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Review article

Toxicity and health effects of ultrafine particles: Towards an understanding of the relative impacts of different transport modes

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ABSTRACT

Exposure to particulate matter (PM) has been associated with a wide range of adverse health effects, but it is still unclear how particles from various transport modes differ in terms of toxicity and associations with different human health outcomes. This literature review aims to summarize toxicological and epidemiological studies of the effect of ultrafine particles (UFPs), also called nanoparticles (NPs, <100 nm), from different transport modes with a focus on vehicle exhaust (particularly comparing diesel and biodiesel) and non-exhaust as well as particles from shipping (harbor), aviation (airport) and rail (mainly subway/underground). The review includes both particles collected in laboratory tests and the field (intense traffic environments or collected close to harbor, airport, and in subway). In addition, epidemiological studies on UFPs are reviewed with special attention to studies aimed at distinguishing the effects of different transport modes. Results from toxicological studies indicate that both fossil and biodiesel NPs show toxic effects. Several *in vivo* studies show that inhalation of NPs collected in traffic environments not only impacts the lung, but also triggers cardiovascular effects as well as negative impacts on the brain, although few studies compared NPs from different sources. Few studies were found on aviation (airport) NPs, but the available results suggest similar toxic effects as traffic-related particles. There is still little data related to the toxic effects linked to several sources (shipping, road and tire wear, subway NPs), but *in vitro* results highlighted the role of metals in the toxicity of subway and brake wear particles. Finally, the epidemiological studies emphasized the current limited knowledge of the health impacts of source-specific UFPs related to different transport modes. This review discusses the necessity of future research for a better

Abbreviations: ALI, Air-liquid interface; A549, Lung carcinoma epithelial cells; BEAS-2B, Human bronchial epithelium cells; BD, Biodiesel; BAL/BALF, Bronchoalveolar Lavage Fluid; CB, Carbon black; CeO_2, Cerium oxide; CVD, Cardiovascular disease; CHF, Congestive heart failure; CHD, Coronary heart disease; COPD, Chronic obstructive pulmonary disease; COX-2, cyclooxygenase-2; DEP, Diesel exhaust particles; DE, Diesel exhaust; DLS, Dynamic Light Scattering; DPF, Diesel particle filter; ECE-NAO, European market NAO brake pads; FAME, Fatty acid methyl ester; HaCaT, Human epidermal keratinocyte cells; HBECs, Human bronchial epithelial cells; HVO, Hydrogenated vegetable oil; HO/HO-1, Heme oxygenase; HR, Hazard ratio; HF, Heart failure; IARC, International Agency for Research on Cancer; IL, Interleukin; IHD, Ischemic heart disease; ICD, International Classification of Diseases; IQR, Interquartile range; LUR, Land-use regression; MCP-1, Monocyte chemoattractant protein-1; MI, Myocardial infarction; MSHA, Mount St. Helens Ash; NP/NPs, Nanoparticles; nm, Nanometer; NF-κB, Nuclear factor kappa B; NAO brake pads, Non-asbestos organic brake pads; NTDE, New-technology diesel exhaust; nNOS, Neuronal nitric oxide synthase; NIST, National Institute of Standards and Technology; NuLi-1, Human airway epithelial cells; OB, Olfactory bulb; OR, Odds ratio; PBECs, primary bronchial epithelial cells; PM, Particulate matter; PCO, Pure canola oil; PNC, Particle number concentration; PTB, preterm birth; RAW264.7 cells, Murine macrophage cells; ROS, Reactive oxygen species; RME, Rapsmetylester; RD, Risk difference; SOA, Secondary organic aerosols; SHB, Synthetic hydrocarbon biofuel; SOD, Superoxide dismutase; SSBs, Single-strand breaks; TiO_2, Titanium dioxide; TNF, Tumor necrosis factor; TP1/TP2, Tire particles from different sources; TP2.5, Tire particles <2.5 μm; TP10, Tire particles <10 μm; UFP, Ultra fine particles; ULSD, Ultralow sulfur diesel; μg, Microgram.

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understanding of the relative potencies of NPs from different transport modes and their use in health risk assessment.

1. Introduction to toxicity and health effects of nanoparticles

Particles in the air have historically often been measured as PM2.5 or PM10 (Particulate Matter with an aerodynamic diameter of less than 2.5 and 10 μm, respectively). In recent decades the focus has increased on the smallest size fraction (less than 100 nm) often called ultrafine particles (UFPs) or nanoparticles (NPs). Throughout this review, both terms are used interchangeably since original papers use both terms and the fields (toxicology and epidemiology) to some extent have different traditions. The European air quality directives regulate concentrations of PM10 and PM2.5, but not UFPs/NPs. In 2021, the World Health Organization (WHO) decided on new, lower guidelines for PM2.5 and PM10, and based on new scientific findings in e.g. epidemiological studies they also recommend integrating UFP monitoring, measured as the total number of particles, into the existing air quality monitoring stations (WHO, 2021). While numerous epidemiological studies have shown significant associations between health effects of both long- and short-term exposure to PM2.5 and PM10, the lack of regular monitoring data on NPs has restricted epidemiological studies.

PM in the air will deposit in the lungs to varying degrees, and this deposition depends on many factors, including the size, shape and hygroscopicity of the particles, and the individual breathing pattern. A large proportion of micrometer-sized particles deposit in the upper respiratory tract, whereas NPs with a size of around 10–100 nm are estimated to deposit most efficiently in the alveolar region (ICRP, 1994; Geiser and Kreyling, 2010). Furthermore, the deposition is often higher at tracheobronchial bifurcations, therefore considered as "hotspots" for particle deposition (Balashazy et al., 2003). In the upper respiratory tract, clearance is facilitated by cilia transporting the particles to the oral cavity, being finally swallowed (Geiser and Kreyling, 2010). Particles that reach the alveoli are not cleared as efficiently and, in this case, macrophages play an important role in their removal. Some particles are also cleared from the lung via dissolution while sparingly soluble particles can remain for a longer time. A fraction of NPs may cross the epithelium and translocate to the blood, although the extent of translocation is assumed to be rather small, probably only a few percent of the total mass (Geiser and Kreyling, 2010; Kermanizadeh et al., 2015). Furthermore, if the inhaled NPs are agglomerated they are also expected to translocate to a small extent (Creutzenberg et al., 2022). Studies using animals suggest that particles that deposit in the nose can pass on to the brain via the olfactory nerve (Oberdorster et al., 2009), and NPs may also reach the brain by crossing the blood-brain barrier (Qi et al., 2022). Magnetite NPs and other metal-rich NPs have been found in the brain of humans (Calderon-Garciduenas et al., 2020; Maher, 2019).

A vast variety of health effects have been linked to NPs inhalation, including respiratory and cardiovascular diseases as well as cancer. Many of these effects have been linked to the ability of NPs to cause oxidative stress, inflammation, and genotoxicity, the latter being particularly important in carcinogenic processes (Leikauf et al., 2020; Miller and Newby, 2020; Stone et al., 2017). All these endpoints can be analyzed using cultured cells, such as lung epithelial cells and macrophages, thus allowing the relative toxicological potential of NPs from different sources to be estimated. Cardiovascular effects have also been linked to the stimulation of lung sensory receptors, leading to changes in neural activity, as well as to the "direct" interaction of NPs with blood components, particularly platelets (Miller and Newby, 2020). A review with focus on both combustion-derived PM and nanomaterials suggested that systemic inflammation seems not necessary for the development of atherosclerosis and vasomotor dysfunction (Møller et al., 2016).

Oxidative stress can be caused by various mechanisms, including a reactive surface of the NPs forming reactive oxygen species (ROS) via interaction with mitochondria, and the so-called "respiratory burst" from inflammatory cells. Genotoxic effects can also be induced by the formation of ROS-mediated DNA strand breaks or by the formation of DNA adducts with different polyaromatic hydrocarbons (PAHs). When the damage to cellular DNA exceeds the capacity of DNA repair, mutations may occur constituting a critical part of cancer development. In general, NPs display a larger (by mass) and often more reactive surface area than larger particles, which makes them more prone to elicit toxic effects (Leikauf et al., 2020; Miller and Newby, 2020; Stone et al., 2017).

One can suspect that particles from different sources have different potencies regarding their ability to cause oxidative stress, inflammation, and genotoxicity. This review focuses on studies on the actual particles, but some studies instead use extracts from particles. For example, an interesting study by Park and colleagues compared the toxicity of extracts from fine (<2.5 μm) particles produced from various combustion sources (diesel and gasoline engines, biomass burning, coal combustion) to others from non-combustion sources (road dust, sea spray aerosols, ammonium sulfate, ammonium nitrate, and secondary organic aerosols (SOA) (Park et al., 2018).

While the current understanding of the health effects of NPs/UFPs to a large extent has been based on toxicological data, the importance of the UFP fraction in the adverse cardiopulmonary effects has also been investigated in epidemiological studies. The latest comprehensive review of epidemiological studies on the health effects of UFPs published in 2019 concluded that the evidence regarding associations of mortality and morbidity with UFPs is inconsistent or insufficient (Ohlwein et al., 2019). The authors emphasized one of the most important issues in the evaluation of UFP-related health effects is the quality of exposure assessment. While epidemiological studies evaluate the role of realistic exposure conditions in humans, they cannot always provide accurate or complete estimation of true exposures, often relying on a limited number of measurements made at sites located far from the population(s) of concern or modelling techniques with underlying assumptions. Furthermore, contrary to toxicological studies that can produce precise measures of exposure concentrations and durations and assess the relative contribution to response of components of pollutant mixtures, epidemiologic investigations cannot always disentangle independent biological responses due to specific pollutants, often because of high correlation between pollutants, as well as potential confounding factors that cannot always be adjusted for. Therefore, an integrated review of evidence from both toxicological and epidemiological research is needed to better understand the effects of UFPs on human health (Lippmann and Schlesinger, 2000).

This review aims to compile data on toxicity (*in vitro* and *in vivo* studies) and health effects (controlled human exposure studies and epidemiological studies) of particles from different sources with a focus on road traffic emissions as well as particles from shipping, aviation, and rail. The purpose was to summarize findings and assess whether current knowledge can support the use of different exposure-response functions of NPs from different sources or, more generally, how to consider the relative toxicity (potency) of the NPs, when performing health risk assessments. The review focuses on NPs/UFPs and does generally not refer to studies using larger particles (see more details in "search strategy"). As already indicated, NPs and UFPs are used interchangeably and the term UFPs is e.g. used more in the epidemiological part since this is the more common term used in the field. Also, a certain part of the review focuses on comparing DEP and biodiesel exhaust particles.

2. Toxicity studies on nanoparticles

2.1. The search strategy

Medline was used with different combinations of search terms of "nanoparticles" or "ultrafine particles" and different transport modes. Since particles collected from combustion in a laboratory setting would mainly (but not always) consist of NPs, a second search strategy was performed to include "diesel exhaust" as a term. This led to an overwhelming number of studies (not possible to summarize here), which led us to focus on studies that compared diesel and biodiesel exhaust particles and to mainly focus the search on three toxicity endpoints: inflammation, oxidative stress, and genotoxicity. Although this review focuses on nanoparticles (NPs, <100 nm), the cut-off size of 100 nm was not strictly used and in general, also studies describing sizes below approx. 300 nm were included (often called quasi-UFPs). This notwithstanding, and due to the scarcity of studies on NPs, important studies and reviews on larger-size fractions were sometimes also considered. We also screened the literature to find relevant reviews and additional studies that were included in reference lists or recommendations. The main part of the search was performed during spring 2022 although additional studies were added during the preparation of the manuscript during autumn 2022. Studies investigating extracts from particles were, in general, not included.

2.2. General results

Based on different search terms a total of 583 articles were found, among them 61 were found relevant and chosen based on the toxicological parameters considered. See Table 4 in supplement materials for more information on the search strategy and the obtained results, in terms of the number of articles found as well as the number of those considered relevant. The most relevant studies are summarized in Tables 1–4 (SI Tables 1–3), as well as in the text below.

2.3. Diesel exhaust

Many studies have been published regarding the toxic effects of diesel exhaust. In a recent review, it was concluded that 104 studies had been performed on controlled human exposure experiments with diesel exhaust (Long and Carlsten, 2022). Exposure sessions in these studies were typically 1 h or 2 h in duration, with participants alternating between exercise and rest, and most studies targeted a PM concentration of 300 $\mu g/m^3$ (Long et al., 2022). The review concludes that several studies consistently demonstrated increased levels of biomarkers for oxidative stress and pulmonary inflammation after exposure, thus providing evidence that such mechanisms are likely important for the health effects observed in humans. Inflammatory markers, however, were not consistently increased in asthmatic subjects, although NPs exposure worsened asthma physiology. Several other effects that can be linked to cardiovascular effects were repeatedly observed, including impairment of vasomotor function and endogenous fibrinolytic function. The review also presents data showing that ozone exposure may worsen the effect of diesel exhaust. The effects found in these studies are summarized in Fig. 1 (Long and Carlsten, 2022).

With regard to effects related to long-term exposure, IARC (International Agency for Research on Cancer) has classified diesel engine exhaust (the full exhaust) as "carcinogenic to humans" (Group 1) (IARC, 2014). Indeed, the diesel PM as a component of traditional diesel exhaust has been considered to be the primary driver of lung tumorigenesis in rats exposed chronically to diesel emissions (Iwai et al., 1997). However, many of these studies were performed with "old" diesel engines and using the whole exhaust, not only the particles. Therefore, the relevance of these results for the new engines, and the contribution of NPs to the total toxicity are still not clear. A study performed by the Health Effects Institute in the USA and its partners carefully investigated carcinogenic effects in rats (16 h/day, 5 days/week for up to 30 months) of "new-technology diesel exhaust" (NTDE) and compared it to traditional technology diesel exhaust (McDonald et al., 2015). The study showed less severe toxic responses to NTDE compared to that observed previously for traditional diesel exhaust. For example, NTDE-exposed rats did not show the extensive accumulation of particles within alveolar macrophages or "the inflammation leading to neoplastic transformation of epithelial and lung tumors", as described in early studies with DEP. Interestingly, the effects of NTDE were similar to those observed in rats exposed chronically to NO_2 alone, suggesting that NO_2 could have been responsible for most of the effects observed for the NTDE. It should be noted that this study compared the full exhaust and not the same mass of particles from the two different techniques (as many other studies in this review). In a further systematic review, the authors concluded that "exposure to different components in diesel exhaust can have distinct and independent health effects" and called for more studies comparing the effects of particle-filtered and whole diesel exhaust (Weitekamp et al., 2020).

Because an overwhelming number of toxicity studies were found when searching for "diesel exhaust" and various terms for toxicity, we decided to focus on studies comparing either different types of diesel exhaust (e.g., Euro 4 and Euro 5 engines, SI Table 1), studies including also other NPs for comparison, or on studies comparing diesel and biodiesel (discussed in the next section, Table 1). Two studies showed no clear differences in toxic effects between NPs from Euro 4 and Euro 5 engines (Mastrofrancesco et al., 2014; Pierdominici et al., 2014) or from Euro 3 and Euro 4 engines (Rossi et al., 2021). The observed effects, both *in vitro* and *in vivo*, included cytotoxicity and intracellular ROS production. An *in vivo* study (rats) showed that the NP-enriched particles from a Euro 4 engine increased the vulnerability to cardiac arrhythmias in rats (Rossi et al., 2021). Another study focused on cardiovascular effects showed that DEP and carbon black particles were taken up by platelets, but only DEP (from 6 μg/mL) induced platelet aggregation and Ca^{2+} release *in vitro* (Solomon et al., 2013). DEP also caused collagen-induced platelet aggregation *in vivo* following i. v. injection. In the following section, we discuss studies comparing NPs from diesel and biodiesel exhaust more specifically.

2.4. Diesel vs biodiesel/renewable diesel

Biodiesel can be regarded as fuels from biological sources and is typically produced by so-called "transesterification" of lipids with alcohols (mainly methanol) into fatty acid methyl esters (FAME) in which the lipid can come from plant oils from rapeseed, soybean, and animal fats, among other sources (Bunger et al., 2012). In the case of rape seed the biodiesel is called rapeseed methyl ester (RME). Alternatively, the newly developed hydrogenated vegetable oil (HVO) uses hydrogen instead of methanol to produce biodiesel-like fuel. In a review from 2012 (Bunger et al., 2012) it was concluded that, when compared to diesel, the combustion of biodiesel reduces emissions of carbon monoxide, hydrocarbons, PM, and PAHs, while increasing nitrogen oxides and aldehydes emissions. The authors concluded that "most biological *in vitro* assays show a stronger cytotoxicity of biodiesel exhaust, and the animal experiments reveal stronger irritant effects". Both findings are possibly caused by the higher content of nitrogen oxides and aldehydes in biodiesel exhaust (Bunger et al., 2012).

A recent study comparing acute cardiovascular effects of controlled human exposure to diesel (or so called petrodiesel since it is made from petroleum) and biodiesel exhaust in healthy volunteers concluded that both exhausts showed similar cardiovascular effects, despite the differences in PM composition and particle reactivity between both exhausts (Unosson et al., 2021). In another controlled human exposure study, the participants were exposed to relatively low concentrations of PM1 emissions from the HVO combustion (~1 $\mu g/m^3$ and ~90 $\mu g/m^3$) for vehicles with and without exhaust after-treatment systems, respectively. It was concluded that short-term exposures did not increase urinary

Table 1
Summary of toxicological studies on diesel/biodiesel exhaust NPs.

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Diesel oil (DE) Biodiesel (BD) Sub-10 nm NPs	NPs were collected at the exhaust of a three-cylinder, two valves diesel engine fueled with a biodiesel (BD) and a commercial diesel oil	Skin epithelial cells (HaCaT) and Lung epithelial (A549) cells	Concentrations used: 1.2–12.0 ppm for 24 and 48 h	BD was less cytotoxic compared to DE in both cell types. Less cytokine release in a panel of 27 cytokines was observed for both DE and BD in A549 cells.	Malorni et al. (2017)
Ultralow-sulfur-diesel (ULSD) BD (B100, fatty acid methyl ester (FAME) produced from new canola seeds. 20% BD (B20, mixture of 20% FAME and 80% ULSD PCO (Pure canola oil)	Fuels were combusted using a light-medium duty diesel engine (Isuzu 4BD1-T, 3.9L)	Human airway epithelial (NuLi-1) and (10 KT) cell lines	Exposed to exhaust particles for 1 h in a specific chamber. Cultures were incubated in an atmosphere with 5% CO_2 for 6, 12, or 24 h at 37 °C	All exposures caused significant cellular apoptosis. ULSD exhaust showed the highest apoptosis in both cell lines. A significant reduction in cell viability was observed for all exhaust exposures. PCO > B100 > ULSD In most cases, the highest inflammatory response was observed for B100 exhaust	Mullins et al. (2016)
DE BD100 (derived from coconut oil) BD20 (20% BD + diesel) BD50 (50% BD + diesel) BD90 (10% Triacetin +90% biodiesel)	Emissions were generated using a 6 cylinder, common-rail, turbo-charged diesel engine	Bronchial epithelial cells (Primary HBECs) Air-liquid interface	Exposed for 30 min D100 (PM, 0.79 mg/m^3) B100 (PM, 0.09 mg/m^3) B20 (PM, 0.42 mg/m^3) B50 (PM, 0.19 mg/m^3)	B20, BD50, BD100, and BD90 exposures significantly increased the cell death compared to D100 BD20, BD50, and BD90 exposures significantly upregulated HO-1 mRNA expression. BD100 and BD90 exposure significantly increased IL-8 levels compared to D100. Overall, biodiesel and triacetin/ biodiesel can increase the adverse effects of diesel emissions	Vaughan et al. (2019)
D100 (Diesel) RME20 (BD blends with 20% animal fat methyl ester and D100) AFME20 (BD blends with 20% rapeseed methyl ester and D100)	PM collected from two light-duty diesel engines, complying with Euro 2 or Euro4 emission standards	Lung epithelial (A549) cells, monocyte derived (THP-1) and umbilical vein endothelial cells (HUVECs)	Exposed to 0.78–100 μg/mL for 3 and 24 h	Euro4/D100 p.m. generated higher ROS levels compared to RME20 and AFME20 in THP-1 cells. All types of PM caused rather similar levels of DNA damage in A549 cells Overall: particles emitted from combustion of biodiesel blends were larger in size and less or equally potent than particles emitted from combustion of D100	Hemmingsen et al. (2011)
Petroleum based diesel Soy BD Animal BD Renewable hydro-treated diesel	Particles collected from a heavy-duty vehicle; (2000 Freightliner Truck equipped with a 2000 Caterpillar C15 engine)	Human U937 monocytic cells (differentiated to macrophages)	Exposed to 5, 10 and 50 μg/ mL for 25 h	Diesel and BD exposures induced COX-2, IL-8, and CYP1A1 in human U937-derived macrophages. Exposures significantly increased Nrf2 activity (marker of oxidative stress)	Vogel et al. (2019)
DF0 (Standard diesel fuel) BD7 (7% RME + diesel fuel) BD30 (30% RME + diesel fuel)	Direct injection diesel engine (Euro 3 standard) The exhaust was sampled downstream from the diesel engine (P1), after diesel oxidation catalysis, DOC (P2) or after DOC + diesel particulate filter, DPF, devices (P3)	Lung epithelial (A549) cells (ALI)	3 h exposure to a continuous flow of 10% diluted exhausts (flow rate 2 L/min)	An increase of 8-oxodGuo was observed in cells exposed to DF0 in all sampling points, and to BD7 in P1 and P3 Overall, no clear differences in pattern of toxicity were found between fuels	Barraud et al. (2017)
D100 (Diesel fuel) B10W20 (10% butanol, 20% waste-cooking oil and 70% diesel) B10W40 (10% butanol, 40% waste-cooking oil and 50% diesel)	The diesel engine (Robin SDG 2200, manufactured by Subaru Co. Ltd.) was used	Lung epithelial (A549) cells and Chinese hamster ovary epithelial (CHO–K1) cells	5, 10 and 20 μg/mL for 3 or 24 h	All PM caused genotoxicity in terms of micronuclei formation and DNA strand breaks. D100 (DEP) was most potent	Yang et al. (2017)
Diesel fuel (DF) RME100 or RME30 HVO100 or HVO30 CNG (compressed natural gas)	Evaluated PM emissions from a heavy-duty EURO IV diesel engine (also operated with or without an oxidative catalyst) A bus powered by CNG was included for comparison with the liquid fuels	Murine macrophage cells (RAW264.7)	15, 50, 150 and 300 μg/ml of each sample for 24 h	All PM were cytotoxic, DEP had the highest cytotoxicity in the lowest dose. All samples at least at some PM doses significantly increased TNF-α levels, with no clear difference between the PM. DEP and 30% HVO (blended with DEP) induced the strongest genotoxic responses. CNG bus showed the weakest genotoxic potency, but had the strongest	Jalava et al. (2012)

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Table 1 (*continued*)

Particles	Source	*In vitro*/*In vivo*	Conc./doses	Results	Ref.
				oxidative potency. RME fuel generally decreased toxicity, but the largely increased particulate mass counteracts this effect	
D100 (pure diesel) BD10 (10% biodiesel mixed in diesel). Biodiesel obtained from spent coffee grounds DE10 (9% ethanol and 1% biodiesel mixed in diesel)	Four-wheel drive medium sport utility vehicle (SUV) having a 4-cylinder, naturally aspirated diesel engine with an indirect injection system.	Murine macrophage cells (RAW264.7)	160 μg/mL for 4 h	BD10 showed decrease in cell death (−60.8%) compared to D100, while DE10 led to increase in cell death (84.1%) compared to PM from diesel fuel. biodiesel or ethanol have no significant impact on changes in ROS, TNF-α and IL-6.	Wong et al. (2022)
Petrodiesel B20 (blended at 20%)	Exhaust was collected from a 4-cylinder, 1.9 L Volkswagen light-duty diesel engine	Monocyte derived (THP-1) cells (differentiated to macrophages) Lung epithelial (BEAS-2B) cells Female C57BL/6 mice	*In vitro*: 10 and 20 μg/mL *In vivo*: ~84 μg/treatment for 3 days	THP-1: B20 exposure increased G-CSF, IL-8 and TNF-α levels BEAS-2B: B20 exposure increased IL-8 levels, and both petrodiesel and B20 induced MCP-1 levels. *In vivo*: B20 exposure increased G-CSF, IP-10, and IL-6 cytokine levels	Fukagawa et al. (2013)
DE and BD PM2.5	Metropolitan DE (50 ppm of sulfur with a blend of 5% BD) 100% BD - B100 (sewage methyl esters)	Male Balb/c mice	600 and 1200 μg/m^3 (PM2.5) for 2 h	HRV was increased for BD-600, D-600 and D-1200. BALF from BD exposure groups exhibited an increase in neutrophils. In the bronchial epithelium, BD exposure showed an increase in the receptor of endothelin-A (ET-Ar), endothelin-B (ET-Br), and VCAM-1. An increase in iNOS was observed at D600	de Brito et al. (2018)
Petroleum diesel (DE) BD ~720 nm and larger After sonication BD and DE particulates had a diameter of ~216 nm and ~312 nm, respectively	Particulates collected from exhaust of a mechanically controlled, naturally aspirated directly injected Isuzu C240 diesel engine	Adult female C57BL/6 mice	9 μg and 18 μg of total carbon/mouse of BD particulates for 24 h, 7 d and 28 d	Increase in LDH levels and MPO activity in lung homogenates for BD exposure, indicating cell damage and inflammatory response compared to DE. Upon 24 h exposure to BD and DE, cytokine, chemokine/growth factors were significantly increased (IL-6, IL-1α, MCP-1, RANTES, G-CSF etc.) in BALF. Overall, cytokine levels were prominent both in BALF and lungs for BD exposures compared to DE	Yanamala et al. (2013)
Metropolitan diesel (500 ppm of sulfur with a blend of 3% biodiesel) BD100 (from soybean ethyl esters) BD50 (50% diesel and 50% BD)	Exhaust particulates were collected from a stationary diesel electrical generator (BD-2500 CFE; Branco), fitted with a simple connection on its exhaust tube	Adult male Balb/c mice	PM2.5 inside the chamber:45.08 μg/m^3 (control), 556.41 μg/m^3 (diesel), 551.61 μg/m^3 (BD50), and 550.13 μg/m^3 (BD100) during 1 h exposure	24 h after exposure, neutrophils increased with BD50 and DE, macrophages increased with BD100, BD50, and DE exposures Overall, the study suggested BD was more toxic than diesel as it promoted cardiovascular alterations as well as pulmonary and systemic inflammation.	Brito et al. (2010)
D100 (Diesel fuel) BD100 (soy biofuel) A mass median aerodynamic diameter of 168 nm and 113 nm for D100 and B100 was estimated	Exhaust was generated using a single cylinder, 0.320-L displacement, Yanmar L70 V diesel generator	Female BALB/cJ mice	50, 150, or 500 μg/m^3 for 4 h/d, 5 d/wk for 4 wk)	In both lung and liver, IL-6 and IL-12p70 levels were significantly increased. TNF-α levels were increased only in liver. IL-10 and MCP-1 increased only in the lungs upon exposure to BD100. D100 exposure increased IFN-γ levels in the lungs and IL-12p70, and TNF-α increased both in the lung and liver	Shvedova et al. (2013)
Petrodiesel BD100 (soy biodiesel) BD20 (20% BD and 80% petrodiesel)	Exhaust was generated using Yanmar L70 diesel engine (Adairville, GA) and Pramac E3750 generator (Marietta, GA).	Normotensive Wistar–Kyoto and spontaneously hypertensive rats	Exposed to 0, 50, 150 and 500 μg/m^3, 4 h/day for 2 days or 4 weeks (5 days/week)	All three exhausts produced only modest effects in both strains BALF γ-glutamyl transferase (GGT) activity was increased in both strains (Diesel > BD100 > BD20) Overall, results showed modest cardiovascular and pulmonary effects at low concentrations of all exhausts	Bass et al. (2015)

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Table 1 (*continued*)

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Petroleum diesel (DEP_{13}, $DEP_{9.7}$ and DEP_{17}) Hydro-treated vegetable oil (HVO_{13}) RME_{13} Soot agglomerates size (diameter ~ 50–300 nm) Smaller primary particles (diameter ~ 10–30 nm)	Particles were generated using a modern heavy-duty diesel engine	Female C57BL/6Tac mice	A single dose of collected particles was applied (6 μg, 18 μg or 54 μg per mouse) All cohorts were euthanized on day 1, day 28 and day 90 post-exposure	RME_{13} showed the mildest effect. 28-day post-exposure in mice revealed more lung particle retention for DEP_{13} and HVO_{13} exposures 90-day post-exposure to DEP_{13} exhibited a significant increase in neutrophils and lymphocytes. DEP_{17} and HVO_{13} exposures increased tail length in both lung and liver cells On day 90, DEP_{17} and HVO_{13} exposures increased tail length in both lung and liver cells	Bendtsen et al. (2020)
Petrodiesel B7 (7% FAME in petrodiesel) SHB20 (7% FAME, 13% hydrotreated vegetable oil in petrodiesel)	DE from a Euro 5-classified diesel engine	Adult male Fisher 344 rats	Exposed for 7 consecutive days (6 h/day) or 28 days (6 h/day, 5 days/week), both with and without a diesel particle filter (DPF). B7 and SHB20 p.m. conc. In exposure chambers were ~2 mg/m^3 without DPF, 0.17 and 0.19 mg/m^3 with DPF	Histological and cytokine analysis in BALF did not reveal adverse pulmonary effects from B7 or SHB20 exposures. Significantly different gene expression levels (for B7 compared to SHB20) indicated e.g., disturbed redox signaling. Further, PM presence induced a higher expression of redox than no-filtered exhaust. No genotoxic effects in the lungs were observed	Magnusson et al. (2019)
Gasoil BD30 (Gasoil with 30% of rapeseed methyl ester)	Aerosols were generated from Euro4-compliant supercharged common rail direct injection diesel engine Upstream: with oxidation catalyzer (P1) Downstream: with oxidation catalyzer and particles filter (P2)	Male Wistar rats	Exposed for 3 h every day for 5 days for 3 weeks PM (mg/m^3): P1: Gasoil- 23 BD30- 24 P2: Gasoil- <.1 BD30- <.1	Gasoil exposure showed an increase in phosphorylation of H2AX histone in nuclei of rat lungs (P2 group) Only SOD was induced when rats were exposed to BD exhaust collected upstream of the particle filter (P1 group)	Douki et al. (2018)
Alcohol blended diesel (AD, 30% mol n-butanol and 70% mol n-dodecane) Biofuel blended diesel (BD, 30% mol methyl decanoate and 70% mol n-dodecane) Median particle size: AD (41 ± 5 nm), and BD (26 ± 3 nm	PM were generated with fuels AD and BD using a flat-flame burner (FFB) which operates at atmospheric pressure	Lung epithelial (NCI–H441) cells Monocyte derived (THP-1) cells	Particles: 50 $\mu g/cm^2$ (200 μg/mL), particle extracts: 12.5 $\mu g/cm^2$ (50 μg/mL) Exposed for 24 h	BD exposure in H441 cells induced upregulation of CYP1A1 and 1B1 mRNA levels, which was higher compared to AD A significant increase in IL-8 levels (in H441 cells) was observed (BD > AD) Significant increase in TNF-α levels (in THP-1 cells) was observed (BD > AD)	Jaramillo et al. (2018)
Fossil diesel (B0) 20% RME (B20, 20% rapeseed-methyl ester and 80% fossil diesel) Pure RME (B100)	Exhaust was generated using an Opel Astra X20DTL engine. The exhaust was sampled at the tailpipe, diluted ten-fold with filtered ambient air, MSA.	3D *in vitro* model of the human epithelial airway barrier	Exposed at the ALI for 2 or 6 h Particle numbers observed at around 50 nm diameter: B0: $1.14x10^7/cm^3$ B100: $1.57x10^7/cm^3$	The highest cytotoxic effect was observed for B100 exhaust upon 6 h exposure. Pro-inflammatory responses were induced by all exhaust types. B100-exhaust (6 h exposure) has the highest pro-inflammatory potential (TNF and IL-8) compared to B0 and B20	Steiner et al. (2013)
First-generation BD: B7 (7% FAME in diesel oil) B20 (20% FAME in diesel oil) Second-generation BD: SHB fuel (13% hydrotreated vegetable oils and 7% FAME in diesel oil)	DEP were collected from the exhaust of a Fiat Panda (2014), equipped with a 1.3 L Euro 5 diesel engine	Lung epithelial (BEAS-2B) cells	10, 50, and 100 μg/mL for 4 and 20 h	Exposure to all particles indicated a significant increase in IL-6, CYP1A, and HO-1 expression after 4 h 20 h exposure showed further increase in IL-6, CYP1A1 expression. However, HO-1 levels were decreased compared to 4 h exposure	Skuland et al. (2017)
First-generation biodiesel fuel: B7 (7% vol. FAME in diesel oil) B20 (20% vol. FAME in diesel oil) Second-generation biodiesel fuel: SHB (13% vol. Synthetic HVO and 7% vol. FAME in diesel oil)	DEP were generated from A Fiat Panda with compression ignition engine 1.3 JTD, common rail third-generation injection system (fulfilling the requirements of the Euro V stage)	Lung epithelial (A549 and BEAS-2B) cells	1, 10, 25, and 50 μg/mL for 6, 24 or 48 h	A significant induction in single-strand breaks (SSBs) in both cell lines after exposure to all DEPs. B7-DEP were most effective in inducing SSBs None of the tested exposures showed significant induction of oxidative DNA damage No significant induction in double-strand breaks (by γ–H2AX foci) was observed for all	Kowalska et al. (2017)

(*continued on next page*)

Table 1 (*continued*)

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Hydrodynamic diameter in LHC medium (nm): B7 (126 ± 64), B20 (107 ± 49) and SHB (113 ± 48)				DEPs after 24 h However, the frequency of MN formation was increased for all after 48 h in both cell lines	
Biodiesel exhaust	PM collected from the exhaust pipes of a bus engine	C57BL/6 mice	Exposed to 250 μg or 1000 μg of BD over 5 consecutive days	The BD particle number in pulmonary parenchyma was 175 and 300-fold higher for 250 μg and 1000 μg exposure, respectively. Exposures increased macrophage number and TNF-α protein levels in murine lungs Further, exposure to 250 μg BD increased Nrf2, p–NF–kB, and HO-1 protein levels	Cattani-Cavalieri et al. (2019)

exposure biomarkers or inflammatory markers in plasma, but that exposures without after-treatment systems caused a slight increase in lipid peroxidation, which was therefore associated with the particle fraction (Krais et al., 2021). Furthermore, a recent review summarized articles from 2002 to 2019 describing the toxicological effects (genotoxicity, oxidative stress, and inflammation) of biodiesel exhaust exposure in humans, animals and cell cultures, and concluded that "combustion products from biodiesel and petrodiesel fuel may evoke similar toxicological effects on genotoxicity, oxidative stress and inflammation" (Møller et al., 2020). This review included both studies on PM as well as extracts.

When reviewing studies that compare diesel and biodiesel particles, we found nine *in vitro* studies, eight *in vivo* and one study using both *in vitro* and *in vivo* models (see Table 1). Overall, six *in vitro* studies were carried out in lung cells; two studies highlighted that DEP had higher potency to cause DNA damage than biodiesels (Barraud et al., 2017; Yang et al., 2017), and one study showed more/equal potency to induce ROS and DNA damage of DEP compared to biodiesel particles (Hemmingsen et al., 2011). However, two studies indicated that biodiesel exhaust particles had a higher inflammatory response than DEP, measured as IL-6 and IL-8 secretion (Mullins et al., 2016; Vaughan et al., 2019). The study using both *in vitro* and *in vivo* models showed that biodiesel exhaust exposure resulted in higher IL-8 levels in BEAS-2B cells, and IL-6 in mice lung tissues, compared to diesel particles (Fukagawa et al., 2013). Also, two other *in vivo* studies (Brito et al., 2010; Yanamala et al., 2013) indicated that biodiesel exposures showed a higher inflammatory response (IL-6, IL-1α, MCP-1, increase in neutrophils etc.) than diesel ones. In a recent study comparing the toxicity of diesel (three different types), HVO, and RME exhaust particles following intratracheal instillation in mice, the results indicated that RME induced the least toxic response (Bendtsen et al., 2020). For example, neutrophils in BAL (bronchoalveolar lavage) fluid were not significantly higher compared to vehicle control, but higher in the case of HVO, see Fig. 2.

There are also some comparative studies with different mixtures of biodiesel. For example, one study with 30% butanol-blended diesel and 30% biofuel-blended diesel suggested that the biofuel exhaust induced more inflammation in lung cells (IL-8) and monocytes (TNF-α) than a butanol mixture (Jaramillo et al., 2018). Further, exposure to exhausts from first- and second-generation biodiesels (with 7–20% blending in diesel) increased IL-6 and CYP1A levels in lung cells at 10–100 μg/mL doses. Results showed that exhausts from B20 (20% FAME in diesel oil) and SHB (Synthetic Hydrocarbon Biofuel) fuel (13% hydrotreated vegetable oils and 7% FAME in diesel oil) were more potent inducers of IL-6 expression than B7 (7% FAME in diesel oil) (Skuland et al., 2017). Similarly, another study showed that a first-generation B7 was more effective in inducing single-strand breaks than B20 or SHB (Kowalska et al., 2017), although all blend exhausts caused single-stranded breaks in lung cells. Finally, an *in vivo* study using biodiesel exhaust collected from a bus engine showed the presence of particles and an increase in inflammatory and oxidative stress markers in the lung parenchyma (Cattani-Cavalieri et al., 2019).

Taken together, there is some evidence that biodiesel exhaust particles could have a weaker genotoxic potential compared to DEP, but the general conclusion is that biodiesel NPs cannot be regarded as low toxicity particles and that they show rather similar effects as DEP particles. It should be noted that today's real-world diesel vehicle particle emissions are due to a mix of biodiesel and diesel combustion, that varies in time and from place to place.

2.5. Gasoline

IARC has classified gasoline engine exhaust as "possibly carcinogenic to humans" (Group 2B), but this refers to the full exhaust. As mentioned above, Park et al. (2018) compared the toxicity of extracts from DEP (from heavy and light-duty engines) and gasoline exhaust particles in BEAS-2B cells. Whereas the study showed similar cytotoxicity for all exhaust sources, the mutagenic activity was highest for the DEP from heavy-duty engines, followed by gasoline and by DEP from light-duty engine exhausts. The ability to form DNA breaks (comet assay), however, was highest for extracts from gasoline particles, whereas extract from DEP exhibited the highest mutagenicity (Ames test) and ability to generate ROS (Park et al., 2018).

One interesting study by (Diaz et al., 2012) compared fresh and aged gasoline exhaust particles. This was done by generating diluted car exhaust from a single vehicle and since "the modern gasoline vehicle exhaust contains virtually no primary particle mass" they used so-called seed particles (in this case ash particles "Mt St Helens Ash") with or without "aging". The aging was performed by irradiating the mixture with simulated sunlight, which resulted in formation of secondary products (mostly organic) that condensed onto the seed particles. The results on rats showed that the breathing pattern was affected for both exposures, but inflammatory effects were mainly seen for the animals exposed to the aged aerosol with secondary organic particles. It was concluded that atmospheric photochemical processes enhance the toxicity of exhausts emitted from motor vehicles (Diaz et al., 2012). In a recent *in vitro* study, the 3D cell model MucilAir™ as well as BEAS-2B cells cultured in an air liquid interface were exposed to gasoline exhaust (Cervena et al., 2020). The estimated mass deposited on the cells was approximately 10 ng PM after one day and 50 ng after five days (growth area 0.33 cm^2, thus 30 ng/cm^2 and 151 ng/cm^2, respectively). Despite the relatively low dose, evidence of DNA damage (as induction of gamma-H2AX) in the MucilAir™ was observed after five days. The changes in gene expression, however, were weak and more pronounced in the BEAS-2B cells although the authors state that they "assume that it

Table 2
Summary of toxicological studies on traffic-related NPs from ambient environments.

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Ambient PM0.2 Traffic Aerodynamic diameter <200 nm	nPM (nano-sized particulate matter) were collected from an urban area in central Los Angeles, mostly from traffic emissions	Mice	Average nPM mass conc. After 150 h exposure was ~330 ± 25 μg/m^3. The number conc. Was 1.6 ± 0.3 x 10^5 particles/cm^3 Exposure for 5 h/day, 3 days/week and for a period of 10 weeks	Neuroinflammatory effects in the brain (corpus callosum) Also, serum TNF-α levels were elevated by 28% in exposed mice compared to filtered air	Babadjouni et al. (2018)
Ambient PM0.2 Traffic Aerodynamic diameter <200 nm	nPM were collected from an urban area in central Los Angeles, mostly impacted by traffic emissions	Male C57BL/6J mice	Target mass concentrations 300–350 μg/m^3 (particle conc. 5.6 x 10^4 ± 1.1 x 10^4 particles/cm^3) Exposure for 5 h/day, 3 days/week for 3 weeks	Following cerebral ischemia/reperfusion, mice exposed to NPs showed significantly larger infarct volumes and less favourable neurological deficit scores An increase in markers of inflammation and oxidative stress in the region of the ischemic core was observed	Liu et al. (2016)
Ambient PM0.2 Traffic <200 nm in diameter	nPM were collected in an urban area in Los Angeles primarily impacted by traffic-related emissions	Male C57BL/6J mice	Average mass concentration 330 ± 25 μg/m^3 (particle number conc. 1.6 (±0.3) x 10^5 particles/cm^3 Exposed to 10 weeks (150 h). 5 h/day, 3 days/week, for 10 weeks	Exposure to NPs caused brain effects such as significantly increased iNOS-expressing microglia in the brain (corpus callosum) of mice Overall, results suggested NP-exposure can result in white matter damage, axonal degradation and increased inflammatory microglia in corpus callosum of mice	Connor et al. (2021)
Ambient PM PM2.5 (<2.5 μm) Ultrafine particles (UFP) (<0.18 μm)	Concentrated ambient particles, Los Angeles	ApoE$^{-/-}$ male mice	Mice were exposed to UFPs and FPs (PM2.5), for a total of 75 h over 40 days Number concentration in UFP chamber (particles/cm^3) 5.59 (±1.23) x 10^5	UFPs caused significantly larger early atherosclerotic lesions in mice compared to PM2.5 UFPs also resulted in greater systemic oxidative stress compared to PM2.5	Araujo et al. (2008)
Ultrafine particles (UFP) (<0.18 μm) Thermally denuded UFP	Concentrated ultrafine ambient particles and thermally denuded concentrated ultrafine ambient particles, Los Angeles	ApoE$^{-/-}$ male mice	Mice were exposed to concentrated ultrafine ambient particles (CAP), thermally denuded CAP (deCAP) and purified air 5 h/day, 4 days/week for 8 weeks	Mice exposed to CAP showed accelerated atherosclerosis and progressively reduced heart function whereas mice exposed to deCAP showed no adverse effects The study showed that the absence of organic constituents reduced particle toxicity and cardiovascular effects CAP exposure also resulted in increased markers of oxidative stress	Keebaugh et al. (2015)
Ambient PM Ultrafine particles (UFP)	Collection of UPFs was conducted at the University of Southern California, Los Angeles	LDLR$^{-/-}$ mice	Approx. 360 μg/m^3 for 5 h/day and 3 days/week for 10 weeks	UFPs exposure increased LDL oxidation, triglycerides, free oxidized fatty acids, serum amyloid A, TNF-α, accompanied with atherosclerotic lesions The study showed that changes in lipid metabolism and HDL functionality are likely to be involved in UFP-mediated atherogenesis	Li et al. (2013)
Ultrafine ambient particulate matter >0.025–0.25 μm	Ambient particles were concentrated from Chapel Hill air, North Carolina	34 middle-aged individuals with metabolic syndrome Further individuals carrying null allele for GSTM1 (antioxidant gene) were identified by genotyping	Average particle concentration in the chamber: 189,000 particles/cm^3 Exposed to UFPs for 2 h, and with clean air for 2 h. Blood was obtained prior to, and at 1 and 20 h after exposure	UFPs exposure altered cardiac repolarization in GSTM1 null individuals Blood plasminogen and thrombomodulin were decreased, CRP and SAA were increased in whole population Overall, the study stated ambient ultrafine particles can cause cardiovascular changes in individuals with metabolic syndrome	Devlin et al. (2014)
PM from two road tunnels paved with different stone materials in the asphalt (two tunnels Marienborg and Hell in Norway)	PM was collected with a vacuum pump and a high-volume cascade impactor sampler Sampled during winter, both during humid and dry road surface conditions	Bronchial epithelial (HBEC3-KT) cells	Exposed to coarse (2.5–10 μm), fine (0.18–2.5 μm) and UFP (≤0.18 μm), Concentarions used: 12.5, 25, 50, 100 and 200 μg/mL (equivalent to 0, 1.3, 2.6, 5.2, 10.4 and 20.8 μg/cm^2)	The release of the pro-inflammatory cytokines CXCL8, IL-6, IL-1α and IL-1β was markedly increased, for the fine PM fraction compared to coarse and UFP fraction for most of the conditions. However, at Marienborg, humid surface UFP exhibited similar or greater cytokine response compared to fine particulates. In particular, the ratio of organic carbon to total PM mass was associated with the pro-inflammatory potential	Skuland et al. (2022)

was probably induced by cultivation conditions in the exposure system (the impact of airflow) rather than by the effects of emissions". Thus, firm conclusions seem hard to draw.

2.6. Ambient nanoparticles (road traffic environment)

Table 2 compiles several studies on mice showing different brain effects following exposure to traffic-related ambient NPs at an average mass concentration of 300 μg/m^3 (Babadjouni et al., 2018; Connor et al.,

Table 3
Summary of toxicological studies on aviation-related (airport) NPs.

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Ambient PM0.25 Collected close to the airport Collected close to the highway Turbine engine particles Diesel exhaust particles (DEP)	Urban air samples collected from 1) Los Angeles International Airport (LAX) 2) Central Los Angeles Turbine and diesel samples were directly collected from diluted exhaust (of a Fighting Falcon turbine engine) and a low-sulfur fuel diesel (EN 590) engine.	Bronchial epithelial (16HBE) cells	5–100 μg/mL for 1, 2, 4 and 24 h Cells were also allowed to recover for 20 h (after 4 h exposure)	No cytotoxicity. City particles showed a most potent oxidative potential. City (road traffic emissions) slightly higher ROS compared to airport emissions. Airport PM induced a higher release of inflammatory mediators (IL-6, IL-8 and TNF-α) compared to city/traffic PM	He et al. (2018)
Airport UPF Road traffic emissions UFPs from a turbine engine	Airport and non-airport UFPs samples were collected using impactors from the airport and non-airport emissions, respectively UFPs were also collected directly from the exhaust of a turbine engine	Lung epithelial (Calu-3) cell model in combination with ALI	UFPs at low doses ranging from 0.09 to 2.07 μg/cm^2 for 24 h	An increase in LDH release was only observed at the highest exposed dose around 1.5 μg/cm^2 for most of UFPs samples. 24 h exposure to UPFs promoted the production of IL-6 and IL-8 at the highest concentration Overall, airport and non-airport (road traffic) UFPs, as well as UFPs samples from a turbine engine, have similar toxic properties	He et al. (2020)
Non-commercial airfield particles (JEP) Commercial airport particles (CAP) Average hydrodynamic size: 143–196 nm for JEP, and 136–269 nm for CAP (Large agglomerates were also observed) DEP CB	JEP collected from a non-commercial airfield Samples collected at the apron of a commercial airport NIST standard reference diesel exhaust particles (2975) CB (Printex 90)	Female C57BL/6Tac mice	6, 18 and 54 μg/mouse, euthanized after 1, 28 or 90 days	JEP and CAP caused similar effects as DEP and CB, e.g., dose-dependent increases in neutrophils after 1 day as well as increased levels of Saa 3 mRNA in lung tissue. After 28-day exposure, lymphocytes were increased for both JEP and CAP as well as neutrophils for the CAP	Bendtsen et al. (2019)
Non-volatile PM (nvPM) emissions	Emissions from a commercial CFM56-7B26 turbofan engine was collected, running on Jet A-1 base fuel or 32% HEFA blend at 85% and ground-idle thrust conditions	Lung epithelial (BEAS-2B) cells ALI condition	The aerosol was applied to BEAS-2B cell cultures for 60 min	Overall, exposure to nvPM from Jet A-1 induced cytotoxicity (LDH release) and oxidative stress (HMOX-1 increase), while HEFA blend caused increased cytokine release (IL-8 and MCP-1).	Jonsdottir et al. (2019)

Table 4
Summary of toxicological studies on rail-related (subway) particles.

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Ultrafine particulate from underground railway	Quasi-UFPM (PM0.18) collected from a European mainline underground railway station	Primary bronchial epithelial cells (PBECs)	PBECs were exposed apically (ALI) to 25 μg/mL	ROS generation confirmed. Exposure for 6 h showed 52 differentially expressed genes (DEGs) associated with epithelial maintenance and 24 h exposure led to 23 DEGs, involved with redox homeostasis and metal binding. Upregulation of metallothionein factors at both time points	Loxham et al. (2020)
PM from underground railway station Coarse (PM10–2.5) Fine (PM2.5) Quasi-ultrafine (PM0.18)	PM collected at a European mainline underground railway station	Primary bronchial epithelial cells (PBECs) Bronchial epithelial cells (16HBE) cells Cells were cultured as monolayers or at ALI	Monolayer and mucociliary ALI cultures of PBECs exposed to 1.1–11.1 μg/cm^2	Underground PM increased IL-8 release from PBECs, but was diminished in mucus-secreting ALI cultures Fine and ultrafine PM generated a greater level of ROS than coarse PM. Also, ALI cultures displayed increased HO-1 expression despite the presence of mucus	Loxham et al. (2015)
PM from underground railway station	Collected coarse (5–10 μm) and fine fractions (1–2.5 μm; 0.5–1 μm; 0.25–0.5 μm)	Lung epithelial (NCI–H727) cells	Exposed to PM solutions of 70 μg/mL for 3, 6 and 24 h	PM from an underground railway environment did not display higher cytotoxicity and oxidative stress levels compared to outdoor air	Spagnolo et al. (2015)
Diesel exhaust	Diesel-powered trains, and electric trains	29 healthy volunteers Exposed to DE while sitting as passengers in diesel-powered trains Exposure in electric trains was used as a control	The concentrations of black carbon and ultrafine particles were 8.5 μg/m^3 and 1.2–1.8 × 10^5 particles/cm^3 higher, respectively, in diesel compared to electric trains	Exposure to DE inside diesel-powered trains for 3 days caused reduced lung function and systemic effects in terms of altered heart rate variability and increased levels of DNA strand breaks in PBMCs compared to electric trains	Andersen et al. (2019)

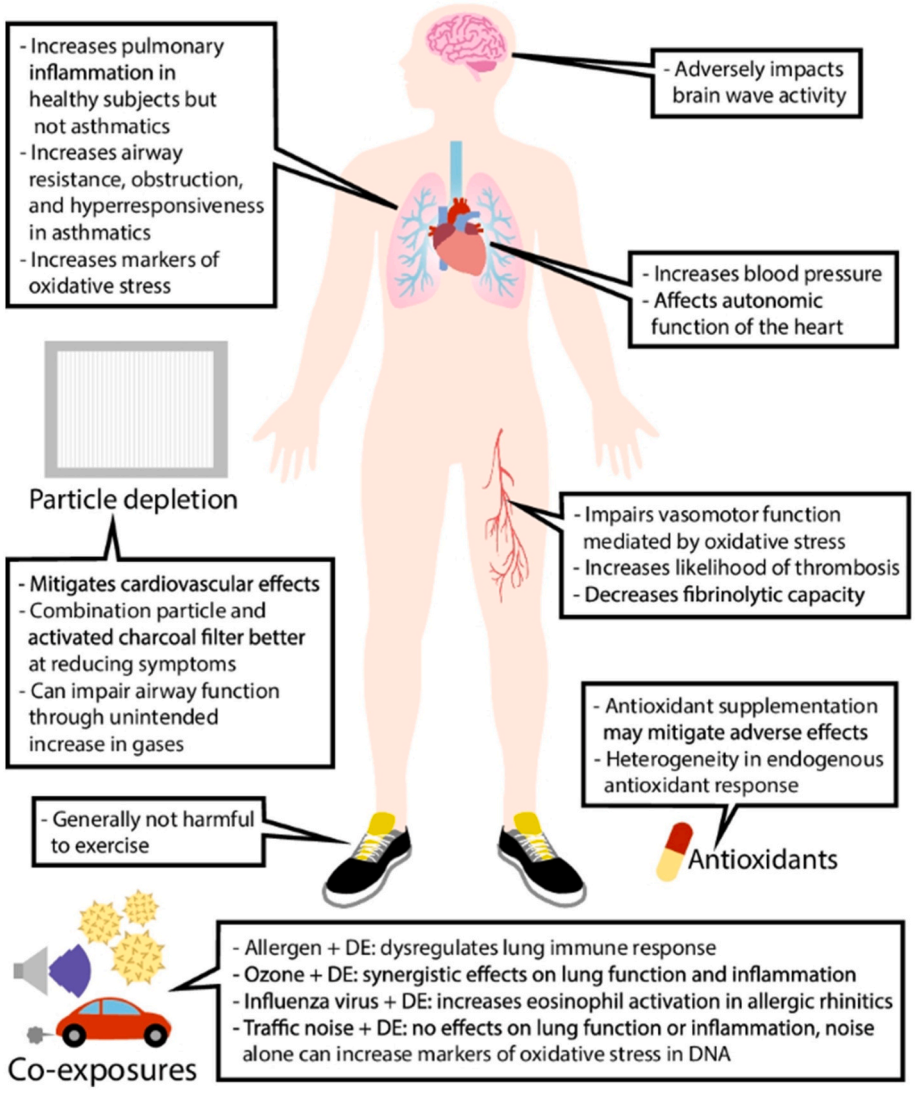

Fig. 1. Health outcome findings from controlled human exposure to diesel exhaust (DE) as summarized in Long and Carlsten (2022). Adapted from (Long and Carlsten, 2022).

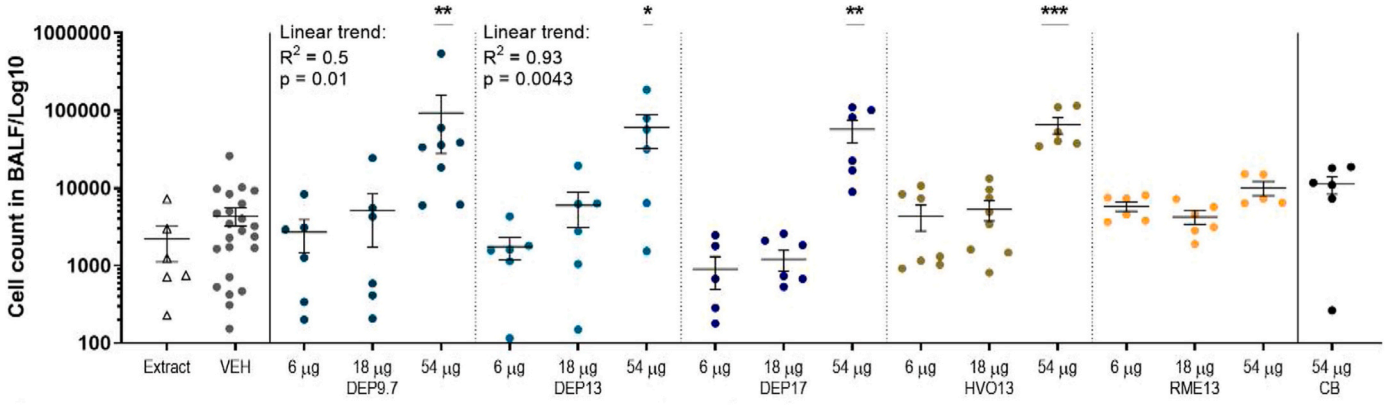

Fig. 2. Neutrophils in broncho-alveolar lavage of mice day 1 post-exposure to diesel exhaust particles (DEP) or exhaust particles from renewable fuels. The mice were exposed to 6, 18, and 54 µg of three different types of DEP (DEP9.7, DEP13, DEP17), and exhaust particles from the two renewable diesel fuels hydrotreated vegetable oil (HVO13) and rapeseed methyl ester (RME13). Adapted from (Bendtsen et al., 2020).

2021; Liu et al., 2016). It should be noted that this is a very high concentration, in the order of × 100 times the concentration of NPs/UFPs in many cities or × 15–20 times the concentration reported at different Asian locations (Phairuang et al., 2022). These studies were performed by collecting quasi-NPs (<200 nm) on Teflon filters in an urban area in Los Angeles, removing the NPs into a water solution followed by resuspension (vortex and sonication), and then freezing. Particles were then re-aerosolized using a nebulizer and mice were exposed in a chamber (repeatedly for several weeks). The effects found included an increase in inflammation and oxidative stress markers in the ischemic core region. The authors suggest that the effect could be triggered either by direct toxicity from NP transport to the brain via the olfactory bulb or through their dissemination in the bloodstream and crossing of the blood-brain barrier. An alternative toxicity mechanism could be a systemic inflammatory response that would permeate the blood-brain barrier. One weakness of these studies is the use of collected NPs leading to the need for re-aerosolization. In contrast, in an interesting study on freshly produced UFPs in a "real" environment, mice (genetically susceptible, apolipoprotein E–deficient) were exposed to concentrated UFPs (exposure approx. 113 μg/m^3, 5.6 × 10^5 particles/cm^3), concentrated PM2.5 (exposure approx. 438 μg/m^3, 4.6 × 10^5 particles/cm^3), or filtered air in a mobile animal facility close to a Los Angeles freeway (Araujo et al., 2008). The animals were exposed 5 h per day, 3 days per week, for a combined total of 75 h. The results showed that mice exposed to the UFPs exhibited significantly larger early atherosclerotic lesions when compared to mice exposed to PM2.5 or filtered air. Exposure to UFPs caused also other effects such as the upregulation of Nrf2-regulated antioxidant genes in the liver indicating systemic oxidative stress (Araujo et al., 2008). The study showed higher potency of traffic-related UFPs/NPs compared to larger ones. Importantly, the UFPs/NPs contained higher amounts of organic carbon (OC) and PAH and thus, this was proposed to explain the higher reactivity of UFPs. In another study, using similar exposure and same type of mice as in the study by Araujo et al. (2008), the authors compared the effects of concentrated UFPs to UFPs from which organic constituents had been removed by thermal denuding (i.e. the aerosol is heated up to a temperature sufficient to volatilize semi-volatile organic compounds). The results showed that the concentrated UFPs caused various effects related to atherosclerotic disease (e.g. increased size of arterial plaque and decreased HRV) but interestingly, such effects were not observed in the UFPs with less organic constituents (Keebaugh et al., 2015). It should be noted that the thermal process of denuding also led to lower mass concentration of UFP (28.6 μg/m^3) compared to the non-denuted (58 μg/m^3), and a role of this difference in mass cannot be totally ruled out. The role of organic carbon has also been reported when UFPs are collected at other locations. In a recent *in vitro* study, different sizes of PM were collected in Norway from two different road tunnels paved with different stone materials (Skuland et al., 2022). Both acellular ROS formation and inflammatory effects (cytokine release following exposure of HBEC3-kt cells) were then compared. Whereas the coarse and fine fractions had relatively similar ROS-forming potential, it was considerably lower for UFPs in nearly all samples. When tested up to 200 μg/mL (20.8 μg/cm^2) during dry road conditions, the particles showed no cytotoxicity and the fine particles were most potent in causing inflammatory effects. It was in general concluded that the "pro-inflammatory potentials of the road tunnel were not correlated to hydrodynamic size distributions, endotoxin content or acellular ROS generation" but that they appeared to be correlated to the content of organic carbon. The authors further discuss that the "large contribution of OC in our study could be explained by lower temperatures in the tunnels/Nordic countries during winter due to increased binding of volatile or semi-volatile species to particles (secondary OC)" (Skuland et al., 2022). It should be noted that the characterization of the PM was limited, as morphology and metal content were not analyzed.

A few controlled human exposure studies have been performed on concentrated UFPs collected from ambient air (Chapel Hill ambient air). The results showed e.g. an increase in markers of heart rate variability, a modest elevation in IL-8 levels in lavage fluid, and an increase in D-dimer (a fibrin degradation product) levels in blood following 2 h exposure of healthy volunteers (Samet et al., 2009). Different effects, such as an increase in C-reactive proteins were also noted in individuals with metabolic syndrome (Devlin et al., 2014). Similarly, adult volunteers (healthy or with mild asthma) exposed to concentrated UFPs for 2 h (with intermittent exercise) experienced a slight fall in arterial O_2 saturation, a small drop in forced expiratory volume in 1 s (FEV1), and a decrease in low frequency (sympathetic) power (Gong et al., 2008).

2.7. Aviation exhaust

Only a few studies have investigated the toxicity of aviation exhaust (see Table 3). A recent *in vitro* study exposed BEAS-2B cells cultured in the ALI to PM from one of the most commonly used aircraft turbines (a CFM56-7B26) either to conventional Jet A-1 aviation fuel or to 32% hydro-processed esters and fatty acids (HEFA) blend fuel (Jonsdottir et al., 2019). The study showed different toxic effects, like an increase in cell membrane damage, oxidative stress and pro-inflammatory response. A comparison was made to previous studies and it was concluded that "the cytotoxic effects of a single exposure of 1–2 h to PM from combustion of gasoline, diesel, and aviation fuel in BEAS-2B cells are moderate and comparable for similar doses" (Jonsdottir et al., 2019). Another study collected ambient PM0.25 from an airport in Los Angeles and at a central Los Angeles site, as well as exhausts particles (PM2.5) directly from a turbine engine (Fighting Falcon turbine engine) and a low-sulfur fuel diesel (EN 590) engine. The oxidative potential of the particles as well as toxic effects in human bronchial cells were then compared (He et al., 2018). The results showed that the traffic-related transition metals (Fe and Cu) in the airport and city samples mainly affected the oxidative potential. Furthermore, none of the particles caused cytotoxic effects, however, airport PM at 10 μg/mL induced a higher release of inflammatory cytokines (IL-6, IL-8 and TNF-α) than traffic emissions. It was concluded that "aviation emissions were the major contributor to the total mass of PM0.25 collected downwind a major airport" and that "airport-related PM0.25, even at relatively low exposure concentrations, possess toxic properties similar to the PM0.25 emissions from urban traffic". Furthermore, an *in vivo* study using mice instilled with particles from different sizes and origins concluded that "Pulmonary exposure of mice to particles collected at two airports induced acute phase response, inflammation, and genotoxicity similar to standard diesel exhaust particles and carbon black nanoparticles, suggesting similar physicochemical properties and toxicity of jet engine particles and diesel exhaust particles", see Fig. 3 (Bendtsen et al., 2019). The content of PAHs and metals in the airport particles in this study was similar to that of the NIST2975 material.

Taken together, the number of studies on aviation-related NPs is few and thus it is difficult to draw firm conclusions related to their toxicity compared to other sources. The studies published so far indicate, however, rather similar toxicity compared to other traffic-related particles.

2.8. Shipping exhaust

There is a limited number of studies in the area of shipping exhaust NPs. One study showed that PM2.5 emissions from the combustion of heavy fuel oil showed cytotoxicity and ROS generation in lung cancer cells (A549) (Wu et al., 2018). Another study comparing PM from different sites in the Netherlands showed that the fine (<2.5 μm) and quasi-ultrafine (<0.18 μm) particles collected from a harbor caused a significant increase in TNF-α in murine macrophages (Steenhof et al., 2011), and the quasi-ultrafine fraction was more potent compared to fine particles. Furthermore, a comparison between heavy fuel oil (HFO) and diesel fuel (DF) shipping emissions showed that HFO predominantly contained particles <50 nm and the DF contained mainly larger particles (>200 nm) (Oeder et al., 2015). Overall, reactions to HFO emissions

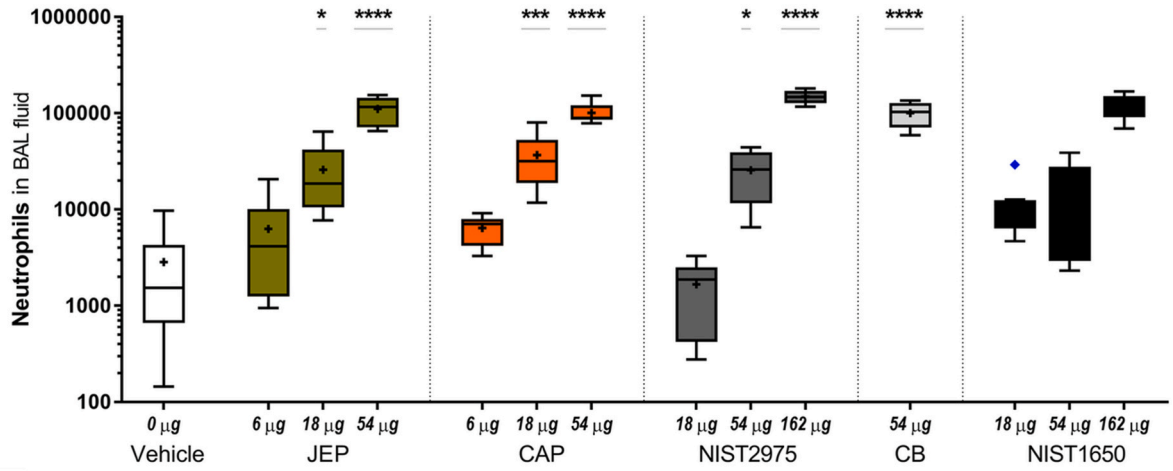

Fig. 3. The neutrophil influx in BAL fluid on day 1 following exposure to jet engine particles (JEP), commercial airport particles (CAP), and reference particles NIST2975, NIST1650, and Carbon black Printex 90 (CB). Mice were exposed to 6, 18, and 54 μg of JEP and CAP, to 54 μg of CB, and 18, 54, and 162 μg of NIST particles with 6 mice in each group. Adapted from (Bendtsen et al., 2019).

were dominated by oxidative stress and inflammatory responses, whereas DF emissions induced a broader biological response, affecting essential cellular pathways such as protein synthesis, energy metabolism, and chromatin modification (Oeder et al., 2015). Similarly, ambient PM0.25 were collected in three contrasting locations; central Los Angeles (USC), north Long Beach, and the Port of Long Beach and the aim was to identify the sources that contributed to the oxidative potential of the ambient particles in these locations. Results from an alveolar macrophage assay and multiple linear regression analysis indicated that vehicular emissions (39 ± 2%) and secondary organic aerosols (SOA) (40 ± 5%) from PM0.25 were the main contributors to the oxidative potential, and suggested that "emissions of PM0.25 related to port activities, (including emissions from ships, locomotives, and heavy-duty vehicles operating at the port) accounted for 16 ± 3% of the overall oxidative potential of the ambient PM0.25 samples" (Mousavi et al., 2019).

2.9. Rail/subway

Various studies of relevance for subway-related health effects, including *in vitro* studies using cell lines, studies on chemical composition, controlled short-term exposure studies in humans, and epidemiological studies of underground railway workers exposed chronically, were reviewed in 2019, concluding that "Studies aimed at understanding the effects of exposure to underground railway air pollution have generally found no consistent, convincing evidence for significant effects on the health after either acute (2–8 h) or chronic exposure" (Loxham and Nieuwenhuijsen, 2019). This notwithstanding, several *in vivo* and *in vitro* studies performed on PM10 particles showed a high capacity of subway PM to cause oxidative stress and DNA damage (Karlsson et al., 2005, 2006). Table 4 summarizes the results from studies focusing on NPs (see Table 4). One study analyzed the effects of the quasi-ultrafine (<0.18 μm) fraction of collected particles at different locations, including an underground railway station, an urban background site, a farm, three traffic sites (continuous traffic, stop-go traffic, and truck traffic), a harbor, and an area near a steelwork. Comparing coarse, fine, and quasi-ultrafine fractions, and using the same PM mass, the underground PM showed greater cytotoxic effects on RAW 264.7 macrophage viability (Steenhof et al., 2011). Another study with focus on UFPs exposed primary bronchial epithelial cells (PBECs) to size-fractionated underground (subway) particles (1.1–11.1 μg/cm^2), and measured cytotoxicity (as the release of lactate dehydrogenase), inflammation (IL-8), and ROS generation. The results showed fine and UFPs caused ROS generation, and induced IL-8 release in PBECs, but not in mucus-secreting ALI cultures (Loxham et al., 2015). Interestingly, the UFPs were more potent inducers of IL-8 release in cells from healthy individuals than from asthmatic ones.

2.10. Non-exhaust road traffic (road-brake and tire wear)

Non-exhaust road traffic particles originate from brake-, clutch-, tire-, and road wear. Based on mass, they are typically at the micron-size scale, although NPs are also formed in the process and they can dominate in terms of particle number (Fussell et al., 2022) and mass for certain hot spot operating conditions (Nosko and Olofsson, 2017). Since exhaust emissions have been decreasing in recent years, the proportion of particles from non-exhaust sources in urban air has become more significant. A health risk assessment on "tire and road wear particles (TRWP)" concluded that there were uncertainties in the risk assessment stemming from both the hazard and exposure assessments, but that "the current weight of evidence suggests that TRWP presents a low risk to human health" (Kreider et al., 2020). This risk assessment was mainly based on an inhalation study in rats (Kreider et al., 2012) testing concentrations up to 112.2 μg/m^3 (showing no effect), and for particles at the micron-size range. Thus, it seems difficult to draw firm conclusions. However, *in vitro* studies on micron-sized road-wear particles have indeed shown, for example, inflammatory effects (Karlsson et al., 2006; Lindbom et al., 2007). Furthermore, different studies have shown an association between coarse PM and daily mortality, and the association was stronger for November through May when road dust is the most important (Meister et al., 2012).

Regarding brake wear particles, there are some studies available, also including particles in the nano-size (see Table 5). One study investigated the toxicity of particles from four different brake pads (BW1-4), tires, and road pavement, as well as DEPs (Gerlofs-Nijland et al., 2019). All particles were collected as PM2.5, and the results showed that PM from tire/road wear and BW-1 and BW-2 appeared less potent in inducing pulmonary inflammation than BW-3, BW-4, or DEP. The authors suggest that the copper content possibly could explain the differences in the brake wear toxicity, but also discussed that "copper-free brake pads seem to emit more PM per volume of air and, albeit less toxic, the increased exposure concentrations may result in a higher health risk compared to the conventional brake pads with copper" (Gerlofs-Nijland et al., 2019). The toxicity of the copper content was also noted in another study on four different PM2.5 samples from brake wear. Here, A549 cells were used to show a good direct correlation between Cu content and toxic effects. A potential role of TiO_2 was suggested in another study investigating the toxicity of micron-sized

Table 5
Summary of toxicological studies on non-exhaust traffic particles (- brake wear).

Particles	Source	*In vitro/In vivo*	Conc./doses	Results	Ref.
Brake wear debris (BWD) NPs Fine crystalline particles below 100 nm	Air borne BWS generated in a laboratory by a full-scale brake dynamometer	Human lymphocytes	3, 15 and 75 μg/cm^2 (equivalent to 5.4, 27 and 135 μg/mL)	Only at 3 μg/cm^2 increase in frequencies of micronucleated binucleated cells (MNBNCs) observed after 48 h	Kazimirova et al. (2016)
Brake wear particles (BWP) Nano- to micrometer range	BWP harvested in two test facilities operated in France, providing samples from different braking systems and driving/testing conditions.	Lung epithelial (Calu-3) cells	Nano-sized fraction (50–400 nm) represents 26% by mass of the initial BWP. Cells were exposed to BWPs and to its nano-sized fraction at conc. 1–100 μg/cm^2 for 4–24 h	Cell viability was decreased with both samples, but BWP exhibited a stronger effect Both BWP and NPs exhibit a similar increase in ROS generation. Overall, results indicated nano-sized fraction appeared to be less toxic than BWP	Puisney et al. (2018)
Brake wear particles Different size fractions (2–4, 1–2, and 0.25–1 μm)	Brake wear particles generated from commercial low-metallic and non-asbestos organic automotive brake pads used in mid-size passenger cars	Human 3D multicellular model of lung epithelial (A549) cells and human primary immune cells (macrophages and dendritic cells) mimicking human epithelial tissue barrier.	Non-airborne samples: ~12, ~24, and ~48 μg/cm^2 Brake wear: ~3.7 μg/cm^2 Exposed for 24h applying a pseudo-air–liquid interface approach	Brake wear debris with low-metallic formula does not cause any adverse biological effects to the lung model However, brake wear particles from non-asbestos organic formulated pads (non-airborne) induced IL-8 release	Barosova et al. (2018)
Brake wear particles Nanoparticles (<100 nm) and fine particles (100 nm - 2.5 μm)	Different braking behaviours used in the study 1) Normal deceleration 2) Full stop 3) No stop run	Lung epithelial (A549) cells (ALI)	Total particle number (Number/cm^3) 1) Normal deceleration 8x (333x10^3) 2) Full stop 8x (1130x10^3) 3) No stop run (335x10^3)	Brake wear particles were non-cytotoxic in the doses tested. The tight junction's protein occludin density decreased significantly with increasing concentrations of metals on the particles (iron, copper, and manganese)	Gasser et al. (2009)
Brake wear (PM2.5) (Likely also containing NPs but not shown)	Four different brake pad/disc particulates PD1, PD2, PD3, and PD4 contained 13.98, 7.47, 3.21, and 0.18% Cu respectively	Lung epithelial (A549) cells	1–500 μg/mL for 3–48 h	The brake debris with lowest Cu content (P4) only caused minor effects whereas PM2.5 containing higher Cu quantities induced cell toxicity, correlated with Cu concentration	Figliuzzi et al. (2020)
Brake abrasion dust (BAD) Diesel exhaust particles (DEP) Average diameter (nm): BAD: 616.2 ± 1.6 DEP: 1228.2 ± 115.8	Mixed BAD sample was extracted from a filter collected from drum brakes used in buses and trucks representative of urban driving and highspeed braking conditions SRM-2975 DEPs from NIST	Monocyte-derived macrophages (U937)	4–25 μg/mL for 4–48 h	Both DEP and BAD perturbed phagocytosis and promoted inflammatory responses in U937 cells with similar potency Both particles induced IL-8, TNF-α, and IL-10 levels after 24h exposure	Selley et al. (2020)
PM2.5 from different types of brake pads Tire/road wear particles	1) ECE low-metallic pads containing copper (BW-1) 2) Semi-metallic pads without copper (BW-2) 3) Non-asbestos organic (NAO) brake pads (BW-3) 4) ECE-NAO hybrid brake pads ((BW-4) 5) Tire/road wear	Female BALB/cOlaHsd mice	Mice were exposed for 1.5, 3, or 6 h by inhalation (nose-only) up to 9 mg/m^3.	Cytotoxicity and oxidative stress were not observed Exposure to wear emissions (BW-3, BW-4) provoked inflammation evident by neutrophil chemoattractant, KC and MIP-2 and lung neutrophil influx, compared to other brake wear or tire/road wear particles.	Gerlofs-Nijland et al. (2019)
Tire particles *Note: micron-size*	TP1: fresh tire material sold under the trade name of "recycled SBR" (styrene butadiene rubber) TP2: a mixture of two scrap tire samples	Male Wistar Kyoto rats	Exposure 1: TP1 or TP2 (5 mg/kg) Exposure 2: soluble Zn, Cu (0.5 μmol/kg), or both Pulmonary toxicity and cardiac mitochondrial enzymes analyzed after 1 d, 1 wk, or 4 wk (for TP), and 4 or 24 h for metals	An increase in inflammation markers (in lavage fluid) was observed at day 1(TP2 > TP1), however, these changes were reversed by week 1. Effect on cardiac enzymes was not observed- Results indicated that the observed acute pulmonary toxicity of TP could be due to the presence of metals.	Gottipolu et al. (2008)

particles from brake pad particles in an ALI system (Barosova et al., 2018). The study concluded that brake wear particles with a "low--metallic formulation" were non-toxic whereas a sample with a "non--asbestos formula" triggered inflammation and cytotoxicity, which was linked to the presence of anatase (TiO_2). Furthermore, a recent study showed that brake wear particles (around 600 nm) caused a very similar inflammatory response as DEPs and that the effects were already observed in the lowest dose tested (4 μg/mL) (Selley et al., 2020). A study focusing on brake wear dust collected in brake drums in passenger vehicles showed that these dust samples were heterogeneous in size with few NPs and with a composition of primarily iron and copper. ROS were detected in the cell-free fluorescent test, but cellular oxidative stress response to the brake wear particles remained low (Zhao et al., 2015). Furthermore, lymphocytes exposed to brake wear NPs at 3 μg/cm^2 exhibited an increase in micronucleus frequency, suggesting chromosomal damage at a relatively low dose (Kazimirova et al., 2016). No

information regarding the toxicity of NPs from clutches was found.

No studies were found on the toxicity of tire wear particles at the nano-size scale, although, two *in vivo* studies investigated the inflammatory effects of tire wear particles (TP) in micron-size (Gottipolu et al., 2008; Mantecca et al., 2009). In one of them, tire wear particles were produced by milling forming particles <5 μm and one of the materials (called TP1) was sold as "recycled SBR" (styrene butadiene rubber), and the second (TP2) used a mixture of two scrap tire samples. Zn and Cu were detected at high levels in water-soluble fractions of the materials (TP2 > TP1). Both materials increased different markers of inflammation after one day of exposure and the results suggest a possible role for leached Zn and Cu, as TP2 was more potent than TP1. Another study investigated pro-inflammatory and toxic effects of two different sizes of tire particles, PM2.5 and PM10, following suspensions of these materials (Mantecca et al., 2009). The results showed toxic effects from both materials, concluding that "lung toxicity induced by PM10 was primarily due to macrophage-mediated inflammatory events, while toxicity induced by PM2.5 appeared to be related more closely to cytotoxicity" (Mantecca et al., 2009).

3. Epidemiological studies

The latest comprehensive review of epidemiological studies on the health effects of UFPs was published in 2019 including a systematic literature research in MEDLINE and Web of science up to May 11, 2017 (Ohlwein et al., 2019). The authors identified 85 original studies, conducting short-term (n = 75) and long-term (n = 10) investigations. As many as 8 (of which 7 were short-term) and 10 (of which 6 short-term) studies examined mortality and morbidity outcomes, respectively.

Only two out of eight studies on mortality have source-apportioned UFP exposure, using Positive Matrix Factorization (PMF) method described in detail elsewhere (Hopke, 2016). One cohort study with over 100,000 female participants from the California Teachers Study reported significant positive associations between ischemic heart disease (IHD) mortality and interquartile range (IQR) increase in long-term UFP exposure. Estimated hazard ratio (HR) values were 1.12 (95% CI: 1.04–1.22) for on-road gasoline, 1.14 (1.04–1.24) for off-road gasoline, 1.13 (1.03–1.24) for on-road diesel, and 1.14 (1.05–1.23) for off-road diesel (Ostro et al., 2015). In addition, no statistically significant associations were observed for either all-cause or pulmonary mortality. The second study from London, U.K., found no associations between daily cause-specific mortality and traffic-specific particle number distribution used as a measure of UFPs (with diameter <0.6 μm) (Samoli et al., 2016). The other six included studies on mortality considered short-term ambient UFP exposure without source apportionment and their results were inconclusive. Three out of these six studies reported positive associations with cardiovascular mortality, two studies demonstrated null effect, while one study observed inverse associations. Similarly, the results for respiratory mortality were also mixed.

Out of the six studies investigating the short-term effects of UFPs on various morbidity outcomes, including respiratory symptoms, arrhythmia events, and mental discomfort, only one study observed a significant association with an increase in Perceived Stress Scale score, a widely used stress appraisal measure (Mehta et al., 2015). It is worth noting that none of these studies implemented source apportionment. Three out of four long-term morbidity studies investigated the relationships between source-specific UFP exposure and adverse pregnancy outcomes in Los Angeles County, California, USA. In a cross-sectional analysis, increased odds for low birthweight, as well as preterm birth frequency were linked to UFP exposure from both diesel and gasoline exhausts, with no association with the exposure to shipping sources (Laurent et al., 2014, 2016a, 2016b). None of these long-term studies adjusted for co-pollutants, thus precluding the evaluation of independent effects. This brings us to the conclusion that there is inconsistent or insufficient epidemiological evidence for effects of UFPs on mortality and morbidity (Ohlwein et al., 2019).

3.1. Search strategy

For the current project, we systematically searched the MEDLINE database for eligible studies written in English investigating the health effects of UFPs for the period from May 12, 2017 up to April 1, 2022. We applied the search strategy from Ohlwein and colleagues (Ohlwein et al., 2019), which consists of three different concepts, operationalized with multiple synonyms and combined with the operator "AND": 1) ambient air pollution, 2) ultrafine particles/nanoparticles and 3) health and epidemiology. Inclusion criteria were: 1) epidemiologic studies, 2) reporting of health outcomes, such as mortality, or International Classification of Diseases (ICD)-coded diseases, symptoms, or adverse pregnancy outcomes, and 3) describing at least one quantifiable effect-measure association with UFPs or quasi-UFPs. The UFP measure/metric as well as exclusion criteria are more specified in the supplement. We included studies on mortality and morbidity outcomes, leaving out preclinical endpoints (e.g., lung function, inflammatory biomarkers, etc.). Further, a special focus was put on studies that considered UFP pollution source apportionment in an attempt to disentangle possible effects of UFPs attributed to different transport modes.

3.2. General results

The Medline search yielded 1423 references that were examined for inclusion and exclusion criteria. Out of them, 1403 were disqualified due to the exclusion criteria, leaving an overall number of 20 relevant original research articles. We additionally included one article that was not captured by our search strategy described above (Fig. S1). The majority of studies were related to the investigation of long-term effects (n = 14) assessing exposures averaged over a period of months to several years. These consisted of 12 cohort studies, one cross-sectional, and one case-crossover study. The remaining seven time-series studies investigated short-term associations using exposure estimates during hours to weeks. All these 21 studies are compiled in Tables 6 and 7, and the main findings are summarized below. Major differences in study group characteristics and exposure assessment techniques, leaving too few studies per single health endpoint, hindered a formal meta-analysis.

3.3. Exposure assessment

UFPs were most commonly assessed as PNC, but a few studies also measured particle mass concentration. Most of the included studies investigated particles <100 nm, while some considered accumulation mode (aerodynamically stable, typically 100–1000 nm) PNC. Only seven out of 20 included studies estimated source-specific UFP exposures, categorizing them into traffic/non-traffic, and one study focused specifically on aircraft emissions. Most of the remaining studies claimed local vehicle exhaust emissions as a main contributor to UFPs in a study area, although without having done source apportionment. Other air pollutants were assessed in all studies, but only 11 studies adjusted for co-pollutants in the statistical analysis. Three studies additionally considered transportation noise as a potential confounder.

3.4. Studies with source apportionment (ambient ultrafine particles)

Two cohort studies investigated associations of traffic-related UFPs with diabetes. In the population-based German Heinz Nixdorf Recall cohort, long-term residential levels of traffic-specific accumulation mode particle number concentration (PN_{AM}; aerodynamic diameter between 0.1 and 1.0 μm; particles/mL) were estimated using a chemical transport model (Lucht et al., 2020). Traffic PN_{AM} exposure was associated with increased 10-year diabetes mellitus incidence, RR = 2.11 (1.04–4.28) per 500 particles/mL increase, after adjustment for noise exposure. In a study of the entire Danish population followed up over 2005–2017, long-term residential exposure to traffic contributions of

Table 6
Summary of epidemiological studies on mortality.

City, country & Study period	Study design	Sample size, study population	Exposure assessment, Size fractions and UFP source	Covariate adjustment	Outcome & Outcome assessment	Exposure time windows & Exposure levels, counts/cm3 Mean/median (min-max)	Effect sizes (95% CI) per increment	PM2.5 adjusted UFP effect	NO2 adjusted UFP effect	Reference
Eight European urban areas 1999–2013	Time-series		Fixed monitor PNC≤100	Long-term and seasonal trend, weekday, influenza epidemics, and population dynamics due to summer vacation and holidays	Mortality Administrative database	Short Median PNC across eight study areas ranging 4685–29,168	**Natural mortality** increase per 10,000 particles/cm3 PNC_lag6 by low temp = 0.08% (−0.44, 0.61); medium temp = −0.49% (−1.08, 0.11); high temp = 1.24% (−0.72, 3.24). **CVD mortality** = −0.18 (−0.97, 0.62); −0.81 (−1.92, 0.32); 2.51 (0.39, 4.67)	The effects of PNC on mortality across air temperature levels decreased after adjustment for PM2.5 (presented in figure)		Chen et al. (2018)
Ruhr Area, Germany 2009–2014	Time-series	Inhabitants of 3 cities within the Ruhr Area	Fixed monitor PNC≤100	Time trend, temperature, humidity, day of the week, holidays, period of seasonal population decrease, and influenza	Mortality Administrative database	Short 9870.6/9871 (NA)	No clear associations for natural mortality, e.g. increase per IQR = 4900.2 for lag 4–7 = 2.01% (95% CI: −1:41, 5.55). For respiratory mortality, associations at lag2 and lag6: 3.50% (95% CI: −0:77, 7.95) and 4.51% (95% CI: 0.37, 8.81), respectively.		Effect estimates for natural mortality became more negative overall considered lags	Hennig et al. (2018)
Barcelona, Huelva, Tenerife, Spain 2008–2014	Time-series	Administrative database	Dedicated monitoring campaigns PNC Total/vehicle exhaust/ nucleation	Long-term trends, temperature and population dynamics	Mortality Administrative database	Short Mean across 3 study areas ranging 4514.6–6593.1; median ranging 4020.3–5626.7	Total: no significant association; Barcelona: % increase per IQR 3277.4 = 1.63 (0.74–2.52). Similar for lag1 and lag2			Tobias et al. (2018)
Stockholm, Sweden 2000–2016	Time-series	Residents of Stockholm	Monitor PNC4 mainly road traffic exhaust emissions	Temperature	Mortality Administrative database	Short 9177/NA (NA)	No clear associations			Olstrup et al. (2019)
Shanghai, China 2009–2011	Time-series	Residents of the Pudong New Area	Fixed monitor PNC (0.25–10 μm)	Time trend, day of the week, holiday, temperature, and relative humidity	COPD mortality Administrative database	Short PNC0.25–0.28: 2360/1921 (2–8264); PNC2.5-10: 3/2 (0–98)	% increase PNC0.25–0.28 per IQR 2053 particles/cm3 = 7.51 (2.45–12.81)		8.82 (1.50–16.66)	Peng et al. (2020)
Barcelona, Helsinki, London, and Zurich 2009–2016	Time-series		Fixed monitor PNC Total/Traffic (Fresh traffic, Urban with size mode around 30 and 70 nm)/ photonucleation/ secondary	Temperature, weekday, bank holiday	Mortality Administrative database	Short Fresh traffic PNC: Mean ranging 3264–6530; Median ranging 2600–5868	Fresh traffic PNC and natural mortality in London (lag 0 = −1.18% [-1.95, −0.41]), CVD mortality in Zurich (lag 0 = 3.54% [0.06, 7.05]), and respiratory mortality in Zurich (lag 1; 8.42 [0.43, 16.53]).The urban source (mostly aged traffic) was associated with natural mortality in Zurich (lag2 = −2.93 [-5.34, −0.48]) respiratory mortality in Zurich (lag 0 = 10.55 [0.43,			Rivas et al. (2021)

(*continued on next page*)

Table 6 (*continued*)

City, country & Study period	Study design	Sample size, study population	Exposure assessment, Size fractions and UFP source	Covariate adjustment	Outcome & Outcome assessment	Exposure time windows & Exposure levels, counts/cm3 Mean/median (min-max)	Effect sizes (95% CI) per increment	PM2.5 adjusted UFP effect	NO2 adjusted UFP effect	Reference
Seoul, Korea 2013–2016	Time-series		Fixed monitor PNC size range 10.6–478.3 nm	Temperature, relative humidity, air pressure, time trends, day of the week and holidays	Mortality Administrative database	Short PNC10.6–30: 2815/2554 (0.364–21,131); PNC100–200: 954.8/851.7 (6.756–3841)	21.51]) and London (lag 3; 2.4% [0.90%, 4.0%]) PNC100-200: excess risk for CVD mortality indicated the highest effect of 2.15% (95% CI: 0.12–4.23) at lag 1 per IQR 811.5 particles/cm3. No clear associations with other PNC fractions			Park et al., (2022)

Abbreviations.
COPD=Chronic obstructive pulmonary disease; CVD = cardiovascular disease.
IQR=Interquartile range; 95% CI = 95% Confidence interval.
UFP=Ultrafine particles; PNC = particle number concentration.
Exposure time windows: short = up to 1 month prior to the outcome assessment; long = longer than 1 month prior to the outcome assessment.
NA=Not available.

UFPs was estimated using the Danish DEHM/UBM/AirGIS modelling system followed by calculation of time-weighted 1-, 5- and 10-year running means (Sorensen et al., 2022). The authors reported 5-year time-weighted mean exposure to UFPs to be associated with a higher risk of type 2 diabetes (HR per 1698 particles/cm^3 increase = 1.049 (1.040–1.058). Noteworthy, UFP pollution originating from traffic was associated with higher risks than non-traffic contributions. Another study based on the German Heinz Nixdorf Recall cohort investigated long-term exposure to traffic PN_{AM} and the incidence of cardiovascular events (Rodins et al., 2020). Statistically significant associations were observed with stroke, HR = 1.27 (1.05–1.55) per 100 n/cm^3 increase in PN_{AM} exposure estimated at baseline, while no consistent associations were seen with coronary heart disease or total cardiovascular events.

Two time-series studies investigated day-to-day variations in traffic UFP exposure on daily mortality. In one study, the impact of UFP emitted by vehicle exhaust on daily mortality in three Spanish cities was investigated (Tobias et al., 2018). An association was observed in Barcelona, by increasing approximately 1.5% between lags 0 and 2, per interquartile increase of 3277 particles/cm^3. A similar pattern was found in Santa Cruz de Tenerife, although none of the associations were significant. More recently, another study assessed the impact of UFP sources on daily mortality in four European cities (Rivas et al., 2021). Using the PMF method (Hopke, 2016), the authors determined two traffic-related sources, i.e., fresh traffic (particles that nucleate, <20 nm) and urban (aged traffic emissions, 40–150 nm). Fresh traffic PNC was associated with natural mortality in London: lag0 = −1.18% (−1.95, −0.41), cardiovascular (CVD) mortality in Zurich: lag0 = 3.54% (0.06, 7.05), and respiratory mortality in Zurich: lag1 = 8.42% (0.43, 16.53) per city-specific IQR that ranged between 1818 and 3582 particles/cm^3. The urban source PNC was associated with natural mortality in Zurich: lag2 = −2.93% (−5.34, −0.48), as well as respiratory mortality in Zurich: lag0 = 10.55% (0.43, 21.51) and London: lag 3 = 2.4% (0.90%, 4.0%) per city-specific IQR that ranged between 1429 and 1744 particles/cm^3.

3.4.1. *Gasoline and diesel ultrafine particles*

In a study among pregnant women in Beijing, China, maternal exposure to fine and UFP in size fractions of 5–560 nm (PNC5–560) was analyzed for the risk of preterm birth (PTB) (Fang et al., 2022). Further, sources of (PNC5–560) were apportioned using positive matrix factorization models. In this study, higher levels of number concentrations of size-fractioned particles in size ranges of 5–560 nm (PNC5–560) from gasoline and diesel vehicle emissions during the third trimester were significantly associated with the increased risks of PTB, with ORs of 1.14 (95% CI: 1.01–1.29) and 1.11 (95% CI: 1.04–1.18), respectively, per 1000 particles/cm^3 increase in exposure.

3.4.2. *Aviation ultrafine particles*

One US study evaluated whether UFP from jet aircraft emissions were associated with increased rates of PTB among pregnant women living within 15 km downwind of Los Angeles International Airport (Wing et al., 2020). *In utero* exposure to aircraft-originated UFP was significantly associated with 4% higher risk of PTB per interquartile range increase in UFP exposure. When comparing the fourth quartile of UFP exposure to the first quartile, the OR for PTB was 1.14 (95% CI: 1.08–1.20). Further, exposure to aircraft-origin UFP was independently associated with PTB after accounting for co-exposure to NO_2 and aircraft noise.

None of the present epidemiological review studies investigated exposure to shipping exhaust, rail, road, or brake wear nanoparticles.

3.5. *Studies without source apportionment*

In this section we first discuss studies with morbidity and then mortality outcomes.

Table 7
Summary of epidemiological studies on morbidity.

City, country & Study period	Study design	Sample size, study population	Exposure assessment, Size fractions and UFP source	Covariate adjustment	Outcome & Outcome assessment	Exposure time windows & Exposure levels, counts/cm3 Mean/median (min-max)	Effect sizes (95% CI) per increment	PM2.5 adjusted UFP effect	NO2 adjusted UFP effect	Reference
Toronto, Canada 1996–2012	Ontario Population Health and Environment Cohort (ONPHEC)	1,105,258 (age 30–100 yrs)	LUR PM0.1 mainly traffic emissions	Comorbidities, area-level SES, BMI, smoking	COPD, asthma, lung cancer Administrative database	Long (3-yr moving average) 28,473/26,000 (6772–109,759)	**COPD:** HR per 10,097 particles/cm3 = 1.06 (1.04–1.08); **Asthma:** HR = 1.00 (1.00–1.01); **LC** = 1.00 (0.97–1.04)	**COPD:** HR per 10,097 particles/cm3 = 1.07 (1.05, 1.09); **Asthma:** HR = 1.01 (1.00, 1.02); **LC** = 1.00 (0.97, 1.04)	**COPD:** HR per 10,097 particles/cm3 = 1.01 (0.98, 1.03); **Asthma:** HR = 1.00 (0.99, 1.01); **LC** = 0.98 (0.94, 1.01)	Weichenthal et al. (2017)
Toronto, Canada 1996–2012	Ontario Population Health and Environment Cohort (ONPHEC)	1,1 mln; (age 30–100 yr)	LUR PM0.1 mainly traffic emissions	Age, sex, neighborhood-level SES, comorbidities, smoking, obesity, co-pollutants	Diabetes Administrative database	Long (3-year moving average) 28,383.1/25,877.9 (6697.0–10,531.0)	HR per IQR 9948.4 particles/cm3 = 1.06 (1.05, 1.08)	HR per IQR 9948.4 particles/cm3 = 1.06 (1.05, 1.08)	HR per IQR 9948.4 particles/cm3 = 1.04 (1.02, 1.05)	Bai et al. (2018)
Brisbane Metropolitan Area, Australia 2010–2012	Cross-sectional	655 children (8–11 yrs)	LUR (home); measured (schools) PNC≤100 mainly road traffic emissions	Air conditioning at home, carpet, a garage, gas cooking, and gas heating; home flooded or had visible mold within the last 12m; the child's blood cotinine levels	Wheeze in the past 12m, Cough in the past 12m; Current asthma Questionnaire	Long 14,330.8/12,530.0 (NA)	**Wheeze**: OR per 1000 particles/cm3 = 1.06 (0.91–1.25); **Cough** = 1.00 (0.86–1.18); **Asthma** = 0.96 (0.84–1.11)	**Wheeze**: OR per 1000 particles/cm3 = 1.05 (0.87–1.25); **Cough** = 0.99 (0.84–1.18); **Asthma** = 0.97 (0.82–1.16)		Clifford et al. (2018)
The Netherlands 1993–2010	Cohort EPIC-NL	33,831 (mean age at baseline 50 yr)	LUR PM0.1	Smoking, diet, alcohol consumption, BMI, recruitment year, gender, marital status, education, area-level SES	CHD, AMI, HF Administrative database	Long (1993–1997) 11,110/NA (7190–29,470)	**CHF:** HR per 10,0000 particles/cm3 = 1.12 (0.94, 1.33); **MI:** HR = 1.34 (1.00, 1.79); HF = 1.76 (1.17, 2.66)	**CHF:** HR per 10,0000 particles/cm3 = 1.27 (1.04, 1.57); **MI:** HR = 1.59 (1.13, 2.24); HF = 3.10 (1.89, 5.10)	**CHF:** HR per 10,0000 particles/cm3 = 1.09 (0.82, 1.47); **MI:** HR = 1.22 (0.74, 2.02); HF = 1.75 (0.89, 3.45)	Downward et al. (2018)
Toronto, Canada 1996–2012	Ontario Population Health and Environment Cohort (ONPHEC)	1,1 mln; (age 30–100 yr)	LUR PM0.1	Age, sex, neighborhood-level SES, comorbidities, smoking, obesity, co-pollutants	AMI, CHF Administrative database	Long (3-year moving average) 28,441.7/25,943.3 (6697.0–110,531.0)	**CHF:** HR per 10,004.8 particles/cm3 = 1.03 (1.02–1.05); **AMI:** HR per 10,029.4 particles/cm3 = 1.05 (1.02–1.07)	CHF: HR per 10,004.8 particles/cm3 = 1.03 (1.02–1.05); AMI: HR per 10,029.4 particles/cm3 = 1.04 (1.02–1.06)	CHF: HR per 10,004.8 particles/cm3 = 1.02 (1.00–1.03); AMI: HR per 10,029.4 particles/cm3 = 1.05 (1.03–1.07)	Bai et al. (2019)
Toronto, Canada 2006–2015	Cohort	160,641 singleton live births in 2006–2012	LUR PM0.1	Distributed lag weekly exposures during pregnancy, distributed lag monthly exposures after birth, maternal age at delivery, infant sex, parity, breastfeeding, maternal smoking during pregnancy, maternal atopy, gestational age, birth	Asthma up to age 6 yrs Administrative database	Long (trimester-specific and during first 6 yrs of life) 28,910/29,000 (NA)	HR per IQR of 10,862 particles/cm3 during 1st trimester = 1.01 (0.97–1.05); 2nd trimester 1.09 (1.06–1.12); 3rd trimester 1.04 (1.00–1.08); entire pregnancy 1.03 (0.99–1.07); during first 6 yrs of life 1.03 (1.00–1.06)	HR per IQR during 1st trimester = 0.99 (0.96–1.02); 2nd trimester 1.07 (1.04–1.10); 3rd trimester 1.02 (0.99–1.06); entire pregnancy 1.01 (0.99–1.04); during first 6 yrs of life 1.01 (0.97–1.04)	HR per IQR during 1st trimester = 1.00 (0.97–1.03); 2nd trimester 1.07 (1.04–1.10); 3rd trimester 1.00 (0.96–1.04); entire pregnancy 1.00 (0.97–1.03); during first 6 yrs of life 0.99 (0.96–1.02)	Lavigne et al. (2019)

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Table 7 (*continued*)

City, country & Study period	Study design	Sample size, study population	Exposure assessment, Size fractions and UFP source	Covariate adjustment	Outcome & Outcome assessment	Exposure time windows & Exposure levels, counts/cm3 Mean/median (min-max)	Effect sizes (95% CI) per increment	PM2.5 adjusted UFP effect	NO2 adjusted UFP effect	Reference
				weight, residential greenness exposure during pregnancy, area-level SES, and random effects for clustering within families						
Augsburg, Germany 2005–2015	Case-crossover	5898; (age 25–74 yrs)	Fixed monitor PNC10–30; PNC10–100; PNC30–100; surface area (PSC) mainly traffic emissions	Temperature and relative humidity	Nonfatal MI Administrative database	Long (2005–2015) PNC10–30: 3825/2828 (0–934,944); PNC10–100: 8387/6333 (0–1,009,8); PNC30–100: 4561/3320 (0–291,5)	**MI** increase per IQR increase in PNC30–100, PNC10–100, and PSC at lag 6h 3.02% (0.36, 5.75), 3.27% (0.27, 6.37), and 5.84% (1.04, 10.87), respectively. For all size fractions of PNC, no increased risks of MI were found for daily average exposures (24, 48, and 72 h).	PNC10–100 = 2.67% (−0.47, 5.90), PLC = 5.61 (0.72, 10.75)	PNC10–100 = 3.31% (−0.11, 6.84); PSC = 6.49 (−0.01, 13.41)	Chen et al. (2020)
Ruhr Area, Germany 2000–2015	Heinz Nixdorf Recall cohort	4814 (ages 45–75 years)	Dispersion model (EURAD) PNC(100–1000 nm) Total/traffic/industry	BMI, education, alcohol consumption, age, chronic noise exposure, nutrition index, physical activity, sex, smoking, ETS exposure, cumulative smoking, and statin use	Diabetes self-reported DD/glucose-lowering medication	Long (10 yrs) NA/336 p/mL (NA)	RR per 500 particles/mL increase = 2.11 (1.04–4.28)			Lucht et al. (2020)
Ruhr Area, Germany 2000–2015	Population-based Heinz Nixdorf Recall cohort	4105 (aged 45–76 at baseline)	Dispersion model (EURAD) accumulation mode PNC Total/traffic/Industry	Age, sex, and individual and neighborhood SES, BMI, smoking, alcohol consumption, physical activity, nutritional pattern, and nighttime traffic noise exposure	Stroke, CHD, and total cardiovascular events self-reports, physician and next-of-kin interviews, and medical records	Long (baseline (2001–2003); follow-up (2006–2008) Traffic PN_{AM}: NA/402 (167–933)	Traffic: HR per 100 n/cm3 increase = 1.27 (1.05–1.55)			Rodins et al. (2020)
Los Angeles, USA 2008–2016	Cohort	Mothers living within 15 km of LAX (174,186)	Dispersion model PM0.1 aircraft emissions	Maternal age, maternal education, SES, maternal race, smoking, NO2, and airport noise	Preterm birth (<37w) Administrative database	Long (trimester-specific) 12,000/NA (2500–120,000)	OR per 9200 particles/cm3 = 1.04 (1.02–1.06). Comparing the fourth quartile of UFP exposure to the first quartile, OR = 1.14 (1.08–1.20)			Wing et al. (2020)
Boston, USA 2002–2009; 2011–2013	ACCESS and PRISM	376 mother–child dyads	Spatial–temporal model PNC	Maternal age, education, race, and obesity, child sex,	Asthma Questionnaire	Long (trimester-specific/throughout	OR = 4.28 (1.41–15.7) per doubling of the			Wright et al. (2021)

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Table 7 (*continued*)

City, country & Study period	Study design	Sample size, study population	Exposure assessment, Size fractions and UFP source	Covariate adjustment	Outcome & Outcome assessment	Exposure time windows & Exposure levels, counts/cm3 Mean/median (min-max)	Effect sizes (95% CI) per increment	PM2.5 adjusted UFP effect	NO2 adjusted UFP effect	Reference
	pregnancy cohorts		mainly road traffic exhaust emissions	NO2, temperature, and postnatal UFP exposure		pregnancy) NA/27,842 (NA)	estimated UFP across pregnancy			
Oakland and San Jose, California, USA 2013–2015	Cross-sectional	8823 singleton births	Google vehicles PM0.1 mainly traffic	SES, conception season, city of residence, maternal age, nulliparity; and other pollutants	Preterm birth (<37w) Administrative database	Long NA/25,500 (NA)	RD in Black mothers per 16.4 n x10^3/cm3 = 5.4% (−10.5 to −0.3), in White mothers RD = 3.7 (−8.6 to 1.1), in Latina mothers RD = 1.5 (−1.2 to 4.2)			Riddell et al. (2022)
Beijing, China 2014–2017	Cohort	24,001 singleton live births	Fixed monitor PNC5–560 Nucleation/ gasoline vehicle emissions/diesel vehicle emissions/ secondary aerosols	Temperature, relative humidity, maternal age, ethnicity, gravidity, parity, gestational weight gain, fetal gender, the year, and season of conception	Preterm birth (<37w) Administrative database	Long (trimester-specific) 20,617/NA (NA)	OR = 1.14 (1.01–1.29) and 1.11 (1.04–1.18) per 1000 particles/cm3 increase in emissions from gasoline and diesel vehicles during the third trimester, respectively.			Fang et al. (2022)
Denmark 2005–2017	Cohort	2.6 mln aged >35 yrs	DEHM/UBM/ AirGIS modelling system PM0.1 Total/Non-traffic/ Traffic	Age, sex, calendar year, civil status, individual and family income, country of origin, occupational status, education, and area-level SES	Diabetes Administrative database	Long (5-yr time-weighted running mean) Traffic: Median ranging from 1378 in 2005 to 776 in 2015	Total: HR per IQR 4248 particles/cm3 = 1.052 (1.042–1.063); Non-traffic: HR per 2769 particles/ cm31.027 (1.019–1.036); Traffic: HR per 1698 particles/cm3 = 1.049 (1.040–1.058)			Sorensen et al. (2022)

Abbreviations.
MI = myocardial infarction; CHF = congestive heart failure; HF = heart failure; CHD = coronary heart disease; COPD=Chronic obstructive pulmonary disease; LC = lung cancer.
HR = hazard ratio; OR = odds ratio; RD = risk difference; IQR=Interquartile range; 95% CI = 95% Confidence interval.
UFP=Ultrafine particles; PNC = particle number concentration; PN_{AM} = accumulation mode particle number concentration.
LUR = land-use regression; SES=Socioeconomic status.
Exposure time windows: short = up to 1 month prior to the outcome assessment; long = longer than 1 month prior to the outcome assessment.
NA=Not available.

3.5.1. Morbidity

Riddell and coworkers linked birth-registry data with UFPs number concentration measured using a condensation particle counter for singleton births in Oakland and San Jose, California (Riddell et al., 2022). After stratification by race/ethnicity, they found negative associations between UFP and preterm birth for Blacks and Whites, null associations for Asians, and some evidence of positive associations among Latinas. Given that UFP exposure levels were spatially clustered in this study, the authors speculated that estimated negative associations might at least partly be attributed to confounding if women living in areas with higher UFP are at otherwise lower risk of preterm birth due to other factors, e.g., socio-economic status. Two further studies from North America investigated the association between prenatal exposure to ambient UFPs number concentration determined using spatiotemporal modelling, and childhood asthma. In the cohort from Toronto, Canada, IQR increase in exposure during the second trimester of pregnancy was associated with an increased risk of developing asthma in children before age 6 by 9%, independent of other air pollutants (Lavigne et al., 2019). Similarly, a more recent U.S. study linked prenatal UFP exposure with asthma development in children during the first 6 years of life, independent of NO_2 exposure and temperature, with OR = 4.28 (1.41–15.7) per doubling of the UFP concentration across pregnancy (median = 2784 particles/cm^3; IQR = 2403–3230) (Wright et al., 2021). Conversely, a study among 8 to 11-year-old school children in the Brisbane Metropolitan Area, Australia, found no association between exposure to PNC, measured at schools and modelled at homes using land-use regression, and respiratory symptoms or asthma diagnosis (Clifford et al., 2018). For adults, a cohort study of 1.1 million Toronto, Canada, residents found a positive correlation between the residential exposure to UFP and the incidence of chronic obstructive pulmonary disease: HR = 1.06 (1.05–1.09) per 10,097 particles/cm^3, using a single pollutant model. This association was attenuated after adjustment for exposure to NO_2. In the same study, no clear association was observed with lung cancer or adult-onset asthma (Weichenthal et al., 2017).

In the German registry-based time-stratified case-crossover study, elevated concentrations of PNC10-100 (particle number concentration of particle sizes 10–100 nm) were linked to a 3.27% (0.27–6.37) increase of risk of myocardial infarction (MI) 6 h after high exposure episodes (estimated IQR increase of 6528 particles/cm^3) (Chen et al., 2020). In the same study, but using two-pollutant models, exposure to PNC10–100 remained positively associated with the hourly onset of MI, although effect estimates slightly decreased and became less precise (Chen et al., 2020). These results were in line with the previous Dutch prospective cohort EPIC-NL study, showing significant associations between long-term UFP exposure and increased incidence risks for all CVD (18%), myocardial infarction (34%) and heart failure (76%) per an estimated IQR increase of 10,000 particles/cm^3 (Downward et al., 2018). The observed association remained in the two-pollutant models.

Bai et al. investigated the effects of UFP exposure estimated through land use regression (LUR) on the incidence of diabetes, as well as major CVD events among all long-term residents of Toronto, Canada aged 30–100 years. Each IQR increase of 10,000 particles/cm^3 in exposure to residential UFP was associated with an increased risk of diabetes: HR = 1.06 (1.05–1.08), congestive heart failure (CHF): 1.03 (1.02–1.05), and acute MI: 1.05 (1.02–1.07), which remained elevated after adjusting for PM2.5 and NO_2, as well as traffic-related noise (Bai et al., 2018, 2019).

3.5.2. Mortality

Studies on mortality show mixed results. In a time-series analysis of associations between PNCs of particles between 250 and 10000 nm in diameter and COPD mortality in Shanghai, China, the researchers found that daily COPD deaths were significantly associated with PNCs of particles <500 nm. The magnitude of associations increased with decreasing particle size, from 4.15% mortality increment for a PNC450-500 IQR increase of 126 particles/cm^3 to a 7.51% mortality increment for a PNC250-280 IQR increase of 2053 particles/cm^3 (Peng et al., 2020). The observed associations remained robust after adjustment for other pollutants. A multi-city analysis of the correlations between total mortality, air pollution, and air temperature in eight European urban areas showed that an increase of 10,000 particles/cm^3 in PNC corresponded to a 2.51% (0.39–4.67) increase in cardiovascular mortality on days with area-specific high air temperatures (>75th percentile), which was significantly higher than the values corresponding to days with area-specific low air temperatures (<25th percentile) (Chen et al., 2018). No clear association between PNC100 and natural or cardiovascular mortality was found in a time-series investigation based on three German cities, while a significant 4.51% increase in respiratory mortality was observed with IQR increase in PNC100 of 4900 particles/cm^3 at lag6 (Hennig et al., 2018). Conversely, no link between excess of daily mortality and UFP exposure (estimated as PNC for particles >4 nm) was found in a study based on data from an urban background measuring station in Stockholm (Olstrup et al., 2019). Further, no statistically significant excess risk for daily mortality in relation to PNC in size-fractions ranging from 10.6 to 478.3 nm was observed in a time-series study in Seoul, Korea, with exception of 2.15% (95% CI: 0.12–4.23) excess risk for cardiovascular mortality per IQR 811.5 particles/cm^3 increase in PNC100-200 at lag 1 (Park et al., 2022).

3.6. Conclusions related to epidemiological studies

Epidemiological studies included in the present review add further evidence to the adverse morbidity effects of UFP exposure, including preterm birth, childhood asthma, diabetes and myocardial infarction, whereas studies on mortality show mixed results. However, there is still limited knowledge regarding the health impacts of source-specific UFP related to different transport modes, as very few existing studies focused on source apportionment. Therefore, there is a need for more studies that could estimate UFP contributions from various transportation-related sources, as well as incorporate multi-pollutant models to elucidate the independence of effects of UFPs.

4. Discussion

In this study, we have reviewed studies on toxicity and health effects of NPs/UFP from different sources with a focus on vehicle exhaust and non-exhaust, as well as particles from shipping (harbor), aviation (airport), and rail (mainly subway). The search strategy was challenging, as it was difficult to know which search terms to use to collect all relevant studies without getting an overwhelming number of not relevant studies. There is, for example, substantial literature available considering the toxic effects of “manufactured nanoparticles” as well as studies related to “diesel exhaust”. It is also difficult to define the size cut-off for “nanoparticles”, with the additional difficulty that reported size distributions usually include agglomerated NPs. Moreover, comparing the potency of NPs from various sources is easier if they are included in the same study or if at least harmonized protocols are used (same cell types, similar doses, and time-points, possibly also benchmark particles to compare with). For example, since several studies have compared diesel and biodiesel, this comparison was more straightforward, and the main conclusion is that biodiesel NPs cannot be regarded as low-toxicity particles and their effects are rather similar to those observed with diesel NPs. It should be noted that this is only based on relative toxic potency and that the final health outcome will indeed depend on exposure and thus how many NPs are generated. Clearly, the rat study performed on exhaust from “new-technology diesel” showed that this was less toxic and carcinogenic, compared to traditional technology diesel exhaust, presumably due to the lower content of NPs of the new-technology diesel exhaust (McDonald et al., 2015). One other factor to consider for diesel PM is that the composition and effect will depend on the conditions during the collection. For example, the NIST diesel material called SRM2975 was collected under very hot conditions and thus contained far less VOC and more oxidized species compared to

other diesel PM, which also impacts the mutagenicity (DeMarini et al., 2004). Considering aviation-related NPs, one comparative *in vivo* study (Bendtsen et al., 2019) concluded that they had similar toxic effects as traffic-related particles, DEP and CB. Furthermore, several studies highlight the toxic effects of particles from the subway, but few studies focused on NPs. In a comparative *in vitro* study (Steenhof et al., 2011) underground (subway) UFPs had the highest oxidative potential and were most cytotoxic whereas pro-inflammatory markers were more pronounced following harbor and truck traffic-related UFPs. Several *in vitro* studies also highlighted the role of copper in the toxicity of brake wear particles. However, on the other hand if such brakes generate fewer NPs, this must be taken into account when assessing health risks. Disc brake pad material composition differs for different market segments. The European market demands higher performance, i.e., higher coefficient of friction, whereas the US and Asian market prefers low noise emission. This affects the chemical composition of the brake pad materials that also have been influenced by the ban of asbestos and lately copper. In a study from 2016 by Alemani et al. the copper content for typical European pad materials varied between 20 and 2% (Alemani et al., 2016). The replacement materials for the copper contents differ with so far very limited studies of the health effects of the replacement materials.

The review of epidemiological studies adds evidence to the current knowledge on the adverse health effects of UFPs but emphasizes the scarcity of studies regarding the health impacts of source-specific UFPs originating from different transport modes. Different sources can generate UFPs with different chemical compositions that may lead to different health effects. Current epidemiological studies focusing on differences in the chemical composition of PM are largely based on studies investigating larger PM fractions (Samet, 2022). For instance, mortality effect estimates for 25 U.S. communities were higher in the communities where PM2.5 content was enriched for aluminium, arsenic, sulfate, silicon, and nickel (Franklin et al., 2007). In another US study, a higher risk of hospitalizations related to short-term exposure to PM2.5 was found in areas with a higher PM2.5 content of nickel, vanadium, and elemental carbon and/or their related sources (Bell et al., 2009). However, WHO does not recommend using different exposure-response functions for PM2.5 particles from different sources (WHO, 2006; WHO, 2013). On the other hand, there is a growing number of studies indicating significantly different exposure-response functions for different sources of particles, a circumstance that will have very important implications for health impact assessments (Segersson et al., 2021). Using the same exposure-response functions irrespective of particle source, local actions in cities will be estimated to have none or very small benefits for public health, while adopting the higher exposure-response functions for local fresh, particle sources, local abatement strategies will have large health benefits (Segersson et al., 2021). The indications that exposure to local, fresh particles is more hazardous than secondary, long-range transported particles is also consistent with the higher exposure-response functions seen for black carbon compared to PM2.5 (Grahame et al., 2014; Janssen et al., 2011). UFPs is a good indicator of primary, freshly emitted particles, and thus using exposure-response functions for UFPs could be a good way to demonstrate relative benefits in health impact assessments of actions reducing emissions from transports. This assessment should rely in any case on epidemiological and toxicological evidence of the health effects of emissions from different transport modes. As shown in this review, current evidence does not consistently support the use of different exposure-response functions for different UFP sources – neither for mortality nor morbidity outcomes, and studies on source-specific UFP are very limited.

An alternative approach to using particle source-specific exposure-response functions for UFP exposure could be the use of relative potential toxicities of UFPs or their constituents based on toxicological evidence (Park et al., 2018). This is in a similar way used in assessments of impacts on cancer associated with exposure to different mixtures of PAHs using different "Toxic Equivalency Factors". One relevant question for studies on extracts is to what extent the extracted organics will be bioavailable in a physiological environment and the role of the particle itself in the observed toxic outcomes. An interesting study on dogs showed, for example, that up to 90% of the PAH (benzo[a]pyrene) adsorbed to carbon particles translocated unmetabolized into circulation within a few minutes.

One difficulty when comparing the toxic potency of NPs from different sources is that, as discussed in this review, they could induce different types of effects depending on their composition. For example, DEP may be more genotoxic than biodiesel particles whereas the inflammatory effects may be more similar or even higher for the biodiesel exhaust. Another example is that the subway particles show higher ROS generation and cytotoxicity, but not higher inflammatory potential when compared to traffic-related UFPs (Steenhof et al., 2011). This makes it hard to firmly conclude which sources generate the overall "most toxic" NPs. Another difficulty is to compare studies using the NPs/UFPs as such (as was reviewed here) to those on extracts. The interesting study by Park et al. (2018) used extracts (using a mixture of dichloromethane and methanol, 1 h sonication) and included experiments using several cell types and analyzed cell viability, genotoxicity, oxidative stress, and inflammatory effects. The general conclusion was that the chemical components from combustion aerosols showed higher toxicity than those from non-combustion aerosols. Furthermore, extracts from the diesel exhaust particles (DEP) had the highest mutagenic potency, with large differences between the heavy-duty diesel engine (higher mutagenicity) and the light-duty diesel engine (lower mutagenicity). In contrast, the two different diesel extracts displayed similar inflammatory potential (Park et al., 2018). The composition of the extracts will also differ depending on the size of PM collected. For example, the composition and toxicity of organic extracts (dichloromethane) from fine (PM0.3–2.5) and quasi-UFPs (in this case particles with aerodynamic diameter below 0.3 μm, PM0.3) was compared (Badran et al., 2020). The results showed that PAHs were 43 times more predominated in the organic fraction of the quasi-UFPs compared to the larger particles. In line with this, the organic fraction of the quasi-UFPs also caused more pronounced changes in proteins related to genotoxicity following exposure of BEAS-2B cells. A comparison was also made between the extract and the complete PM0.3–2.5 (the PM0.3 was not included). Interestingly, the particle fraction caused more changes in genes related to the metabolic activation of PAHs, compared to the extract. Neither of them caused much changes in protein expression in terms of genotoxicity (Badran et al., 2020). In another study, focusing on genotoxicity and cancer risk of PM extracts (toluene), cultured cells (HepG2) were exposed to extracts from PM10 collected in Stockholm and levels of two proteins involved in DNA damage signaling (pChk1 and γH2AX) were analyzed using Western blotting (Dreij et al., 2017). The response of the extracts was compared to that of several PAHs and the use of mixture potency factors was explored. The results suggested that airborne PAHs have a significantly higher carcinogenic potency than estimates based on B[a]P alone thus emphasizing the role of the mixture.

For the studies using NPs collected on filters and not extracts, some methodological aspects need to be considered. One aspect is the method used for removing the NPs from the filter matrix before exposing cells and the fact that certain filters, such as glass fiber filters, could release fibers that may cause an inflammatory response (Karlsson et al., 2006). One alternative and novel approach is to use air-liquid interface (ALI) strategies, in which particles are directly deposited onto cells, therefore eliminating the need for filters (Upadhyay and Palmberg, 2018). This is, however, more technically demanding and it requires more equipment compared to submerged exposure. In both approaches it is important to understand the actual mass of particles deposited on the cells (the cell dose). Finally, there is often a question of whether the observed inflammatory effects could be explained by endotoxins (a component of the outer membrane of gram-negative bacteria) possibly present in the particle samples.

One challenge in the epidemiological studies is that when comparing incremental risks associated with NPs it is important to consider the different instruments/techniques/size ranges being used to quantify particle number concentrations. Ideally, to be comparable risk increases should be expressed per e.g. 10,000 particles/cm^3 using the same size range. The total number of particles will be very different depending on the lower cut-off diameter of the instrument (it can range from 4 nm to 20 nm, which might give several times lower concentrations depending on the size distribution of the sources). Incremental risks for particle numbers larger than 100 nm cannot be compared to risks based on exposure to particle numbers smaller than 100 nm. The fact that the exposure data related to UFPs is "sparse and diverse" leading to larger measurement error, in combination with the fact that UFPs have a larger spatial and temporal variability compared to fine PM, was also discussed as problematic in a recent systematic review and meta-analysis with focus on respiratory hospital admissions and emergency room visits following short-term exposure to UFPs (Samoli et al., 2020).

Our review has emphasized the lack of studies on NPs/UFPs from different transport modes. This missing knowledge is required to evaluate the use of toxic potency scores in health risk assessments. Generation of the relevant toxicological data and the development of methodologies about how to use such data will be further explored in the project nPETS (nanoparticle emissions from the transport sector: health and policy impacts), an EU project within the framework of the "Smart, green and integrated transport" program (Horizon, 2020). The project deals with the biological processes leading to adverse effects of NPs on humans. It evaluates whether and what variation of these effects exists depending on the size of the particles, chemical content, and shape of the particles. It links the effects with specific emission sources to an understanding and quantification of the risks with different types of transport-related NP sources. Two parallel projects are the EU funded projects ULTHRAS and LEON-T. LEON-T focuses on tire to road emissions and ULTHRAS has, similar as nPETS, a wide transport source perspective. Specifically, it addresses the impact of different transport modes, fuel technologies and wear components, including atmospheric ageing processes, on the physicochemical characteristics of particulate and gaseous emissions. Yet another EU-funded project with a focus on UFPs is the TUBE project. This project aims to discover the harmful components of air pollution as well as to identify biomarkers for early detection of brain disease related to air pollution.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116186.

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