Biodiesel, fossil diesel and their blends: Chemical and toxicological properties

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Chapter 2

Biodiesel, Fossil Diesel and Their Blends: Chemical and Toxicological Properties

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Abstract

The worlds air pollution problems are increasingly been related to automotive exhaust emissions. The ~~more frequent~~ implementation of diesel and biodiesel blends in passenger vehicle engines have gradually produced a new ecotoxicological profile of urban and rural air pollution, where nanoparticles, volatile exhaust fractions, microparticles and aerosol agglomerates dominate the spectrum of emission species. The effects of these species are increasingly associated with cardiovascular diseases, lung cancer and increase in all-cause mortality in the human population, particularly in urban and other highly trafficked areas. Also, the size of particles and agglomerates from exhaust has been related to particular diseases, risks of contracting types of pathologies and development of cardiovascular complications. ~~$PM_{2.5}$, PM_{10} and nanop~~Particles have therefore selectively been ~~review~~addressed in this literature review for adverse health effects. With particular focus has biodiesel blending been extensively reviewed for chemical species and associated adverse health effects. The reviewed data suggests that the legislatory environmental health ~~organs~~ authorities worldwide are not fully updated with ~~the serious~~ all ~~nature~~ aspects of air pollution and that filtering technologies, fuel types and threshold values for particle content in the air are not up to date with the medical and ~~pathophysiological~~patho-physiological ~~findings that have been acquired pr 2010~~knowledge.

Introduction

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The majority of motorized road vehicles in Europe run on diesel~~.~~, ~~a~~Almost all heavy duty ~~trucks~~lorries, ~~large~~ buses, and an increasing number of passenger cars use this fuel. The advantage of diesel lies in its high fuel efficiency, high power output and its lower price ~~than~~ compared with gasoline. Given the increased ~~of applying~~ use of diesel, the pollution and exhaust ~~eco~~toxicological aspects have changed ~~since the usage of diesel increased~~ considerably since ~~from~~ the mid 90s, particularly ~~in~~for ~~–~~ passenger cars. A vast number of studies has been published on diesel's toxic potential and indentified it as the major source of pollution in metropolitan and trafficked areas [1-8]. The deposition of combustion particles in the human respiratory tract and the inflammatory responses including potential carcinogenic responses have been documented [2-4, 6] and the toxicological characteristics of the combustion particles from the diesel engines are still under ongoing research. Having a great variety in chemical composition, the particles from diesel and biodiesel fuels present toxic aspects which may vary from being an inflammatory threat to carcinogenic factor. This variation depends on dose-dependent exposure, time-dependent exposure and frequency of exposure [5-8].

Durbin and colleagues [9] cited that elemental and organic carbons species (EC and OC) are the primary constituents of diesel particulate matter (DPM), consisting of approximately 73-83% of total mass. Soot, a collective terminology for OC and EC including other pollution components from diesel combustion, has been shown in Taiwan and at the Karolinska Institute in Sweden to cause cancer in children [10, 11], while EC and OC alone have been correlated to respiratory and cardiovascular diseases, and even carcinoma [12, 13]. In addition to these factors, fossil diesel has a high content of polycyclic aromatic hydrocarbons (PAH) and the incomplete combustion of these ~~heavy weighted molecular~~ compounds generate~~s~~ exotic species in the exhaust, in addition to uncombusted PAHs [14-16]. These findings have made several organizations, including the US National Institute of Occupational Safety and Health [17], the International Agency for Research on Cancer (IARC) [18], the World Health organization [19], the US Environmental Protection Agency [20] and the US National Toxicology Program [21] to classify diesel exhaust as a potential human carcinogen. Additional studies characterize the composition of DPM to be highly complex and composed of hundreds of components in particulate, liquid and gaseous form [22].

The gaseous compounds of DPM are nitrogen oxide species, carbon dioxide and monoxide, sulphur compounds and several low molecular weight hydrocarbons such as aldehydes, benzene, PAHs and nitro-PAHs. The DPM core is mainly made of elemental carbon and adsorbed organic compounds, in addition to trace amounts of sulfate, nitrate, metals, and other trace elements. DPM consists of coarse and fine particles, with diameters lower than 10 and 2.5µm respectively (PM$_{10}$ & PM$_{2.5}$)~~–~~, including a number of ultrafine particles of diameters less than 0.1µm, so-called nanoparticles [22]. The nanoparticles have surface that easily adsorb other aerosol compounds and are able to reach deep into the lungs and cross the blood barrier [23-25]. The organic part, encompassing the mentioned compound classes can reach up to 49% of the DPM weight~~,~~ and contain 5% metals (including heavy metals) and 4% sulfates and nitrates [22].

These components have been shown to affect respiration and promote cancer [26-28], through mechanisms of inflammation after deposition in the alveoli.

Public Health Studies

Several studies have been carried out to determine the effects on public health of long term exposure to air pollution with particular emphasis on diesel exhaust [29-46]. In all these, a common theme of increased mortality and morbidity was found in relationship with nanoparticles from traffic exhaust. The effect of the smallest particles was determined to be less known than

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coarser particles [29]. Given the poor availability of studies on nanoparticles, a series of policy options were proposed to be reviewed and modified in accordance with public safety [29]. The expert panel that evaluated the various toxicological scenarios relating to nanoparticles (NP) was consistent in determining a medium to high risk of short-term exposure's cause to promote increased all-cause mortality. The correlation between NP and mortality was centered on the thrombotic and respiratory inflammatory effects of NP on human health. This proved the main theme to introduce improvements in the current public health regulations against NP.

The sensitivity of human health to various health pollutants has also been thoroughly discussed by Brunekreef and Holgate [30] who showed that reactions to pollution both at small and large doses and during short term as well as long term exposure, are of equal importance and must be evaluated consistently for better public health regulations to be implemented. In The probability that we are dealing with new types of particles continuously, because due to of the rising number of motorized vehicles and the photochemical reactions between sun-exposure and particle clouds, gives reason to underline that toxicological studies of traffic pollution must be continuously updated, and new experiments in relation with temperature, weather conditions and city densities have to be considered [30]. The traffic pollution condition is in accord with Brunekreef and Holgate [30] evolving with society, infrastructures and not at least with the new types of combustible fuels. In relation to the high levels of pollutants in cities, life-expectancy of the population exposed has been assessed to be reduced by 1-2 years, which is considerably high compared to other life-style or environmental risk factors [30, 31]. Most of the pathophysiological complications related to this shortened life expectancy reside in the pulmonary system, where the conditions start from asthma, allergies and worsen in late stages of life to pneumonia, lung cancer and other pulmonary disorders. The risk of developing lung cancer in Danish cohorts was found to be present and to request novel strategies to reduce exposure of pollution to the population [32]. Furthermore, in another study, the association of ambient residential exposure to PM_{10}, $PM_{2.5}$, NO_2, SO_2 and mortality was examined in 53,814 men in the US trucking industry [33]. The various ranges of particle diameter, likely indicating various degrees of pathological responses, were assessed in order to provide data to the existing knowledge on the more grave danger of finer particles. In accord with expectations, the risk for lung cancer was substantially higher for finer particles, but not for PM_{10}. The overall increased risk for cardio-pulmonary complications was increased with all particle sizes [33]. In another cohort study recently published in the journal *Stroke* [34], researchers discovered the reduced survival rate of stroke patients in highly trafficked areas in the UK. In this study PM_{10} was identified to increase risk of death in stroke patients by 52%. Additionally, in a study of 21 cities, all-cause mortality was found to increase by 0.6% for every $10\mu g/m^3$ increase in PM_{10} [35]. Admission for asthma and chronic obstructive pulmonary disease among people over 65 years of age was then found to increased by 1% for every $10\mu g/m^3$ increase in PM_{10} [36]. Also admission for cardiovascular diseases increases by 0.5 % for the same increase of PM_{10} and by 1.1 % for each increase of 10 $\mu g/m^3$ of black smoke from diesel engines [37].

The case for smaller particles was surveyed in a study from Boston, where the cases of the onset of myocardial infarction for patients suffering of arrhythmia occurred at times during higher concentrations of $PM_{2.5}$ [38]. $PM_{2.5}$ was also associated with severe cases of arrhythmia leading to therapeutic intervention by an implanted defibrillator [39].

The relationship between nanoparticle diameter and type pathology may therefore be hypothesized in a sense where 1) the finer the particle are, and the longer the duration of exposure is, more likely to cause the development of chronic pulmonary and cardiac pathologies (given the ability to cross the blood barrier) 2) at short but repetitive and intense exposures to larger particles,

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the more chances are to develop abrupt reactions such as pulmonary inflammation, asthma, frequent common colds and immune reactions related to throat and bronchi disorders, including difficulties in recovering from stroke and circulatory disorders and interventions [34]. These hypotheses may be followed up by a third hypothesis where the variation of particle chemistry given changes in weather, sun and urban conditions [30] may increase the risk of lung cancer. This third point is hypothesized accounting for the continuous provocation by new antigens in interaction with the immune system thereby promoting continuous and frustrated inflammatory conditions. Frustrated inflammation [47] activates a series of protein complexes, where some have been involved in the proliferation of cancer [48].

The continuous exposure to traffic pollution particles in cities and large urban areas shapes therefore the epidemiological profile of urban populations in a manner that requires tighter regulations and attention on the issue, particularly monitoring of lung-related conditions. The "frustrated" behavior of the immune system in the alveoli is also observed in the cases of asthma, where asthma is triggered from the immune system upon early exposure in early childhood [40]. This underlines that the immune system's reaction to traffic pollution has the tendency to behave in an "iterative exaggerated" manner in babies and small children, because of their more frequently maturing physiome, promoting stronger and more adverse reactions than an adult immune system. Accounting for the ever-present levels of nanoparticles in urban and trafficked areas, pediatric heath becomes quickly a central aspect with scientific demands and challenges to public health, and therefore studies on pediatric health in urban and highly trafficked areas compared to low pollution areas are needed.

In this context, PM_{10} particles, in addition to NO_x and SO_2, were found to be particularly relevant to the development of asthma in children [41]. This underlines that larger particles may trigger asthma more easily given their larger physical occupation in the alveoli, affecting respiration in a suffocating manner. Smaller particles on the other hand may affect more significantly the circulatory and immune system given penetration of the blood barrier in the alveoli, causing more complications in internal tissue such as in brain and nerve tissue [49]. Indeed, the smaller particles, once crossed the blood-barrier may impair vasomotor function, and cause vascular and circulatory complications [42], thereby exerting more stress on the cardiac system causing complications such as myocardial infarction [45]. The dependency of these reactions evolves eventually on cellular and biochemical responses, which have been thoroughly studied.

In Vivo Studies

Pathophysiological mechanisms of action from nanoparticles on organs have been elucidated by various groups. A group in particular has worked on studying the effects of nanoparticulates and PAH from diesel exhaust on cells from the pulmonary and cardiac system [50-56]. On a study on cardiac cells in particular, Totlandsdal and colleagues [50] applied ultrafine carbon black particles of increasing size on rat cardiomyocytes and cardiofibroblasts. The particle size was at a minimum size of 12 nm and agglomerating in culture, reached sizes up to 100 nm. The effects on the cells were detrimental, and the increasinged release of interleukins was observed in addition ~~with~~ to cell damage. A similar effect was observed on rat lung epithelial cells, where the expression of interleukin- 6 and 1β was found to be the main response to particulate matter of ultrafine carbon black [51]. The mechanisms of release of IL-6 from the lung cells was

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furthermore described [52], where the expression of IL-6 occurred with the expression of IL-1α and IL-1β. The findings indicated also that the reactions occur within hours from the moment of exposure to nano–particles, and that it is facilitated by the initial release of IL-1 after only 4 hours. The exposure to nanoparticles furthermore induced the MAPKs (Mitogen-activated protein kinases) and NF-κB (Nuclear factor kappa-light-chain-enhancer of activated B cells) and p38. These mechanisms of action indicate a significant inflammatory response in the lung tissue and show also the involvement of proliferation factors, which also participate in the mechanisms of apoptosis and anti-cancer reactions. The question on whether pollution particles affect and induce cancer is therefore also relevant and of interest to review. In further studies by the same group [53-56], the carcinogenic aspects of nitro PAHs on human bronchial epithelial cells and murine hepatoma cells were demonstrated. 1-nitropyrene (1-NP) induced DNA damage and cytotoxicity while 3-nitrofluoranthene was particularly toxic to the cells and altered their cell-cycle. The mechanisms of action were through the cytokine/chemokine pathways, activating cell cycle checkpoint factors [53, 54]. These same toxic compounds were furthermore observed to affect the caspase-pathways [56] which can in turn take part in the development of cancer if repetitively disturbed by toxic substances.

Blood Barrier and Inflammatory Responses

At the boundary of the blood barrier, a series of molecular reactions become relevant to the assessment of damage by nanoparticles. These mechanisms of reaction to particulate matter can become involved in "persistent and non-resolving inflammation" [57]. In this review the authors describe how the non-resolving action of inflammatory agents creates long termed diseases which affect the respiratory and cardiac tissue, contributing also to necrosis and cancer. The difficulty the immune system cells experience in decomposing and attempting the decomposition on a continuous amount of nanoparticles results in many cases to deficiencies where the inflammatory system persist either excessively or subnormally. These reactions to nanoparticles trigger therefore mechanisms that may result in harming the body, through many different cell pathways.

The research on these pathways has been conducted over several years, but given the variation of chemical species in exhaust and cellular reactions, many unanswered questions still remain. In a particular study [58], the effects of diesel exhaust showed that DPM induced serum vascular cell-adhesion molecule levels (VCAM)-1 in mice and enhanced vasoconstriction. Affecting the cardiac and respiratory system, the ability of the nanoparticles to enter the blood barrier was shown and the reactions to DPM were identified at the tissue, cellular and the molecular level [58, 59]. The observations showed also that DPM blocked transportation attempts of DPM by the cellular machinery by blocking transcription or protein synthesis. In a fashion which may induce persistency in inflammation, inhibition of NADPH oxidase also occurred. This event took place catalyzed by radical scavengers, which ameliorated the up-regulation of DEP-induced P-glycoprotein. This indicated that reactive oxygen species (ROS), arisen during exposure to nanoparticles, took part in the signaling between cells and disturbed the inflammatory response in a repetitive manner [58, 59].

Once crossed the blood barrier, nanoparticles have also been shown to cause increased expression levels of brain capillary tumor necrosis factor-alpha (TNF-alpha) and leading to P-glycoprotein down-regulation [60]. The down-regulation of P-glycoprotein affects the transport of nutrients and signaling factors to the cell, thereby leading to necrosis. Nanoparticles promote more severe reactions at the blood-barrier, where combined with persistent inflammation may lead to

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more serious conditions. This was observed in a study where emphysema in rat was observed upon exposure to DPM [61]. Here, the proinflammatory response in the lungs of the rats was characterized by a significant infiltration of leukocytes such as macrophages, eosinophils, lymphocytes and an increased level of IL1β in lung homogenates. Lung damage was also observed in this study, which showed characteristics of emphysema-related morphological changes including airspace enlargement and progressive destruction of alveolar wall structures [61, 62]. A group of researchers also found the increased risk of spontaneous abortion for pregnant women given exposure to DPM [63]. The findings were related to a mouse model study where the ability of the embryo to bind to a fibronectin matrix was studied and found to be affected by DPM. DPM have also been shown to amplify the cellular response in the lung tissue to invading agents, thereby promoting "frustrated inflammation" thus yielding excessive phagocytosis [64].

The mechanism of frustrated phagocytosis is of particular relevant in development of asthma and allergies as an epidemic [65 - 67]. A significant exposure to nanoparticles on a continuous basis may lay the foundation for the immune system to promote stronger reactions to the particulate matter such as dyspnoea, respiratory difficulties, heart palpitations; arrhythmia and chest pain on a continuous basis [68-72], making a high-alert mode of the immune system a daily and almost background reference for immune activity causing serious physiological complications in the masses exposed to nanoparticles, $PM_{2.5}$ and PM_{10}.

Physiological Complications and Disease Development from Exhaust Emission Particles

The initial signs of wheezing, coughing, chest tightness and shortness of breath have been reported among a group of 249 subjects that were included in a study to assess the reactions to diesel $PM_{2.5}$ [68]. In this study, the results indicated that the smallest particles of diesel exhaust may confer a greater health risk than only larger PM. PM overall was however enough to increase risk of respiratory symptoms [68]. Asthmatics are more prone to difficulties and graver symptoms than none-asthmatics; however a 6-8 hour daily exposure introduces substantial health risks for both groups [68]. Additional complications promoted by PM in humans are: worsening peak flow, necessity of inhaler usage, respiratory symptoms, and emergency room visits in asthmatic children and adults [67]. Gradually, these complications grow to more serious conditions such as cardiopulmonary mortality and lung cancer [73]. Furthermore, general air pollution has been correlated to lung cancer and mesothelioma [74] and the particulate matter is pinpointed as the central component for particularly the cardiovascular complications developed during a prolonged exposure in urban and trafficked areas [75]. This statement from the American Heart Association states also that life span is reduced by several months up to years by only a few years of exposure to PM; in particular $PM_{2.5}$. Other events occurring from the exposure to particulate matter are stroke, myocardial infarctions, and heart failure exacerbation [75]. The causes for this are changes in prothrombotic and coagulant content in the blood, progression of atherosclerosis and vasoconstriction [75].

Inhalation of exhaust particles is ranked as the 13 leading cause of death in the world and responsible for approx. 800.000 premature deaths pr year [76]. Even short-term exposure to $PM_{2.5}$ in particular is responsible for thousands of deaths pr year in the US alone [77, 78]. Still related to particulate matter, admission at the hospitals increased with only a 10 $\mu g/m^3$ increase, causing cerebrovascular disease increment, and heart failure [79].

In an Italian study, particulate matter has also been shown to be correlated to deep vein thrombosis [80]. Particulate matter has also been correlated cardiac arrest and arrythmia [80, 81].

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Interestingly, heart failure, ischemic stroke, cardiovascular hospitalizations, systemic inflammations and heart-rate variability are reported to be more likely to be contracted during short-term exposure to particulate matter rather than long-term [77]. Long-term exposure to particulate matter is instead more associated with ischemic heart disease and cardiovascular mortality [77].

Therefore, in a general overview of the complications that are involved on exposure to particulate matter, the more grave exposure is short-term, which gives more severe reactions to various clinical conditions as mentioned here. Systemic and pulmonary arterial bypass is also caused in many cases by particular matterDPM on short-term exposure in addition to repolarization abnormalities of the heart [77].

Most of the cardiopulmonary conditions hereby mentioned are seemingly caused ultimately by the effects of particulate matterDPM on unbalancing the brain and the autonomic nervous system [82]. This observation indicates the crucial aspect of particulate matterDPM, its ability to cross not only the blood barrier, but most likely also the membrane barrier of cells with due to its chemical properties and surface components.

Chemical Properties of Nanoparticles

The properties of exhaust nanoparticles vary according to the type of fuel combusted. A series of studies are here reported in the delineation of the differences between biodiesel and fossil diesel fuel nanoparticles, and also nanoparticles from the combustion of fossil/biodiesel blends.

Nanoparticles from Biodiesel/Fossil Diesel Blends and Their Toxicity

Few studies have been conducted on nanoparticles from in the exhaust from the combustion of biodiesel blends in /fossil diesel blends, given the recent public implementation of soy, rapeseed and other types of deriving esters in fossil fuels [83-85]. Today's biodiesel consists of fatty acid methyl esters (FAME) derived from the trans-esterification of the triglycerides in plant oils, ex.g. soya oil (generating soya methyl esters - SME) and rapeseed oil for the production of rapeseed methyl esters (RME). The combustion of FAME the methyl esters has a closed carbon cycle compared to fossil diesels and generates lower levels of CO_2 in its life-cycle [86], however it generates a amount of higher more of the smallest particulate lesmatter. In addition , nor-combusted FAME are emitted, - particularly in colder countries. , vThe exhaust contains ery little if zero polycyclic aromatic hydrocarbons (PAH), and a low amount of aldehydes, and ketones, but more NOx , in comparison with fossil diesel and nitrogen species [87-89].

Fossil diesel on the other hand, is quite rich in toxic combustion products of stronger toxic degrees. The majority of chemical compounds from the soluble organic fraction (SOF) of fossil diesel arranges in agglomerates which contain unburned hydrocarbons, semi-oxidized hydrocarbons and PAHs [90-92]. The level of PAHs in fossil diesel has been documented to be high, reaching as much as 7 times higher concentrations than in biodiesel [88]. Their carcinogenic potential of PAHs is well known [93]. Few Some studies have been done on the toxicological aspects of biodiesel, and even but very few er have been done on the toxicological aspects of on biodiesel in blends with and fossil diesel mixtures. A group of researchers It has been showedn that dust deriving from a biofuel production facility plant caused strong inflammation in mice [94], and the irritation was a similar also response confirmed was found in tractor drivers driving

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employing ~~on~~ biodiesel as fuel [95]. Another group proved a significant cytotoxic effect of combustion products from RME on mouse lung cells and on a mammalian microsome model [88, 96]. In the attempt to further characterize the toxicity of biodiesel and biodiesel blends, the ~~particulate matter~~ PM from biodiesel/fossil diesel blends has been demonstrated to promote a higher cytotoxicity than regular fossil diesel. However, the reason for this observation was not fully understood [89]. Bünger and colleagues suggested in 2000 [97] that the unburned parts of biodiesel represent the main toxicity aspect of biodiesel.

~~The combination~~ Blends of biodiesel ~~and~~ in fossil diesel introduces therefore new toxicological aspects given the mixtures of two different chemistries, giving rise to potentially "toxic partnerships" [97] between biodiesel components and fossil diesel components. ~~, and~~ gGiven the indications in [87-89, 97], the component of interest from biodiesel which is particularly relevant for toxicological assessments is uncombusted FAME as a "Trojan horse" component [98]. Nanoparticles from biodiesel are therefore of different size and composition than those from fossil diesel. Lin and colleagues studied and characterized the particle size and distribution from diesel engines running on palm-biodiesel and fossil fuel blends [99]. Their results showed primarily that pure biodiesel fuel could cause incomplete combustion, thereby generating higher amount of particulate matter and gaseous FAME. Furthermore, blending palm biodiesel with fossil diesel would contribute to an increment in nanoparticle size. The range of nanoparticles diameter was below 0.31µm, indicating that the smallest fractions would be affected by blending biodiesel and fossil diesel [99]. Noting that the smallest fraction has a higher ability to penetrate the lung tissue and cross the blood barrier, the hazardous aspects of blending biodiesel and fossil diesel is therefore important. The B20 category (20 % biodiesel, 80% fossil diesel) generated a larger amount of smaller nanoparticles than the other tested blends [99]. The highest level of nanoparticles emission was generated with 100% biodiesel, then with 100% fossil diesel and ultimately with the B20 blend particularly during the first stages of combustion [99]. Paraffinic emulgation of the blends reduced particle size substantially for all stages of combustion [99].

In a study where soy bean derived biodiesel was assessed for emissions, a higher number of PM concentrations were found for B0, B10, B20 and B50 blends [100]. The highest concentrations of PM were found for B50 fuels, indicating the contribution of biodiesel in the generation of higher amounts of nanoparticles than fossil diesel solely [86, 87]. Biodiesel fuels have also recently been found to contribute with higher concentration of organic carbon and total carbon during light load-conditions [101]. Biodiesel has furthermore been found to increase NO_x emissions by 10% [102, 103], implying a more toxic contribution of blends. CO_2 emissions have also been found to increase with biodiesel blending [102], which is contradictive with earlier reports [86]. Additionally and of interest, the total emission of carbonyl compounds (ketones and aldehydes) increased with biodiesel blends, compared to regular diesel [102]. Ketone- and adehyde emissions have originally been found to be highly generated primarily by alcohol fuels [104, 105], and have not been previously mapped in biodiesel emission studies as clearly as in the study by Karavalakis et al [102]. Interestingly, the PAH-content was found to be slightly higher in biodiesel blend B20 than in 100% fossil diesel [102]. This pattern was also observed in two other studies [106, 107] presenting the intriguing finding that biodiesel may not be as non-toxic as previously presumed [86, 87]. The mixture with fossil diesel appears to generate different chemical exhaust species depending on percentage of mixture, type of biodiesel blended (RME, SME etc.), the cetane number, outside temp-erature, engine type and age of the biodiesel.

Studies on nanoparticles and chemical compositions from the combustion of blends are therefore imminent and require greater attention, given their increased implementation in society

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[83]. Blends of fossil diesel and used cooking oil methyl ester (UCOME) were regarded to be 3-4 times more toxic than the unblended fuels [102]. The level of PAH was found to be 4 times higher in UCOME fuels than in fossil diesel [102]. Karavalakis and co [103] also presented interesting findings on that formaldehyde and acetaldehydes were the dominant aldehydes emitted from rapeseed and palm fuels. Lower emissions of total PAH and nitro-PAH have however been reported for biodiesel [103] but also increases of the low-molecular weight PAHs (anthracene and phenanthrene) and oxy-PAH [108]. Oxy-PAHs and anthracene are known to be toxic and carcinogenic [109, 110].

Blending biodiesel into fossil diesel has also been reported to increase toxicity of the semi-volatile fraction (SVF) in the exhaust [111]. This includes carbonyls, semi-combusted hydrocarbons and emulgates arising with the moisture in the exhaust system. The knowledge of the generation of additional bi-products from the cooling process and interaction with moisture in the exhaust pipe is therefore a topic of interest; however it has not been mentioned in the literature hereby reviewed. Particularly in cold climates this can be a specific problem [86]. This is related to that the higher viscosity of biodiesel contributes to the generation of more viscous combustion emissions, particularly based on the presence of uncombusted FAME particles. This is caused by the known drop in combustion efficiency, particularly in certain blends [111]. Blending biodiesel with fossil diesel can therefore be expected to generate novel exhaust compounds and also more water soluble nanoparticles: hydrosol particles which can be a more hazardous part of pollution than dry particles. In this context, the uncombusted part of biodiesel may be of central importance: uncombusted FAME may play a pivotal role in giving novel toxicochemical properties and pollution-challenges in bioblended diesel. This is stated because uncombusted FAME is reported to be a central aspect for the increase in emissions of the smallest nanoparticles from ~~and PM by~~ combustion of biodiesel blends and pure biodiesel [112-114].

In this context, another point is of interest. The difference between the types of methyl esters (e.g. palm oil, soy etc.) may be of importance to asses which biodiesel generate the highest nano-agglomerating potential (the most poorly combusted at various conditions), SVF and toxicity in combination with fossil diesel. Because of the effect mentioned in the previous paragraph where outside temperatures play a role [86], and ~~not to mention~~ cold start emissions representing 10% of total emissions [115], more analyses on the agglomeration profiles based on different types of methyl esters, temperatures, and engine types, cetane numbers is substantial for new projects. This is particularly explained by ~~disagreeing~~ contrasting results among studies [86, 102, 111-117], where two of these studies [111, 116] report inverse results of the levels of SVF in emissions from combustion of B100 and blends ~~than~~ compared to the other groups [86, 102, 117]. Therefore, there is a need to make affirmations on whether biodiesel contributes to increase or decrease in SVF and PM and in accord with which blends, cetane number, ~~cc volume,~~ iodine number and FAME type.

Therefore, to what extent blends increase toxicity is still not properly determined. It has been ~~n [115] biodiesel it is~~ stated that~~:~~ "Biodiesel was more toxic than diesel because it promoted cardiovascular alterations as well as pulmonary and systemic inflammation~~.~~" [115]. ~~,~~ This is a statement which is contradicting with toxicological expectations based on that fossil diesel contains a higher level of PAH than biodiesel and generates a higher level of hydrocarbon species, while biodiesel reduces these emission components [117-122]. The level of various PM generated from bio-blending may also provide information to explain the toxic aspects of biodiesel such as stated [102, 106-108, 111, 115, 123].

Interestingly, the importance of studying blends and differences within blends has been studied by Lin and colleagues amongst others [123]. In their study, all blends below B15 (B10, B5 etc) were concluded to contribute to increase the PAH content in the nanoparticles with diameters

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between 0.056 μm and 0.31 μm. The PAH content, being the prime cytotoxic, carcinogenic and toxic part in fossil fuels, is therefore a key component to be evaluated for aerosol-potential, hydrosol-potential so the toxicity of nanoparticles generated in general can be better assessed. Its ability to attach to a specific particle type~~s~~ may also explain the differences in results in toxicological assessments of blends. Knowledge of PAH-FAME-PM interactions may therefore aid in developing better exhaust filters.

FAME-PAH: Potential Toxic Partnerships?

The type of FAME applied in the biodiesel fraction may prove crucial for the ~~genera~~interaction ~~of more~~ with PAH, ~~or~~and potentially ~~alternatively~~ a higher toxicity. This is of particular interest because the higher toxicity may be correlated to ~~an increase in PAH when blending, or it can be related to~~ an *increase of delivery* of PAH molecules to the cells in the alveolar passages. This ~~latter~~ point of toxicology research promotes the importance of assessing whether FAME (and which types of FAME) actively carries, or aids in carrying PAHs to the cell nucleus, a theory of a so-called "Trojan-horse mechanism" where micelles are created by merging aromatic (PAH) and semi-polar compounds (FAME). This theory is currently being explored through molecular simulations [124] and promotes for further studies to be carried out on the basis of investigating whether the PAH increase is the prime suspect for increase toxicity of blends, or that PAH is more efficiently delivered. Also, the important points of that toxicity increases in biodiesel blends [102, 106-108, 111, 115, 123] has not been investigated using molecular biology methods, where levels of various cellular factors are monitored while exposed to various concentrations of PAH in combinations with FAME. The significance of relating FAME and PAH to molecular biology studies is founded on that PAH's toxic effects are partly known, but not in context with the chief component in uncombusted biodiesel, FAME, which may be of significant importance given the ~~significant~~ high levels of uncombusted ~~matter~~ fuel in biodiesel ~~running engines~~ exhaust [112-114], and the relatively high part of total exhaust emissions of 10% of cold-start phase [87, 111, 115, 125].

Naturally, hydrocarbons, carbonyls and other combustion products may be of equal importance, however these have been tested for toxicity, particularly carbonyls [102]. Uncombusted FAMEs are more viscous than carbonyls, and may act in the formation of nanoparticles making the emissions hydrosolic and increase their size and ability to bind more pollution components and also more efficiently contaminate water-sources. The action of uncombusted FAMEs in a nanoagglomerate context may therefore be participation in the formation of micelles with PAH and un-/semi-combusted hydrocarbons, and substantiate the formation of "toxic partnerships" between these two molecules, increasing toxicity and carcinogenicity [124] when blending.

Are Blends More Toxic Than Pure Biodiesel / Fossil Diesel?

A recent study from Finland has shown that there is a significant reduction of PM_{10} in the emissions when comparing B100 RME to EN590 diesel, without DOC/POC (Dissolved and Particulate Organic Carbon) filter [126]. However, when using DOC/POC filter the emissions were virtually the same, something that implies the need to improve the understanding of the generation of nanoparticles in blends, and their properties. The same study reported that the levels

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of PAH were reduced by nearly a third, when applying B100 RME compared to EN590 fossil diesel [126], however the cytotoxicity of B100 RME and fossil diesel were still nearly the same. Furthermore, the release of TNF-alpha by macrophages was far lower in RME-combustion exposed cultures, including ROS production. These findings may suggest that the reduction of PAH content may reduce the inflammatory reactions observed in macrophages, but not affect the cytotoxicity. Cytotoxicity may therefore be dependent on the emission species from blends, either carbonyls, hydrocarbons or other species primarily. Genotoxicity was also lower for RME-emission exposed cells, which supports the potential in that fossil diesel does cause more damage to DNA than biodiesel, but blends were not studied in this study [126]. Controversially to these findings, Kado and Kuzmicky [127] report higher mutagenic activity pr. particle from biodiesel/fossil diesel blends, but given reductions in total mutagenic emission rates finds blends to be less toxic than fossil diesel. Not changing the subject on that particles from blends may be more toxic than particles from fossil diesel, the increase of PM in blends [86] debates these findings, and instigates that blends may increase toxicity [102, 106-108, 111, 115, 123] and not decrease it [126]. Biodiesel PM extracts have also been found to be more inflammatory potent than fossil diesel PM extract [128], which correlates well with the observed findings on that biodiesel may irritate the mucous membranes and cause irritation, dizziness, and nausea [128, 130]. A review by McCormick [131] showed that the generation of emission species, ranging from PAH, nPAH, aldehydes and other emission species differ extensively when applying different engines. This review also suggested B20 blends to be the ideal blend to reduce PM, HC, carbonyls and toxic compounds including volatile C1-C12 carbon species.

However, in a report by Krahl and colleagues [132] the mutagenicity of the exhaust from biodiesel and biodiesel blends was measured and gave different results. The results showed that biodiesel blends shows a peak in mutagenicity at B20 and that this mutagenic effect most likely derives from species of molecules and nanoparticles generated in its interaction with fossil diesel. The results by Krahl and colleagues also showed that the fossil diesel condensates from the tested fuels where the most mutagenic, and that these condensates are most likely carried more efficiently in nanoparticle formation when blending at B20. 100% RME showed interestingly 4-5 times lower mutagenicity than B20 which delineates that the potential of mutagenicity derives from blending. Similar findings were found earlier by Fang and McCormick [133]. Published recently from the conference "Euro Oil and Fuel" in Krakow [134] utterly sustaining data to these findings was introduced. The findings by Mayer and colleagues [134] showed that nanoparticle formation in the diameter range from 10-100nm in standard fossil diesel is 5-10 times lower than of B10, B20 and B30 RME biodiesel blend. These results showed also that blends increasing from 0 to 100% biodiesel behave parabolically in the generation of carcinogenic PAHs (highest generations of PAHs at medium concentrations than low and high concentrations). Additionally, the findings by Mayer et al [134] showed that 100% RME had 5-7 fold higher generation of PAHs than regular fossil diesel during the first point of engine operation, indicating poorer combustion efficiency and higher generation of heavy aromatic compounds. The results were similar for particle-bound PAHs and for particle-bound carcinogenic PAHs [134].

Of equal interest, Munack and colleagues presented in 2010 at the XVIIth World Congress of the International Commission of Agricultural and Biosystems Engineering in Canada [132] evidence on that PM content in blends increases from B30 toward B100 RME compared to fossil diesel. Fossil diesel presented higher HC content than blends and B100 however NOx content was higher in blends surpassing B30. In the same presentation [132], Munack and colleagues concluded that particles from B20 blends are the most mutagenic, and that mutagenicity increases with increasing addition of biodiesel in fossil diesel (while fossil diesel particles has the lowest

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mutagenicity). The reason for these findings is based on the tendency of blends to form sediments, particularly if aged (partly oxidized) biodiesel is used [136]. The mutagenicity is therefore hypothesized to be caused when carotenoids in the aged biofuel lose their anti-oxidative effect in the biodiesel fraction forming oligomers and thereby sediments [136]. These reactions may therefore account for the 9.7 – 59 fold increment in mutagenicity of RME compared to fossil diesel as reported by Krahl and co [137, 138], and additionally be co-involved with the higher generation of NOx by RME combustion [139]. RME produces also more particles below 10nm in diameter than fossil diesel and gas-to-liquid fuels [139, 140], which is consistent with the particle formation observed by Manzetti and colleagues using molecular simulations [124]. The results from the simulations [124] showed that FAME gas agglomerates to stable nanoparticles with a mean diameter of 10nm at a pressure of 100bar, as found in the combustion chamber of diesel engines (J. Czerwinski, personal communication).

The evaluation of applying biodiesel in blends should therefore evolve around its toxicological aspects, unanswered questions, and potential increase of toxic and ecotoxic effects, rather before implementation is applied in traffic vehicles. The application of a small percentage of biodiesel in fossil diesel has taken place in Norway, accordingly with EU-regulations, and data show that even low percentages of biodiesel addition in blends increases PAH generation and nanoparticle formation [134]. The effects of blending on public health are therefore important to explore based on the generation of new emission ~~species~~compounds, which also may pass through DPF-systems and not to mention on DPF-devoid vehicles. DPF technologies may also have to be revised accordingly with blending implementations. After all, biodiesel and diesel generate a higher amount of nanoparticles than gasoline [86, 139, 140], and however detrimental for the environment and health, all three options should face more stringent evaluation as sustainable energy options (fossil diesel, biodiesel and biodiesel blends), particularly in large cities. The application of fossil fuels in general does represent such hazardous long term effects, as mentioned particularly in the previous paragraphs, that research focus should be intensified on alternative fuel sources and follow the precautionary principle before implementation of new alternatives is granted.

Diesel Nanoparticles

Diesel nanoparticles have been studied for the past 20 years in particular, with the increasing implementation of diesel engines in cars during the 1990's. The implementation of diesel cars in city traffic occurred primarily due to lower prices of diesel compared to gasoline in Europe. Secondary, lower ~~NOx~~CO_2-emissions from diesel engines was also used as an argument to use diesel instead of gasoline. However diesel engines generate up to 800 times more organic and elementary carbon than gasoline [136] due to the lower grade of ~~raffination~~refining from crude oil. Diesel engines generate also more than 30 times more particulate matter than gasoline engines alone, and include a wide range of toxic components such as PAH, HC and sulphur. Ultra-low sulphur diesel was proposed in the USA in 2006 and in Europe in 2005 with EURO IV standard in order to reduce emissions. DPF filters have been applied in many diesel vehicles during the late 2000's so to reduce PM. However diesel emissions still encompass a large fraction of air pollution [141] and display a higher sum added potential carcinogenicity than gasoline cars [142].

The majority of the carcinogenic components in diesel are PAHs. The levels of PAH emissions in diesel exhaust are to an extent reduced through DPF filtering technologies, urea de-nitration and other purification technologies and outside temperatures [143, 144]. However, the

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emissions from purified exhaust are still highly toxic and present a threat to urban public health in particular.

Bergvall and Westerholm [142] demonstrated the presence of two isomers of the highly carcinogenic PAH dibenzopyrene in nanoparticles from two diesel engines which were tested on ARTEMIS rural road running cycles, urban- and highway cycles. The results show that diesel engines emitted a net sum of ~1µg of PAHs pr kilometer in the urban driving cycles, which encompassed 5-10 times more the equivalent value for rural driving cycles and 5-10 times more than motorway driving. The majority of PAH types in the nanoparticles from the diesel engines used in the tests was represented by benzo(b)fluroanthene, benzo(e)pyrene, benzo(a)pyrene and benzo(ghi)perylene. The National Institute for Occupational Safety and Health [145] considers the first three substances as human carcinogens, whereof the latter, has been involved in studies to measure for carcinogenicity in humans [146]. The apparent reason for the lower PAH emission in rural and motorway driving than urban driving with diesel engines was hypothesized to be linked to the more reduced speeds in urban driving and more frequent starts and stops [142].

Diesel exhaust is a central cause to asthma in large cities and dense traffic areas also [147-149]. The content of diesel particles is akin to tobacco smoke, with a high content of organic and elemental carbon and also sulphates and nitrates. Other particularly health threatening substances are PAHs and related compounds such as quinones which have a high inflammatory potential. The inflammation is mediated through the penetration of diesel particles deep in the lung tissue [150] which are defined into sizes as mentioned in the previous paragraphs. The inflammatory mechanism of diesel particles is at its most potent level when quinones in the polar fraction interact with the immune system. The main cause of the triggering of asthma lies in the enhanced IgE antibody production and airway hyper-responsiveness and oxidative stress as demonstrated in mice and rats [151, 152], increased expression of bronchial adhesion molecule, and the novel finding of bronchoalveolar easinophilia cells [153], which explain the high correlation between asthma and diesel and [146].

~~The composition of d~~Diesel exhaust particles has ~~is~~ high content of ~~in~~ aldehydes, aliphatic ketones and aliphatics [154]. In the study by Jakober and colleagues [154], a collection of more than 60 chemical compounds were identified in diesel exhaust during a five phase analysis of a heavy duty diesel truck engine. The highest concentrations were of the aldehydes butanal and heptanal amongs other aldehydes, both in the gas phase as well as in the particle phase. The concentrations of these two compounds were 27.000 µg/L in the gas phase and 2.100 µg/L in the particle phase for butanal, and 13.000 µg/L and 2.300 µg/L in the respective phases for heptanal. These concentrations were measured during a 17 min creep phase. Other compounds emitted at relative high concentrations were the aliphatic dicarbonyl methyl glyoxal, 2-3 hexanedione and the aromatic aldehyd benzaldehyde. All species of compounds were found at much higher concentrations in the diesel measurements than the gasoline measurements, indicating the significant difference in toxicity between gasoline and diesel fuels.

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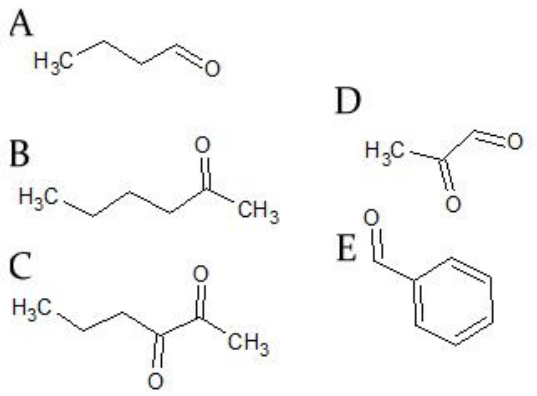

Figure 1. Aldehydes, ketones and dicarbonyls found in diesel exhaust emissions [148]. Alphabetically, A: Butanal; B: Heptanal; C:2-3 hexanedione; D: dicarbonyl methyl glyoxal**;** E: Benzaldehyde.

Further studies on nanoparticles from diesel exhaust have been conducted by Giordano and colleagues, with particular focus on the content of emissions from diesel engines with diesel particulate filter [155]. The content of the nanoparticles from traffic pollution (nowadays represented mostly by diesel cars in urban areas) was found to be composed of a significant amount of the heavy metals of Cu, Ni and Zn based on the biomonitors moss and lichen used in the study, and of Al and Cr in moss, that was significantly increased. Of particular interest, ~~this group~~ found high levels of silica fibers was also found, deriving from the diesel particulate filters from modern diesel cars, and the group exerted alertness towards the potential danger of this material deriving from the catalytic muffler and posing as a possible source very hazardous material to human health. This finding particularly shows how even the filtering solutions themselves may be a source of pollution in these otherwise intricate pollution aspects of diesel combustion. Therefore the aspect of heavy metals in nanoparticles is an equally, if not even more important aspect than carbonaceous compounds from diesel emissions, given the stronger toxic aspect of heavy metals to cells and particularly the nerve system [156]. The application of DPF and in diesel fuel catalysts in the diesel combustion system in modern cars leads to other ecotoxicological aspects, where the application of cerium oxide and silica carbide generates nanoparticles composed of CeO and fibrous compounds of silica, which are found to generate cytoxocity, blood-barrier damage and phospholipidosis with enlarged alveolar macrophages [157]. This aspect of nanoparticles from diesel-cars promotes another important view on the nanoparticle composition from modern diesel cars, where the technologies applied contribute to diversifying the chemical composition of diesel-combustion generated nanoparticles. The size of these ranges is still in the area of PM definitions, PM_{10} and $PM_{2.5}$, however the CeO/CeO_2 particles may also be substantially smaller depending on the fabric of the filter, however laboratory studies applied particles ranging 20nm in diameter for experiments [157].

The health effects of CeO_2 are still ubiquitous. The US National Health Effects Institute [158] reports that inhaled CeO_2 induces enlargement of lymph nodes, increased lung weight, and dose-dependent increases in segmental blood neutrophils. Also, the same institute reports that both pulmonary and systemic toxicity in rats has been provoked by inhaled CeO [158]. Studies were cerium is the major component has also shown to induce rare earth pneumoconiosis with

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pathologic features of granulomas and interstitial fibrosis [159-161]. The direct linkage between pulmonary inflammation in rats and CeO particles has also been recently demonstrated [157] and the inclusion of these durable but toxic materials in DPF filters and catalysts has therefore been questioned [157, 158].

Diesel nanoparticles are otherwise, except from being substantially represented by a solid elemental carbon core, covered by a multitude of known and unknown organic compounds. A study performed in 2001 by Tobias and colleagues [90], revealed the composition of such particles to be mostly made of branched alkanes and alkyl-substituted cycloalkanes. The size of the smallest nanoparticles analyzed was in the range of 50-80nm and a second group in the range between 30-70nm. Within these classes, the unknown carbon compounds were estimated to be composed of chains of a minimum of 17 carbons, and reach up to 25 carbon long. The correlation between length of carbon chains and engine load seem to be poor, showing that there were no particular patterns of chain length of alkanes and engine load. The same accounts for the diameter of the nucleus of the nanoparticles, which was largely 7-13 nm overall. Fuel additives and oil content alternated the composition of the particles to reach up to 45 carbon atoms in length, still encompassing structurally and chemically unknown molecular arrangements. Also in this study, the vast number of PAHs have been discussed and partly mapped, and of particular importance, the PAHs have been reported to exist mostly separated from the particulate ~~masses~~ material as found in earlier studies [162, 163].

Conclusions

Particulate matter and exhaust emissions present a significant environmental threat and hazardous aspects to health. The classification the exhaust species and their detrimental effects need to be furthermore emphasized in the toxicological studies and assessments of fuel types. Biodiesel and fossil diesel introduce both serious aspects to health and the environment, and their chemical aspects reveal indeed the serious threat to the body's immune system, respiratory system and cardiovascular system. Ultimately, blends seem not to reduce toxicity, and may in many case worsen the toxic aspect of emissions~~, and given their dependency on monocultures and deforestation, may in the long run have to be replaced with less viscous and nanoparticle generating fuel types~~. The nanoparticles formed from biodiesel, and the nanoparticles deriving from fossil diesel may be significantly altered during the combustion of blends, thereby producing novel toxicological aspects. In order to limit these problems, exploration of novel filtering technologies, and the search for alternative fuels must continue. This to avoid disastrous conditions for the increasing population of the world, resulting from the continuous combustion of fuels.

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