is not expected to decline.

Due to the use of recycled crumb rubber from scrapped tires in playgrounds and in artificial turfs, more research on the potential hazard of this practice should be undertaken. A thorough and updated chemical characterization as well as additional toxicological studies of tire rubber and rubber granulates are warranted.

The increasing importance of non-exhaust emissions from traffic and the limited data on the relative contribution of PAHs and related compounds from non-exhaust sources implies that research efforts should be taken to provide insights in the potential health impacts from these particles. This research should include finding reliable marker compounds for the different non-exhaust sources, determination of particle size distributions, as well as include a large set of PACs and preferably should also include determinations of transition metals to better estimate the potential health effects from non-exhaust particles.

Biodiesel fuel combustion generates different exhaust emissions than combustion of petroleum diesel. Biodiesel exhaust emissions generally contained lower levels of PAHs than those from petroleum diesel exhaust, with the exception of some high-molecular weight compounds. The Oxy-PAH emissions were lower using biodiesel as fuel, and biodiesel combustion emits fewer and smaller particles. However, the current standards in exhaust
emission measurements, originally developed for petroleum diesel exhausts, may be hampered with sampling biases as for the HC and PM mass emissions when applied to biodiesel combustion. This issue requires further investigations since both these emissions are regulated.

Lubrication oil may have an important contribution to some PAHs on biodiesel emission particles, in particular of phenanthrene and alkylated phenanthrenes. These compounds make up a substantial fraction of the total PAH content of biodiesel particles. Also when considering the contribution of metals, the importance of lubrication oil to the overall particle chemistry and toxicity needs additional studies. Investigation of other parameters influencing the PAC emission or particle chemical and toxicological properties from biodiesel fuel combustion, such as FAME composition or impurities may be of interest.

Non-occupational exposure to PAHs and related compounds needs to be better characterized. The relative importance of exposures occurring in indoor microenvironments is not fully understood and dietary intake of PAHs is typically limited to the priority lists of EPA and EU. With the back-flush technique developed in Paper IV, it may be possible to use coupled techniques for the determination of low abundant but highly toxic PAHs in edible oils e.g., dibenzo[a,l]pyrene. Currently, there is no published data on the occurrence of this particular PAH in foodstuffs and the occurrence of alkylated PAHs is scarce. However, the initial isolation step using SPE may need further development since the edible oil samples in Paper IV were diluted before the cleanup. A SPE cleanup method that enriches PAHs would increase the method detection limits and may be necessary for the detection of low abundant PAHs.

With better chemical speciation at hand, improvements can be made in toxicological research, risk and exposure assessments and source attribution. Ultimately, this may be beneficial for the environment and for the health of the general public. Altogether, this signifies the need of fundamental research in analytical chemistry.
8. Sammanfattning på svenska

Sambandet mellan luftföroreningar, ökande antalet sjukhusinläggningar och en ökad daglig mortalitet från kardiovaskulära- och lung-sjukdomar är vältablerat utifrån tidigare epidemiologiska studier. Det föreligger även en ökad relativ risk för lungcancer vid exponering för luftföroreningar. Partikulärt material (PM), vilket innefattar luftburna vätskedroppar och fasta partiklar, i förorenad luft har tillskrivits en stor betydelse för negativa hälsoeffekter. Varför PM ger dessa effekter är inte helt känt då det råder kunskapsluckor kring de underliggande biologiska mekanismerna för hur PM orsakar ohälsa. PM är kemiskt sett komplex och kan genomgå förändringar i atmosfären. Halterna av PM i luft mätts som PM$_{10}$ (partiklar med diameter mindre än 10 µm) och PM$_{2.5}$ (partiklar med diameter mindre än 2.5 µm) och används som ett mått på luftkvalité.

Polycykliska aromatiska föreningar (PAC) är en stor grupp av föreningar vilket även inkluderar polycykliska aromatiska kolväten (PAH). PAC bildas vid ofullständig förbränning av organiskt material i luft och är ofta associerade med PM. Flertalet PAC-föreningar är kända för sina mutagena och carcinogena egenskaper, samt förmågan att inducera oxidativ stress. PAC antas därmed bidra till de negativa hälsoeffekter som associerats vid exponering för PM.

Avgasemissioner från fordonstrafik, i synnerhet dieselfordonsemisioner, anses vara en av de största källorna till partikelassicerade PAH i stadsluft. Utöver avgaspartiklar genereras även partiklar från slitage av däck, asfalt och bromsbeläggningar, samt via återsuspension av vägdamm. Dessa har i regel större partikelstorlek än avgaspartiklar. Bidraget av PAH från slitagepartiklar är betydligt mindre karaktäriserat än för avgasemissioner och hälsoeffekterna från exponering för slitagepartiklar är i stort sett okända. Det är därför viktigt att undersöka bidraget av PAH från dessa källor.

Allt strängare avgaskrav har lett till utveckling inom både motorteknologi och avgasförebyggande, vilket lett till att trafikbidraget av partiklar från avgasemissioner minskat betydligt. Liknande minskning för slitagepartiklar har dock inte ansågs kommit, vilket gör att det relativt lättare att ta till slitagepartiklar än till avgaspartiklar. Det är därför viktigt att undersöka bidraget av PAH från dessa källor.

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I denna avhandling har PAH-halter bestämts i bildäck från olika tillverkare (Paper 1). Ett av syftena med denna studie var att utröna hurvida däck överhuvudtaget utgör en källa till starkt cancerogena dibenspyrener. I

Analyserna av bildäcken visade att PAH innehållet varierar stort mellan olika däck, vilket även var fallet för bensotiasolerna. Baserat på analyser av bensotiazoler på partiklar insamlade vid Hornsgatan i Stockholm beräknades däcksgummimìnnehållet hos de provtagna partiklarna till 0.7 % för PM₁₀ respektive 5.5 % för samtliga luftpurna partiklar. Det uppmätta innehållet av dibenspyrener i bildäck sammantaget med däcksgummibidraget till luftburet PM, beräknat både i Paper III samt rapporterade i tidigare studier, tyder på att bidraget av dessa cancerogenena PAH-föreningar från däckslitage i stadsluft är liten i förhållande till avgasemissioner. Begränsningar hos bensotiasoler som markörer för däckslitagepartiklar i stadsluft diskuteras i avhandling. Däckslitage kan dock tänkas utgöra en betydande källa till 2-merkaptobensotiazol i stadsluften. Denna förening misstänks kunna orsaka cancer hos människor, och tillsätts avsiktligt i gummiblandningen som en viktig komponent vid själva däckstillverkningen.

För att minska importbehovet och användningen av fossila bränslen ökar behovet av förnybara drivmedel för transportsektorn. Biodiesel är ett biobränsle som produceras från växt- och djurfetter och har föreslagits vara ett lämpligt alternativt drivmedel till vanligt fossil dieselbränsle. Redan idag tillåts en inblandning på 7% biodiesel i dieselbränsle inom EU. En stor majoritet av alla de studier som publicerats gällande hälsoeffekter från dieselemissioner har fokuserat på fossil dieselbränsle, medan hälsoeffekterna från exponering av biodieselsemisioner är i stort sett okända.

I Paper II och Paper V så har biodieselmisemissionspartiklar jämförts med vanligt fossil dieselbränsle genom att halvtämma ett fyrtiotal PAH-föreningar. De bränslen som testades var rapsmetylester biodiesel (RME), konventionell diesel baserad på fossil olja, samt en bränsleblandning av dessa som innehöll 30 % RME. I den ena studien (Paper V) inkluderades även haltbestämmningar av fyra oxygenerade PAH (Oxy-PAH).

Avgaserna från förbränningen av RME-bränslet gav upphov till annorlunda emissioner än de för det fossila bränslet med avseende på flertalet olika emissionskaraktäristika. PM i biodieselmisemissionen var både mindre till storleken och färre till antalet än för det fossila bränslet i båda studierna. Den relativa kemiska sammansättningen av flyktigt och oflyktigt
material på partiklarna skiljde sig åt mellan biodieselbränslet och det fossila bränslet, där t.ex. biodieselpartiklarna hade en högre andel flyktigt material än det fossila bränslet. I Paper II utgjordes en del av detta flyktiga material av oförbränt eller delvist förbränt biodieselbränsle. Detta orsakade störningar vid den kemiska analysen av dessa prover. En ny provupparbetningsmetod utvecklades därför i Paper IV för bestämning av PAH i lipidrika matriser.

Användandet av biodiesel som bränsle gav lägre emissioner av PAH (Paper II och Paper V) och Oxy-PAH (Paper V), med undantag för ett fåtal PAH-föreningar med högre molekylvikt. I jämförelse med fossilbaserad diesel så innehöll biodieselpartiklarna en relativt sett större andel PAH-föreningar med en högre molekylvikt, men emissionerna av dessa var i många fall lägre med biodieselbränslet.
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10. References


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generated emissions of ultrafine particles from pavement-tire interface. Atmos. Environ. 40, 1314-1323.
EFSA, 2008. Scientific opinion of the panel on contaminants in the food chain on a request from the European commission on polycyclic aromatic hydrocarbons in food. EFSA Journal 724, 1-144.


Lawrence, S., Sokhi, R., Ravindra, K., 2016. Quantification of vehicle fleet PM10 particulate matter emission factors from exhaust and non-exhaust sources using tunnel measurement techniques. Environ. Pollut. 210, 419-428.


Lindgren, M., Grudemo, S., Ågren, U., Henke, M., Kyrklund, T., Björsell, M., de Serves, C., 2012. Regeringsuppdrag att belysa skillnader i hälso- och miljöpåverkan av att använda diesel av miljöklass 1 och miljöklass 3; Accessed:


