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REVIEW ARTICLE

Particulate matter beyond mass: recent health evidence on the role of fractions, chemical constituents and sources of emission

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Abstract

Particulate matter (PM) is regulated in various parts of the world based on specific size cut offs, often expressed as 10 or 2.5 μm mass median aerodynamic diameter. This pollutant is deemed one of the most dangerous to health and moreover, problems persist with high ambient concentrations. Continuing pressure to re-evaluate ambient air quality standards stems from research that not only has identified effects at low levels of PM but which also has revealed that reductions in certain components, sources and size fractions may best protect public health. Considerable amount of published information have emerged from toxicological research in recent years. Accumulating evidence has identified additional air quality metrics (e.g. black carbon, secondary organic and inorganic aerosols) that may be valuable in evaluating the health risks of, for example, primary combustion particles from traffic emissions, which are not fully taken into account with $PM_{2.5}$ mass. Most of the evidence accumulated so far is for an adverse effect on health of carbonaceous material from traffic. Traffic-generated dust, including road, brake and tire wear, also contribute to the adverse effects on health. Exposure durations from a few minutes up to a year have been linked with adverse effects. The new evidence collected supports the scientific conclusions of the World Health Organization Air Quality Guidelines and also provides scientific arguments for taking decisive actions to improve air quality and reduce the global burden of disease associated with air pollution.

Keywords

Air pollution – adverse effects, particulate matter, toxicology

History

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Introduction

Air pollution is a significant public health problem. A wide and moreover, growing range of health effects associated with ambient air pollution have been well documented by studies conducted in various parts of the world. The detrimental effect on health, elicited by particulate matter (PM) is especially well documented (Kelly & Fussell, 2012). This particular pollutant creates a substantial burden of disease, reducing life expectancy by almost 9 months on an average in Europe. Of concern, very large parts of the population live in cities with levels of PM exceeding World Health Organization (WHO) Air Quality Guidelines (WHO, 2006), and only a slightly decreasing trend in average concentrations has been observed in countries in the European Union (EU) over the past decade (Barmpadimos et al., 2012). Added to this, there is almost no evidence of a safe level of PM exposure or a threshold below which no adverse health effects occur.

Effective management of air quality, with the aim of achieving WHO Air Quality Guideline levels, is therefore imperative to reduce health risks to a minimum, especially since even at relatively low concentrations, the burden air pollution puts upon health is substantial. Exposure to air pollutants is, however, largely beyond the control of individuals, requiring instead, action by public authorities at regional, national and international levels. A multi-sectorial approach, engaging relevant bodies such as transport, housing, energy production and industry, is needed to develop and effectively implement long-term policies and measures to reduce air pollution and the associated risk to human health.

The EU Directive of 2008 on ambient air quality and cleaner air for Europe explicitly states that ''*emissions of harmful air pollutants should be avoided, prevented or reduced and appropriate objectives set for ambient air quality taking into account relevant World Health Organization standards, guidelines and programmes*'' (EU, 2008). Therefore, and in the framework of the European Commission (EC) declaring 2013 the EU Year of Air, scientific evidence on the health effects of air pollutants was collected and reviewed. These not only include evidence on the health aspects of the major air pollutants (PM, ground-level ozone and nitrogen dioxide), but also emissions to the air of individual metals and polycyclic aromatic hydrocarbons are considered. This activity has been done in the framework of the comprehensive review of EU's air quality policies scheduled for 2013, and falls within the project entitled

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''Evidence on health aspects of air pollution to review EU policies – REVIHAAP'', an initiative commissioned by the EC, coordinated by the WHO Regional Office for Europe and conducted by invited experts from top institutions across the world (WHO, 2013).

Scope

This article gives an overview of the evidence review from REVIHAAP, specifically on the latest *toxicological* findings of PM – one of the pollutants deemed most dangerous to health. Under the REVIHAAP project, this evidence was considered alongside epidemiological studies in an attempt to identify specific sizes, sources and constituents of PM associated with adverse health effects as well as to highlight key areas for future research. The review stems from the advice developed by the WHO REVIHAAP project (WHO, 2013), in the form of answers to 24 key policy-relevant questions asked by the EC. The reader is encouraged to consult the final technical report for the project, which provides a description of the evidence reviewed and answers to these 24 questions (WHO, 2013).

Sources of information and methodology adopted by REVIHAAP

A review of health effects elicited by ambient air pollution, such as the one conducted by the REVIHAAP project, is a challenging task, since it necessitates a remarkably large body of evidence to be assessed. Thousands of new scientific papers have been published on this topic in the past few years, covering a growing number of health effects and encompassing research disciplines such as population exposure, observational epidemiology, controlled human exposure, animal toxicology and *in vitro* mechanistic studies. With that in mind, the REVIHAAP review of the literature to support the key policy questions focused on studies that were published after the latest global update of the WHO Air Quality Guidelines (WHO, 2006). When appropriate, it also included earlier publications. A systematic approach was used to review and assess recent individual publications, with a focus on the most significant and relevant studies, and upon meta-analyses when available. In addition, the project made use of recent major reviews, particularly those prepared by relevant international or national organizations. The reviews selected for inclusion were limited to those with clearly stated methodology for literature searching and evidence selection. The evidence presented in this review article is based on the assessments of, and conclusions, formulated from *toxicological* research examined by REVIHAAP. The main sources of evidence are quoted, and the strength of this evidence is explained. Careful wording has been used throughout the document to properly present the strength of the evidence and to determine potential causality related to associations observed between PM and outcomes. This wording is indicative of the state of the evidence on a particular issue.

The REVIHAAP review was conducted by a group of 29 experts from top institutions around the world, representing various relevant scientific disciplines. A scientific advisory committee of eight experienced scientists was put together to guide and oversee the project. In addition, 32 external reviewers provided detailed comments on the completeness of the literature reviewed, the validity of the conclusions reached and the clarity of the answers. The REVIHAAP conclusions reflect the collective judgment of specialists in the field, and the final text of the answers was adopted by consensus of experts present at a WHO experts meeting held in Bonn on 15–17 January 2013.

The role of PM fractions and/or metrics

Evidence presented in the 2005 global update to the WHO air quality guidelines on the health effects of different chemical constituents in PM was based on toxicological studies (WHO, 2006). The conclusion was that specific characteristics could not yet be identified as critical for toxicity, although research findings supported several candidates, including ultrafine particles. The evidence did not lead to the recommendation of any specific indicator beyond either PM_{10} (PM less than 10 μm in aerodynamic diameter) or $PM_{2.5}$ (PM less than 2.5 μm in diameter), both size fractions of particles that enter the respiratory tract. Nevertheless, the concern for other particle sizes such as ultrafine particles ($PM < 0.1$ μm) and certain chemical-specific constituents of PM such as sulfates, transition metals and polycyclic aromatic hydrocarbons remains. The 2009 Integrated Science Assessment (ISA) for PM, published by the US Environmental Protection Agency (EPA) to support the review of national ambient air quality standards, used evidence from both epidemiological and experimental studies. The latter concluded ''there are many components contributing to the health effects of $PM_{2.5}$ but not sufficient evidence to differentiate those constituents (or sources) that are more closely related to specific health outcomes'' (EPA, 2009). Despite the increased number of studies (especially epidemiological) since 2009, the general conclusion remains the same. The following sections discuss the latest evidence to emerge from toxicological studies of PM fractions, which was considered under REVIHAAP. It should be kept in mind that this article does not discuss the epidemiological literature, which is a crucial part of information to draw conclusion on to what extent the recent evidence is ''sufficient'' to derive new components based PM air quality guidelines or standards.

Black, elemental and primary and secondary organic carbon

The health effects of black carbon particles as a component of $PM_{2.5}$ have also undergone a recent systematic review by the WHO Regional Office for Europe (WHO, 2012), concluding that there are not enough clinical or toxicological studies to (a) allow an evaluation of the qualitative differences between the health effects of exposure to black carbon or those of exposure to PM mass or (b) identify any distinctive mechanism of black carbon effects. The review of the results of all available toxicological studies suggested that black carbon (measured as elemental carbon) may not be a major directly toxic component of fine PM (particles smaller than 2.5 μm), but it may operate as a universal carrier of a wide variety of combustion-derived chemical constituents of varying toxicity to sensitive targets in the human body, such as the lungs, the body's major defense cells and, possibly, the systemic blood circulation.

Healthy human subjects exposed for 2 h to ultrafine (generally defined as particles smaller than 100 nm) clean carbon particles (e.g. generated from pure carbon not using a combustion process and without metal or endotoxin impurities) at concentrations of 10 and 25 μg/m^3 showed a high overall deposition fraction in the respiratory system (0.66 ± 0.12 at rest; mean ± SD), which increased with exercise ($0.83 \pm .04$) (Frampton, 2001). Asthmatic subjects showed an even higher deposition (0.76 ± 0.05) than did healthy subjects while breathing at rest (Frampton et al., 2004). The effects of ultrafine carbon particles were observed in both heart rate variability and cardiac repolarization, but there were no changes in soluble markers of either systemic inflammation or coagulation. In a more recent study, no vascular impairment or effect on blood clotting were observed in volunteers exposed for 2 h to 70 μg/m^3 of ultrafine carbon particles (Mills et al., 2011). In this same study, and in Lucking et al. (2011), it was shown that removing the particles from diluted diesel engine exhaust prevented adverse effects on the cardiovascular system – explained by differences in particle composition, with black carbon particles (soot) in diesel exhaust enriched with (semi)volatile organic particles and metals. There are no studies reported that used exposure periods longer than 2 h. In Toronto, there was a $PM_{2.5}$-related mean decrease in brachial artery diameter but no changes in blood pressure in one study exposing healthy volunteers for 2 h to concentrated ambient particles (CAPs); in a follow-up study, involving most of the same subjects, $PM_{2.5}$ exposure produced a significant decrease in diastolic blood pressure (Urch et al., 2004, 2005). In both studies, the effects were significantly associated with organic carbon. There were suggestive, but not significant, associations with elemental carbon and some metals (cadmium, potassium, zinc, calcium and nickel) in the first study (Urch et al., 2005).

Inhalation of ultrafine carbon particles (38 nm, 180 μg/m^3 for 24 h) caused increased heart rate and decreased heart-rate variability in rats, but there was no inflammatory response and no change in the expression of genes having thrombogenic relevance (Harder et al., 2005). In spontaneously hypertensive rats exposed to similar ultrafine carbon particles (172 μg/m^3 for 24 h), blood pressure and heart rate increased with a lag of 1–3 d. Inflammatory markers in lavage fluid, lung tissue and blood were unaffected. That mRNA expression of hemeoxygenase-1, endothelin-1, endothelin receptors, tissue factor and plasminogen activator inhibitor in the lung showed a significant induction (Upadhyay et al., 2008), suggests a cardiovascular (or even systemic) effect without adverse effects at the port of entry (i.e. the lung). Given differences in the deposited dose in the respiratory systems of rats and human beings, the concentration used in this study is high, but not unrealistic when extrapolated to human exposures. Although not a true toxicological study, Biswas et al. (2009) were able to demonstrate that a substantial portion of soot-induced reactive oxygen production (associated with oxidative stress and inflammation) could be attributed to the (semi)volatile organic fraction on the carbon particle core, suggesting that organic particles bound to PM can be responsible for a substantial part of the toxicity of the carbonaceous fraction of PM. Likewise, but not yet studied, other particles (such as sulfates) may also act as carriers.

Coarse particles

Coarse PM (between 10 and 2.5 μm in diameter) includes PM such as black smoke, soil, dust from roads and building sites, large salt particles from sea spray, mechanically generated particles as well as some secondary particles. This size fraction also includes pollen, mould spores and other plant parts. Although the larger particles contribute rather little to particle number, they contribute the major proportion of particle mass. In 2009, the EPA ISA concluded – based on data from epidemiological, controlled human exposure and toxicological studies – that there was ''suggestive evidence of a causal relationship between short-term exposure to coarse PM and cardiovascular and respiratory health effects and mortality'' (EPA (2009). Since 2009, epidemiological evidence of the short-term effects of coarse particles on cardiorespiratory health and mortality has increased significantly, but toxicological evidence remains relatively sparse.

Although not a direct comparison, Graff et al. (2009) arrived at the conclusion that, in their human-controlled exposure studies (2 h, 90 μg/m^3), exposure to coarse PM produces a measurable mild physiological response in healthy young volunteers that is similar in scope and magnitude to that of volunteers exposed to fine PM, suggesting that both size fractions are comparable in inducing cardiopulmonary changes in acute exposure settings. Since 2005, no other new evidence from controlled exposure studies has been published.

Very few studies have compared animal and *in vitro* (i.e. preclinical) toxicity of coarse and fine PM. Available research has used PM collected on filters, in either *in vitro* assays or intratracheal exposures to assess the relative hazard, often in relation to the sources of emission. Since the inhalability and, therefore, the deposition efficiency in the respiratory tract of coarse particles is substantially lower, the interpretation of the risk of coarse versus fine PM has to be considered in that context. Wegesser et al. (2009) compared these two size fractions, collected during wildfires in California, and concluded that the hazard expressed per unit mass is roughly the same – with some evidence that fine PM is more toxic in terms of inflammatory potential and cytotoxic responses. In a different study, these effects were attributed to the insoluble components of the mixture rather than to endotoxins (which are a more common constituent of the coarse PM fraction) (Wegesser & Last, 2008). Intratracheal exposures in rats and mice, as well as *in vitro* studies, suggest that similar effects can be observed for coarse and fine PM in the bioassays of lung cells (Gerlofs-Nijland et al., 2007; Gilmour et al., 2007; Halatek et al., 2011; Happo et al., 2010; Jalava et al., 2008) and that coarse PM can be even more hazardous than fine PM. Again, given that the deposition efficiency and pattern of coarse and fine PM differ largely, the health outcomes in a population can differ at equal mass exposures.

Ultrafine particles

Ultrafine particles stem primarily from combustion processes in urban settings (Peters et al., 2011). Emitted primary ultrafine particles are transformed rapidly due to coagulation, adsorption and secondary particle formation. Furthermore, new particle formation takes place in the atmosphere and may

give rise to a high number concentration of particles in the nucleation and Aitken modes (0–20 nm and 20–100 nm). This is of special relevance in areas (urban, industrial and rural) with high photochemistry. As a consequence, ultrafine particles have greater spatial and temporal variability than the fine particle mass concentrations. Typically, they are characterized by particle number concentration, which is the metric most measurement devices employ. Research on nano-sized material is applicable to assessing the potential toxicity of ultrafine particles and has shown that not only their size but also their composition, surface chemistry and surface charge are important (Bakand et al., 2012). Although ultrafine particles are defined by size and number, this fraction may contain components as metals and polycyclic aromatic hydrocarbons.

A few recently published controlled human exposure research studies support previous conclusions that suggested increasing evidence for ultrafine particles in eliciting health effects during and after 2-h exposure periods (Langrish et al., 2012; Mills et al., 2007, 2011). However, most studies were performed with a mixture of particles and gases, which do not allow statements to be made about specific contributions of the ultrafine fraction. In controlled exposure studies, the removal of very high particle numbers by filters prevented the otherwise occurring arterial stiffness and increases of blood clotting (Bräuner et al., 2008). Similar observations were made in healthy subjects and patients with coronary heart disease that were wearing a very simple, yet highly efficient face mask while walking in highly polluted areas in Beijing, China (Langrish et al., 2009). Observations in healthy young volunteers exposed to pure elemental carbon particles implied that heart function was not affected by these controlled exposures (Langrish et al., 2009). This was confirmed by a very similar exposure in a study (Mills et al., 2011) that looked at measurements of arterial stiffness and blood clotting in healthy subjects. The presence of a susceptible population has not been shown, and no studies could be identified that have applied exposure periods longer than 2 h.

Substantial advances have been made in understanding the action of ultrafine particles, which have the ability to translocate from the alveolar space into tissues and to spread systemically, reaching many organs, including the heart, liver, kidneys and brain (Kreyling et al., 2010). These particles exhibit systemically a multitude of biological responses owing probably to a very small size fraction and large surface area interacting with the biological system. The study of ultrafine particle toxicology has made substantial advances, as properties of particles smaller than 100 nm are intensively studied for engineered nanoparticles. Toxicological actions include impairment of phagocytosis and breakdown of defense mechanisms, crossing tissues and cell membranes, injury to cells, generation of reactive oxygen species, oxidative stress, inflammation, production of cytokines, depletion of glutathione, mitochondrial exhaustion and damage to protein and DNA, most of which also occurs with larger sized PM (Bakand et al., 2012). The effects of ultrafine particles may very well be observed in organs other than those that correspond to the port of entry – for example, the central nervous system (Kleinman et al., 2008; Kreyling et al., 2013). In light of different biodistributions on inhalation and the likelihood that ultrafine particles can escape natural defense mechanisms, such as phagocytosis, it is likely that this size fraction will also be linked to biological pathways and responses that differ from larger sized particles. Whether or not a particle translocates is dependent on several factors including size and solubility. Small particles (<10 nm) translocate with greater efficiency than large particles (<100 nm). Soluble particles can directly diffuse across the lung membrane. While small soluble particles have been shown to translocate into the circulatory system, carbon core ultrafine particles do not diffuse across the lung membranes to the same extent, although soluble coatings from them can. Many translocated particles are taken up by interstitial macrophages, and transported by the lymphatic system to the gastrointestinal (GI) tract. In some cases, these particles enter distant organs through either the lymphatic system or the GI tract. Furthermore, particle solubility plays a role in uptake from the nasal passages and transport along the olfactory nerve. Particle translocation to the brain has not been confirmed in humans to our knowledge.

The recent US Health Effects Institute (HEI) review on the health effects of ambient ultrafine particles included an appraisal of the evidence to emerge from experimental studies in animals and humans (HEI, 2013). Overall conclusions supported the differential patterns of deposition, clearance and potential for translocation exhibited by this fraction. The review also concluded that ''Experimental and epidemiologic studies provide suggestive, but not consistent, evidence of adverse effects of short-term exposures to ambient UFP'' and that ''The current evidence does not support a conclusion that exposure to UFPs alone can account in substantial ways for the adverse effects of $PM_{2.5}$''.

Secondary inorganic aerosols

Sulfate is a major component, together with nitrate, of secondary inorganic particles that are formed from gaseous primary pollutants. Because of their high solubility (and low hazard) and their abundance in the human body, these secondary inorganic particles have been suggested to be less harmful than, for example, primary combustion-derived particles (Schlesinger & Cassee, 2003).

There is an absence of new relevant evidence reported on the role of secondary inorganic aerosols since the review by Schlesinger & Cassee (2003), in which it was concluded that these particles have little biological potency in normal human beings or animals or in the limited compromised animal models studied at environmentally relevant levels. As mentioned in Reiss et al. (2007), toxicological evidence provides little or no support for a causal association between particulate sulfate compounds and a risk to health at ambient concentrations. Limited toxicological evidence does not support a causal association between particulate nitrate compounds and excess health risks either. However, it cannot be excluded that neither the cations associated with sulfates and nitrates (such as transition metals, acidity marked by hydrogen cations), nor absorbed components (such as polycyclic aromatic hydrocarbons) may be the underlying cause of the strong epidemiological associations between sulfate and health effects (Reiss et al., 2007). In the absence of

published toxicological studies investigating the role of sulfates (or nitrates) in the complex mixture of PM, it cannot be excluded that these secondary inorganic components produce an interactive biological effect with constituents of the overall pollutant mix by, for example, influencing the bioavailability of other components, such as metals.

Transition metals and metal compounds

Toxicological examinations of the constituents of ambient air have not identified an individual metal as being a likely cause of human health problems associated with PM (Lippmann & Chen, 2009). Whilst a study by Lippmann et al. (2006), involving six months of weekday CAP exposures reported an association between nickel and cardiac function changes for short-term responses to pronounced daily peaks in nickel; there was no evidence of an association of nickel with chronic effects in this and subsequent sub-chronic CAP exposure studies. In another study, controlled exposure of young, healthy adults to $PM_{2.5}$ caused an elevation in blood fibrinogen at 18 h post-exposure, which was correlated with a copper–zinc–vanadium factor in the PM. Furthermore, despite the fact that zinc ions have many physiological functions, they can also interfere with the body's homeostasis, leading to adverse effects such as oxidative stress and inflammation (Araujo, 2010). With respect to particular species, metal oxides are substances traditionally considered to be relatively inert chemically, but despite this, in very small (ultrafine) size ranges, these particles have been linked with significant oxidative stress-mediated toxicity (Duffin et al., 2007). Some metals, such as zinc oxide, will dissolve in the body (Charrier & Anastasio, 2011; Gilmour et al., 2006). Overall, it appears that the cardiovascular effects of ambient air $PM_{2.5}$ are influenced, if not dominated, by their metal contents, especially the transition metals, and that nickel is likely to be a key component (Lippmann & Chen, 2009). An important role for metals is also evident in a study in which a single dose of dusts from two types of tire were instilled intratracheally in the lungs of rats, and effects were assessed within 24 h and after four weeks. One dust was made from ground tires of recycled styrene butadiene rubber, while a second dust was made from scrap tires. Tests were done with administered saline, the two tire dusts and soluble zinc, copper or both. At very high dose levels (5 mg/kg rat), the exposures induced cardiac oxidative stress (Gottipolu et al., 2008), which was associated with the water-soluble zinc and copper. Whilst it is unlikely that transition metals can explain all of the health effects observed in epidemiological studies at present ambient levels, these components remain a group of components for which reduction measures will most likely lead to improving the health status of the population. For a more extensive overview on the transition metal toxicity, we refer to the US EPA ISA for PM (EPA (2009).

The role of source types

A WHO workshop in Bonn concluded in 2007 that current knowledge does not allow specific quantification of the health effects of emissions from different sources (or of individual components). In 2009, the EPA ISA concluded that ''there are many components contributing to the health effects of $PM_{2.5}$, but not sufficient evidence to differentiate those sources (or constituents) that are more closely related to specific health outcomes'' (EPA, 2009). The ISA further noted that a number of source types – including motor vehicle emissions, coal combustion, oil burning and vegetative burning – are associated with health effects and went on to include crustal material as another potentially toxic component. The limited new evidence accumulated after 2009 does not lead to changes in the conclusions.

Traffic

Motor vehicles are a significant source of urban air pollution in the form of both combustion particles and road dust that originates from the wear of road surfaces, brakes, clutches and tires. Simultaneous emissions of gaseous pollutants and noise make estimation of traffic-related PM effects a challenge. Indeed, current available evidence does not allow discernment of the pollutants or pollutant combinations that are related to different health outcomes, although several toxicological studies have linked exhaust emissions to adverse effects on health. With progressive reductions in the latter, road abrasion, tire wear and brake wear are becoming relatively more important, with toxicological research increasingly indicating that such non-exhaust pollutants could be responsible for some of the observed adverse effects on health.

A critical review of the literature on the health effects of traffic-related air pollution (HEI, 2010) included toxicological evidence of the impact of *traffic-mixture* exposures. Such evidence stems from controlled exposures of animals in areas of high traffic density, real-world exposure design in which subjects spend time in a polluted location (compared with equivalent activities in a location with relatively clean air) and individuals occupationally exposed to traffic and populations (animals or human beings) naturally exposed to polluted urban environments. Of the small number of studies reported (compared with the much larger literature on *specific components* of traffic emissions which is not specifically covered in this article), the main cardiorespiratory findings in humans were that short-term exposures can bring about decrements in lung function and enhanced responses to allergens in adult subjects with asthma (McCreanor et al., 2007; Svartengren et al., 2000), as well as positive and negative effects on vascular function in healthy subjects (Bräuner et al., 2008; Rundell et al., 2007). On-road animal studies, utilizing compromised or allergic rodents, observed mild pulmonary inflammation (Elder et al., 2004), significant alterations in lung structure and elastic properties (Mauad et al., 2008) and systemic inflammation and effects on vascular function and autonomic control of the heart (Elder et al., 2004, 2007). Recently, the HEI concluded in their review of toxicological studies that only suggestive evidence exists that exposure to pollutants that are components of traffic emissions, including ambient and laboratory-generated PM and exhaust from diesel and gasoline-fuelled engines, alters cardiovascular function (HEI, 2010). Evidence for other cardiopulmonary symptoms is less evident; and toxicological evidence relating exposure to traffic-generated air pollution and adverse human health effects remain incomplete.

Since the HEI review, toxicological evidence on the effects of traffic-mixture exposures include increased respiratory symptoms, decreased peak expiratory flow and an inflammatory response in the upper airways in mild asthmatic adults exposed for two hours in a road tunnel (Larsson et al., 2010). Health effects found in studies of acute (20 min to 2 h) real-life traffic exposure on healthy volunteers are limited to a small increase in the percentage of blood neutrophils (Jacobs et al., 2010), modest effects on peak flow, exhaled nitric oxide and airway resistance (Zuurbier et al., 2011a,b). A study by Strak et al. (2012) was specifically designed to evaluate the contribution of different pollutants. They increased exposure contrasts and reduced correlations among pollutants by exposing healthy volunteers at five different locations, including two traffic sites. Changes in particle number concentrations, NO_2 and nitrogen oxides during five-hour exposures were associated with increased exhaled nitric oxide and impaired lung function. These associations were robust and insensitive to adjustment for other pollutants. PM mass concentration or other PM characteristics, including elemental carbon and trace metals, were not predictive of the observed responses. Studies have also investigated acute cardiovascular health effects in volunteers with type 2 diabetes. Passengers on 90–110 min car rides on a busy road demonstrated a decrease in high-frequency heart rate variability and an increase in the ratio of low-frequency to high-frequency components compared with pre-ride measurements (Laumbach et al., 2010). Chronic exposure to urban air pollution (in chambers 20 m from a street with heavy traffic in downtown Sao Paulo) exacerbates the susceptibility of low density lipoprotein to oxidation, atherogenesis and vascular remodeling in hyperlipidemic mice (Soares et al., 2009); and in Swiss mice, it presents as coronary arteriolar fibrosis and elastosis (Akinaga et al., 2009).

Toxicological reproductive outcomes have been investigated in subjects occupationally exposed to traffic. Findings include abnormal sperm count, mobility and morphology (Guven et al., 2008) and a significantly higher percentage of spermatozoa with damaged chromatin and DNA fragmentation (Calogero et al., 2011) in toll-gate workers. In male traffic policemen, lower free testosterone (Sancini et al., 2011) and higher luteinizing hormone (Tomao et al., 2009) and follicle-stimulating hormone (Tomei et al., 2009) plasma levels were reported. Studies on female traffic police observed significantly higher plasma free testosterone (Tomei et al., 2008) and follicle-stimulating hormone levels during the proliferative phase of the menstrual cycle (Ciarrocca et al., 2011).

Evidence continues to accumulate on the role that oxidative stress, as a mode of action through which traffic-related air pollution leads to adverse effects on human health. Ambient PM derived from vehicles has a high oxidative potential (Kelly, 2003), and it has been found that a clear increment in roadside particulate oxidative potential appears to be associated with metals arising from engine abrasion (iron, manganese and molybdenum) or brake wear (copper and antimony) (Schauer et al., 2006; Thorpe & Harrison, 2008). The roadside increments of particulate oxidative potential are significant and the metal components identified as determinants of this oxidative activity have established toxicity in human beings (Kelly et al., 2011). These results are potentially important as they highlight the contribution of currently non-regulated non-exhaust pollutants. The validity of urinary excretion of 8-oxo-7,8-dihydro-2-deoxyguanosine as a biomarker was also recently demonstrated in a meta-analysis (Barbato et al., 2010). Oxidative damage to DNA and the formation of bulky adducts are two mechanisms by which traffic-related air pollution could lead to mutagenesis and, ultimately, cause cancer. Bulky DNA adducts have been detected among traffic-exposed workers (Palli et al., 2008) and – together with micronuclei – in cord blood after maternal exposures to traffic-related air pollution, suggesting that transplacental environmental exposures could induce DNA damage in neonates (Pedersen et al., 2009).

A large database describes all sorts of adverse health effects due to exposure to diesel engine exhaust, which is rich in PM – mostly below 2.5 μm. Exposure in healthy volunteers causes inflammation of the airways (Behndig et al., 2006) and reduces vascular function (Mills et al., 2005), whilst in patients with heart problems (stable myocardial infarction), myocardial ischemia and reduced clot resolving function (Mills et al., 2007) has been observed. Although in certain urban areas, diesel engine exhaust particles can be a substantial part of the total PM to which people are exposed, it is not clear if this fraction is always more potent than PM on a mass basis. For example, diesel engine exhaust (105 $\mu g/m^3$) appeared to be less toxic in inducing plaque development in ApoE(–/–) mice than corresponding exposures to $PM_{2.5}$ (105 $\mu g/m^3$, four days/week, five months) (Quan et al., 2010). In a further study (Lund et al., 2007) and meta-analysis (Seilkop et al., 2012) investigating pro-atherosclerotic responses in ApoE(–/–) mice, filtration of particles from gasoline and diesel exhaust had little effect on toxicity, suggesting that not only the particles but certainly also the gaseous fraction of engine exhaust is related to adverse health effects.

In their recent review, Hesterberg et al. (2011) pointed out that, although there are good reasons for concern for health effects due to diesel engine exhaust exposure, significant efforts have been made to alter the composition of diesel engine exhaust in the past few decades, resulting in a more fuel efficient and complete combustion process and the installation of filter traps with substantial lower mass emissions. It seems very likely that this will have a profound effect on the toxicity of diesel engine exhaust, but there is no systematic review available that allows clear conclusions on an increase or decrease of the toxic potency and associated health risks. Apart from changing technologies, fuel composition is also undergoing modification, with the introduction of biodiesel blends. There is a large knowledge gap with respect to the health effects related to replacing petroleum-based diesel with biodiesel fuel, conflicting evidence about the extent to which biodiesel fuel exhaust emissions present a lower risk to human health relative to petroleum-based diesel emissions (Swanson et al., 2007). German studies have shown significantly increased mutagenic effects, by a factor of 10, of the particle extracts from rapeseed oil in comparison to fossil diesel fuel, whilst the gaseous phase caused even stronger mutagenicity (Bünger et al., 2007). Biodiesel (rapeseed oil methyl ester) has also been shown to have four times higher

cytotoxicity than conventional diesel under idling conditions, while no differences were observed for the transient state (Bünger et al., 2000). Others, however, have not observed differences in the cytotoxic response to fossil- and bio-diesel emissions under idling conditions (Jalava et al., 2010). Comparability of reported findings is hampered by performance under different conditions – e.g. vehicle type, test cycle, fuel type and quality – all of which can influence emissions as well as the observed the health effects.

Coal and oil combustion

Several studies based in the United States have reported toxicological evaluations in rats of short-term exposure to coal-fired power plant emissions (Godleski et al., 2011). In general, these emissions – weather aged and/or oxidized, and diluted or not – showed very little (if any) adverse effects in responses to the inhaled aerosols studied. Godleski et al. (2011) also reported that no specific toxic constituent could be identified that explained the subtle effects. It should be noted with these studies that the exposures were for only a few hours, and also that due to the extensive emissions control equipment installed on these power plants, the exposure levels were very low. The relatively low exposure levels and short exposure durations may explain the lack of clear health effects in these animals. Barrett et al. (2011) reported that downwind coal combustion emissions are able to exacerbate various features of allergic airway responses, depending on the timing of exposure in relation to allergen challenge, and that these symptoms were related to both the particulate and gaseous phase of the emissions. A large number of studies have been published on residual oil fly ash PM. This type of dust is rich in transition metals, such as vanadium and nickel, all of which possess strong redox activity associated with the ability to cause oxidative stress.

Industry

Toxicological and clinical studies have not been performed near sources of industrial emissions other than power plants. The evidence is mainly derived from PM samples of which PM composition has been determined and used for source apportionment. The estimated source contributions have subsequently been linked with measures for toxicity. For example, Steerenberg et al. (2006) identified, in a small data set, that industrial combustion and/or incinerators were associated with respiratory allergy. Specific data on the toxicity of industry emitted PM other than combustion-derived PM has not been published since 2005.

Biomass combustion

Although the interest in the toxicity of wood smoke and other types of biomass burning is increasing, because it is know that this type of smoke contains a mixture of harmful gases and small particles, very view toxicological studies have been published. Wegesser et al. (2009) demonstrated that fine and coarse PM collected during wildfires are considerably more toxic in the mouse lung per unit mass than PM collected in the same area without fires. This was confirmed by Verma et al. (2009), who tested PM collected during Los Angeles wildfires. A more recent controlled human exposure study from Denmark reported that three-hour exposure to wood smoke with up to 354 $\mu g/m^3$ of PM from a good performing modern wood stove had no effect on markers of oxidative stress, DNA damage, cell adhesion, cytokines or microvascular function in atopic subjects, supporting the suggestion that burning conditions are dominant factors that determine the hazard of the combustion-derived particles. On the other hand, Stockfelt et al. (2013) were not able to demonstrate significant adverse responses in healthy adults that were exposed to two sessions of wood smoke for three hours, one week apart. One session used smoke from the start-up phase of the wood-burning cycle and the other smoke from the burn-out phase with mean particle mass concentrations of 295 $\mu g/m^3$ and 146 $\mu g/m^3$. Another Scandinavian study (Bølling et al., 2012) reported that the hazard of wood smoke particles seems, to a large extent, to depend on the type of stove and combustion conditions (oxygen supply and water content). These outcomes suggest that a simple risk assessment for wood smoke is not possible and the toxicity of the emitted PM can vary significantly. Notably, the toxicity seems to be clearly contingent on the organic fraction. Kurmi et al. (2013) assessed the oxidative potential of samples of biomass smoke PM and concluded that capacity of PM to elicit oxidative stress certainly has the potential to contribute toward negative health impacts associated with traditional domestic fuels in the developing world. In general, the highly variable conditions under which the biomass is combusted do not allow to draw a firm conclusion on the toxicity of the PM released during this process. However, incomplete combustion often, if not always, lead to the emission of hazardous substances suggesting that exposure needs to be reduced to a minimum.

Desert dust

Only one study was identified that specifically investigated the adverse effects of an acute exposure to desert dust (Wilfong et al., 2011). Rats received a single dose in the lungs (1, 5 or 10 mg) of PM_{10} collected in Kuwait. At 24 h, 3 d, 7 d and 6 months, the effects on inflammation, cytotoxicity and pathology were very minimal compared with those of silica dust. Although the evidence is limited and obtained in healthy animals, the hazard per gram associated with desert dust will most likely be smaller than that of, for example, combustion-derived PM or soluble transition metals. Of particular interest, Godleski et al. (2011) reported that viable pathogens in breathable dusts were identified in desert dust collected in Iraq and Kuwait, suggesting that the source is more complex in nature than sand. Polymenakou et al. (2008) found a large load of airborne microorganisms and pathogens during an intense African dust event in the eastern Mediterranean, and they concluded that the presence of aerosolized bacteria in small size particles may have significant implications for human health via intercontinental transportation of pathogens. Other researchers hypothesized that soluble metals contribute to the toxicity of desert dust, which, by the way is often irrespirable. Taylor et al. (2013) collected dust samples at military bases near Fort Irwin USA, in Iraq (Camp Victory, Taji and Talil) and Khost Afghanistan. The sand samples were irradiated to eliminate microbiological flora and sieved to

remove pebbles, twigs and other large objects. The soluble fraction of these dust samples were tested for *in vitro* and *in vivo* effects leading to the conclusion that although *in vitro* toxicity showed differences among the samples, *in vivo* lung pathology was remarkably similar after a single intratracheal instillation. This suggests a very similar risk for these dust samples. Overall, no firm conclusion can be drawn on the relative hazard of dessert dust compared to dust collected in for example urbanized areas.

Ocean and sea

In Edinburgh, healthy and age-matched volunteers with stable coronary heart disease were exposed for 2 h to $PM_{2.5}$ ($190 \pm 37\,\mu g/m^3$) and to clean filtered air using a randomized, double-blind crossover study design. After exposure to PM, there were increases in exhaled breath 8-isoprostane, in blood flow and in plasma tissue plasminogen activator ($p < 0.005$). There were no significant changes in markers of systemic inflammation, and there was no effect on vascular function in either group of subjects (Mills et al., 2008). It was noted that most of the particulate mass consisted of sea salt, and far less PM was derived from combustion sources.

The role of sea spray and/or sea salt has not been investigated in any publication since 2005, although sea salt is not classified as a hazardous compound, and it is plausible that at current exposure levels, no harmful effects will occur.

Role of exposure times

Exposure times considered under the review include either a single episode or repeated, short episodes to very high concentrations of pollutants. Given that risk estimates of long-term exposure studies are usually much higher than those of short-term exposure studies, future air quality guidelines for yearly average concentrations will be of higher relevance than those based on 24-hour averages. It should be noted, however, that the EC's Second Position Paper on PM (EC, 2004) showed that, on an European scale, annual PM_{10} averages and 90.4 percentile values of daily concentrations (equivalent to the daily limit value) of the corresponding year are highly correlated. Clinical studies have demonstrated that sub-daily exposures to elevated levels of PM can lead to adverse physiological changes in the respiratory and cardiovascular systems, also suggesting that an averaging time of less than 24 h – for example, 1 h (similar to ozone) could be considered for air quality guidelines. However, the correlation between the 1-h maximum and 24-h average particle concentration is typically high. No studies have evaluated whether, for example, a high 1-h exposure would lead to a different response than a similar dose given for 24 h, and the same is true for repeated very short-term exposures.

Very little data have been published on health effects due to exposures to PM shorter than the usual 1–2-h duration of clinical studies, whereas the animal and *in vitro* toxicological studies have durations between a few hours and several months. In the study by Mills et al. (2007), patients with stable coronary heart disease have immediate ST-segment depression in their electrocardiogram tracings when exposed to diluted diesel exhaust ($300\,\mu g/m^3$) than they are exposed to clean filtered air. ST-segment depression is an important predictor of adverse cardiovascular events, but the magnitude of ST-segment depression in the trial was less than would be conventionally considered clinically significant. It is likely, however, that the magnitude of ST-segment depression would have been greater at higher workloads. It should be mentioned that although exposure levels were high, they may occur in traffic hot spots of tunnels, and may be serious health risk for air pollution susceptible individuals such as those with a cardiovascular disease. Unfortunately, several variables – such as inter-individual variation, diet and the type of test atmosphere – prevent statements to be made on the role of exposure times.

Conclusions

Toxicological studies have shown that PM mass ($PM_{2.5}$ and PM_{10}) comprises fractions that elicit varying types and degrees of detrimental effects, suggesting a role for not only chemical composition (such as transition metals and combustion-derived primary and secondary organic particles) but also physical properties (size, particle number and surface area). Although current evidence does not allow a precise differentiation to be made as to which constituents or sources are most closely related to specific outcomes, some conclusions can be drawn. For example, for three important components, namely black carbon, secondary organic aerosols and secondary inorganic aerosols, substantial research suggests they may provide additional valuable air quality metrics for evaluating the effects of mixtures of pollutants from a variety of sources.

- Although black carbon may not be a major causal agent, evidence suggests the carbon core can act as a universal carrier of a wide variety of combustion-derived chemical constituents such as semi-volatile organic fractions and transition metals.
- Whilst no new toxicological evidence exists to support a causal role for inorganic secondary aerosols, gaps in research mean that we cannot yet rule out a role for the cations or interactions with absorbed components (again metals and organic particles) of the overall pollutant mix.
- There is growing information on the associations of organic carbon with health effects, and moreover, organic carbon primary emissions are one of the important contributors to the formation of secondary organic aerosols – a significant component of the $PM_{2.5}$ mass. Current evidence is insufficient to distinguish between the toxicity of primary and secondary organic aerosols.

With respect to the toxic properties of the different size fractions of PM, data arising from toxicological research on the effects of coarse particles are scarce compared with accumulating epidemiological evidence. Despite this, clinical studies have reported that coarse particles can be as toxic as $PM_{2.5}$ on a mass basis. The difference in risk between coarse and fine PM may, at least partially, be explained by differences in intake and different biological mechanisms. Substantial advances have been made in understanding the action of ultrafine particles, with toxicological studies showing that, in part, this size fraction acts through mechanisms not shared with larger particles that dominate

mass-based metrics, such as $PM_{2.5}$ or PM_{10}. Additional research is, however, required to establish whether exposure to ultrafine particles alone can substantially contribute to the adverse effects of PM.

A variety of air pollution sources have been associated with different types of health effects. The major one throughout the world is the combustion of fossil fuels and this in turn appears to represent the source associated with the most serious outcomes. Evidence is consistently growing for an association between traffic emissions and detrimental effects and the thrust of research in this area is now to identify the culprit components. There is still accumulating evidence for an adverse effect of carbonaceous material from traffic, although a growing toxicological database exists for traffic-generated dust, such as road, brake and tire wear, as another important component.

The REVIHAAP project also looked at the implications of the new evidence for WHO air quality guidelines, which were last revised in 2005. On PM components and size fractions, the review concluded, based on new information from epidemiological, toxicological and other relevant research on health impacts of air pollution, that ''it would be advantageous to develop an additional air quality guideline to capture the effects of road vehicle PM emissions not well captured by $PM_{2.5}$, building on the work on black carbon and/or elemental carbon and evidence on other pollutants in vehicle emissions''. As well, ''although there is considerable evidence that ultrafine particles can contribute to the health effects of PM, for ultrafine particles (measured by the number of particles) the data on concentration-response functions are too scarce to evaluate and recommend an air quality guidelines. The same applies for organic carbon. Current efforts to reduce the numbers of ultrafine particles in engine emissions should continue, and their effectiveness assessed, given the potential health effects'' (WHO, 2013).

In summary, to support the comprehensive review of EU's air quality policies scheduled for 2013, scientific, evidence-based advice on the health aspects of the major air pollutants has been collected. This initiative ensures the latest studies are used to underpin decisions on which measures are needed to achieve levels of air quality that do not result in unacceptable risks to human health. This review summarizes the considerable amount of published information to have emerged from toxicological research in recent years. Accumulating evidence has identified additional air quality metrics (e.g. black carbon, secondary organic and inorganic aerosols) that may be valuable in evaluating the health risks of, for example, primary combustion particles from traffic emissions, which are not fully taken into account with $PM_{2.5}$ mass. The collected new evidence also supports the scientific conclusions of the WHO Air Quality Guidelines, last updated in 2005. However, the lack of epidemiological evidence does not yet allow the derivation of reliable concentration-response functions on which an air quality guideline or standard can be based.

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Declaration of interest

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The views expressed herein can in no way be taken to reflect the official opinion of the European Union. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions or the stated policy of the World Health Organization. Review of evidence on health aspects of air pollution – REVIHAAP Project: Final technical report is available via http://www.euro.who.int/__data/assets/pdf_file/0004/193108/REVIHAAP-Final-technical-report.pdf. This technical report presents detailed answers to 24 questions on the health aspects of air pollution of relevance for the review of European Union policies. The answers were developed by a large group of scientists engaged in the WHO project.

References

Akinaga LM, Lichtenfels AJ, Carvalho-Oliveira R, et al. (2009). Effects of chronic exposure to air pollution from Sao Paulo city on coronary of Swiss mice, from birth to adulthood. Toxicol Pathol 37:306–14.

Araujo JA. (2010). Particulate air pollution, systemic oxidative stress, inflammation, and atherosclerosis. Air Qual Atmos Health 4:79–93.

Bakand S, Hayes A, Dechsakulthorn F. (2012). Nanoparticles: a review of particle toxicology following inhalation exposure. Inhal Toxicol 24: 125–35.

Barbato DL, Tomei G, Tomei F, Sancini A. (2010). Traffic air pollution and oxidatively generated DNA damage: can urinary 8-oxo-7,8-dihydro-2-deoxiguanosine be considered a good biomarker? A meta-analysis. Biomarkers 15:538–45.

Barmpadimos I, Keller J, Oderbolz D, et al. (2012). One decade of parallel fine (PM2.5) and coarse (PM10–PM2.5) particulate matter measurements in Europe: trends and variability. Atmos Chem Phys 12:3189–203.

Barrett EG, Day KC, Gigliotti AP, et al. (2011). Effects of simulated downwind coal combustion emissions on pre-existing allergic airway responses in mice. Inhal Toxicol 23:792–804.

Behndig AF, Mudway IS, Brown JL, et al. (2006). Airway antioxidant and inflammatory responses to diesel exhaust exposure in healthy humans. Eur Resp J 27:359–65.

Biswas S, Verma V, Schauer JJ, et al. (2009). Oxidative potential of semi-volatile and non volatile particulate matter (PM) from heavy-duty vehicles retrofitted with emission control technologies. Environ Sci Technol 43:3905–12.

Bølling AK, Totlandsdal AI, Sallsten G, et al. (2012). Wood smoke particles from different combustion phases induce similar pro-inflammatory effects in a co-culture of monocyte and pneumocyte cell lines. Part Fibre Toxicol 9:45.

Bräuner EV, Møller P, Barregard L, et al. (2008). Exposure to ambient concentrations of particulate air pollution does not influence vascular function or inflammatory pathways in young healthy individuals. Part Fibre Toxicol 5:13.

Bünger J, Krahl J, Baum K, et al. (2000). Cytotoxic and mutagenic effects, particle size and concentration analysis of diesel engine emissions using biodiesel and petrol diesel as fuel. Arch Toxicol 74: 490–8.

Bünger J, Krahl J, Munack A, et al. (2007). Strong mutagenic effects of diesel engine emissions using vegetable oil as fuel. Arch Toxicol 81: 599–603.

Calogero AE, La Vignera S, Condorelli RA, et al. (2011). Environmental car exhaust pollution damages human sperm chromatin and DNA. J Endocrinol Invest 34:e139–43.

Charrier JG, Anastasio C. (2011). Impacts of antioxidants on hydroxyl radical production from individual and mixed transition metals in a surrogate lung fluid. Atmos Environ 45:7555–62.

Ciarrocca M, Caciari T, Ponticiello BG, et al. (2011). Follicle-stimulating hormone levels in female workers exposed to urban pollutants. Int J Environ Health Res 21:391–401.

Duffin R, Mills NL, Donaldson K. (2007). Nanoparticles – a thoracic toxicology perspective. Yonsei Med J 48:561–72.

Elder A, Gelein R, Finkelstein J, et al. (2004). On-road exposure to highway aerosols. 2. Exposures of aged, compromised rats. Inhal Toxicol 16:41–53.

Elder A, Couderc JP, Gelein R, et al. (2007). Effects of on-road highway aerosol exposures on autonomic responses in aged, spontaneously hypertensive rats. Inhal Toxicol 19:1–12.

EPA. (2009). Integrated science assessment for particulate matter (final report). Washington, DC: United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=216546#Download. [Last accessed: 5 April 2013].

European Commission Cafe Working Group on Particulate Matter (EC). (2004). Second position paper on particulate matter. Available from: http://ec.europa.eu/environment/archives/cafe/pdf/working_groups/2nd_position_paper_pm.pdf. [Last accessed: 28 June 2013].

EU. (2008). Directive 2008/50/EC of the European Parliament and of the Council of 21 May 2008 on ambient air quality and cleaner air for Europe. Official J EU 152:1–44.

Frampton MW. (2001). Systemic and cardiovascular effects of airway injury and inflammation: ultrafine particle exposure in humans. Environ Health Perspect 109:529–32.

Frampton MW, Utell MJ, Zareba W, et al. (2004). Effects of exposure to ultrafine carbon particles in healthy subjects and subjects with asthma. Res Rep Health Eff Inst 126:1–47; discussion 49–63.

Gerlofs-Nijland ME, Dormans JA, Bloemen HJ, et al. (2007). Toxicity of coarse and fine particulate matter from sites with contrasting traffic profiles. Inhal Toxicol 19:1055–69.

Gilmour MI, McGee J, Duvall RM, et al. (2007). Comparative toxicity of size-fractionated airborne particulate matter obtained from different cities in the United States. Inhal Toxicol 19:7–16.

Gilmour PS, Schladweiler MC, Nyska A, et al. (2006). Systemic imbalance of essential metals and cardiac gene expression in rats following acute pulmonary zinc exposure. J Toxicol Environ Health A 69:2011–32.

Godleski JJ, Rohr AC, Coull BA, et al. (2011). Toxicological evaluation of realistic emission source aerosols (TERESA): summary and conclusions. Inhal Toxicol 23:95–103.

Gottipolu RR, Landa ER, Schladweiler MC, et al. (2008). Cardiopulmonary responses of intratracheally instilled tire particles and constituent metal components. Inhal Toxicol 20:473–84.

Graff DW, Cascio WE, Rappold A, et al. (2009). Exposure to concentrated coarse air pollution particles causes mild cardiopulmonary effects in healthy young adults. Environ Health Perspect 117: 1089–94.

Guven A, Kayikci A, Cam K, et al. (2008). Alterations in semen parameters of toll collectors working at motorways: does diesel exposure induce detrimental effects on semen? Andrologia 40: 346–51.

Halatek T, Stepnik M, Stetkiewicz J, et al. (2011). The inflammatory response in lungs of rats exposed on the airborne particles collected during different seasons in four European cities. J Toxicol Environ Health A 46:1469–81.

Happo MS, Salonen RO, Hälinen AI, et al. (2010). Inflammation and tissue damage in mouse lung by single and repeated dosing of urban air coarse and fine particles collected from six European cities. Inhal Toxicol 22:402–16.

Harder V, Gilmour P, Lentner B, et al. (2005). Cardiovascular responses in unrestrained WKY rats to inhaled ultrafine carbon particles. Inhal Toxicol 17:29–42.

HEI Panel on the Health Effects of Traffic-Related Air Pollution. (2010). Traffic-related air pollution: a critical review of the literature on emissions, exposure, and health effects. Boston: Health Effects Institute. Special Report 17. Available from: http://pubs.healtheffects.org/getfile.php?u=553. [Last accessed: 14 March 2013].

HEI Review Panel on Ultrafine Particles. (2013). Understanding the health effects of ultrafine ambient particles. Boston: Health Effects Institute. Available from: http://pubs.healtheffects.org/getfile.php?u=893. [Last accessed: 28 June 2013].

Hesterberg TW, Long CM, Sax SN, et al. (2011). Particulate matter in new technology diesel exhaust (NTDE) is quantitatively and qualitatively very different from that found in traditional diesel exhaust (TDE). J Air Waste Manag Assoc 61:894–913.

Jacobs L, Nawrot TS, de Geus B, et al. (2010). Subclinical responses in healthy cyclists briefly exposed to traffic-related air pollution: an intervention study. Environ Health 9:64.

Jalava PI, Salonen RO, Pennanen AS, et al. (2008). Effects of solubility of urban air fine and coarse particles on cytotoxic and inflammatory responses in RAW 264.7 macrophage cell line. Toxicol Appl Pharmacol 229:146–60.

Jalava PI, Tapanainen M, Kuuspalo K, et al. (2010). Toxicological effects of emission particles from fossil- and biodiesel-fueled diesel engine with and without DOC/POC catalytic converter. Inhal Toxicol 22:48–58.

Kelly FJ. (2003). Oxidative stress: its role in air pollution and adverse health effects. Occup Environ Med 60:612–16.

Kelly F, Anderson HR, Armstrong B, et al.; HEI Health Review Committee. (2011). The impact of the congestion charging scheme on air quality in London. Part 1. Emissions modeling and analysis of air pollution measurements. Res Rep Health Eff Inst (155):5–71.

Kelly FJ, Fussell JC. (2012). Size, source and chemical composition as determinants of toxicity attributable to smbient particulate matter. Atmos Environ 60:504–26.

Kleinman MT, Araujo JA, Nel A, et al. (2008). Inhaled ultrafine particulate matter affects CNS inflammatory processes and may act via MAP kinase signaling pathways. Toxicol Lett 178:127–30.

Kreyling WG, Hirn S, Schleh C. (2010). Nanoparticles in the lung. Nat Biotechnol 28:1275–6.

Kreyling WG, Semmler-Behnke M, Takenaka S, Möller W. (2013). Differences in the biokinetics of inhaled nano- versus micrometer-sized particles. Acc Chem Res 46:714–22.

Kurmi OP, Dunster C, Ayres JG, Kelly FJ. (2013). Oxidative potential of smoke from burning wood and mixed biomass fuels. Free Radic Res 47:829–35.

Langrish JP, Mills NL, Chan JK, et al. (2009). Beneficial cardiovascular effects of reducing exposure to particulate air pollution with a simple facemask. Part Fibre Toxicol 6:8.

Langrish JP, Li X, Wang S, et al. (2012). Reducing personal exposure to particulate air pollution improves cardiovascular health in patients with coronary heart disease. Environ Health Perspect 120:367–72.

Larsson BM, Grunewald J, Sköld CM, et al. (2010). Limited airway effects in mild asthmatics after exposure to air pollution in a road tunnel. Respir Med 104:1912–18.

Laumbach RJ, Rich DQ, Gandhi S, et al. (2010). Acute changes in heart rate variability in subjects with diabetes following a highway traffic exposure. J Occup Environ Med 52:324–31.

Lippmann M, Chen LC. (2009). Health effects of concentrated ambient air particulate matter (CAPs) and its components. Crit Rev Toxicol 39:865–913.

Lippmann M, Ito K, Hwang JS, et al. (2006). Cardiovascular effects of nickel in ambient air. Environ Health Perspect 114:1662–9.

Lucking AJ, Lundbäck M, Barath SL, et al. (2011). Particle traps prevent adverse vascular and prothrombotic effects of diesel engine exhaust inhalation in men. Circulation 123:1721–8.

Lund AK, Knuckles TL, Obot Akata C, et al. (2007). Gasoline exhaust emissions induce vascular remodeling pathways involved in atherosclerosis. Toxicol Sci 95:485–94.

Mauad T, Rivero DH, de Oliveira RC, et al. (2008). Chronic exposure to ambient levels of urban particles affects mouse lung development. Am J Respir Crit Care Med 178:721–8.

McCreanor J, Cullinan P, Nieuwenhuijsen MJ, et al. (2007). Respiratory effects of exposure to diesel traffic in persons with asthma. N Engl J Med 357:2348–58.

Mills NL, Törnqvist H, Robinson SD, et al. (2005). Diesel exhaust inhalation causes vascular dysfunction and impaired endogenous fibrinolysis. Circulation 112:3930–6.

Mills NL, Törnqvist H, Gonzalez MC, et al. (2007). Ischemic and thrombotic effects of dilute diesel-exhaust inhalation in men with coronary heart disease. N Engl J Med 357:1075–82.

Mills NL, Robinson SD, Fokkens PH, et al. (2008). Exposure to concentrated ambient particles does not affect vascular function in

patients with coronary heart disease. Environ Health Perspect 116: 709–15.

Mills NL, Miller MR, Lucking AJ, et al. (2011). Combustion-derived nanoparticulate induces the adverse vascular effects of diesel exhaust inhalation. Eur Heart J 32:2660–71.

Palli D, Saieva C, Munnia A, et al. (2008). DNA adducts and PM(10) exposure in traffic-exposed workers and urban residents from the EPIC-Florence City study. Sci Total Environ 403:105–12.

Pedersen M, Wichmann J, Autrup H, et al. (2009). Increased micronuclei and bulky DNA adducts in cord blood after maternal exposures to traffic-related air pollution. Environ Res 109:1012–20.

Peters A, Rückerl R, Cyrys J. (2011). Lessons from air pollution epidemiology for studies of engineered nanomaterials. J Occup Environ Med 53:S8–13.

Polymenakou PN, Mandalakis M, Stephanou EG, Tselepides A. (2008). Particle size distribution of airborne microorganisms and pathogens during an intense African dust event in the eastern Mediterranean. Environ Health Perspect 116:292–6.

Quan C, Sun Q, Lippmann M, Chen LC. (2010). Comparative effects of inhaled diesel exhaust and ambient fine particles on inflammation, atherosclerosis, and vascular dysfunction. Inhal Toxicol 22:738–53.

Reiss R, Anderson EL, Cross CE, et al. (2007). Evidence of health impacts of sulfate- and nitrate-containing particles in ambient air. Inhal Toxicol 19:419–49.

Rundell KW, Hoffman JR, Caviston R, et al. (2007). Inhalation of ultrafine and fine particulate matter disrupts systemic vascular function. Inhal Toxicol 19:133–40.

Sancini A, Tomei F, Tomei G, et al. (2011). Exposure to urban stressors and free testosterone plasma values. Int Arch Occup Environ Health 84:609–16.

Schauer JJ, Lough GC, Shafer MM, et al. (2006). Characterization of metals emitted from motor vehicles. Res Rep Health Eff Inst 1–76; discussion (133):77–88.

Schlesinger RB, Cassee FR. (2003). Atmospheric secondary inorganic particulate matter: the toxicological perspective as a basis for health effects risk assessment. Inhal Toxicol 15:197–235.

Seilkop SK, Campen MJ, Lund AK, et al. (2012). Identification of chemical components of combustion emissions that affect pro-atherosclerotic vascular responses in mice. Inhal Toxicol 24:270–87.

Soares SR, Carvalho-Oliveira R, Ramos-Sanchez E, et al. (2009). Air pollution and antibodies against modified lipoproteins are associated with atherosclerosis and vascular remodeling in hyperlipemic mice. Atherosclerosis 207:368–73.

Steerenberg PA, van Amelsvoort L, Lovik M, et al. (2006). Relation between sources of particulate air pollution and biological effect parameters in samples from four European cities: an exploratory study. Inhal Toxicol 18:333–46.

Stockfelt L, Sallsten G, Almerud P, et al. (2013). Short-term chamber exposure to low doses of two kinds of wood smoke does not induce systemic inflammation, coagulation or oxidative stress in healthy humans. Inhal Toxicol 25:417–25.

Strak M, Janssen NA, Godri KJ, et al. (2012). Respiratory health effects of airborne particulate matter: the role of particle size, composition, and oxidative potential – the RAPTES Project. Environ Health Perspect 120:1183–9.

Svartengren M, Strand V, Bylin G, et al. (2000). Short-term exposure to air pollution in a road tunnel enhances the asthmatic response to allergen. Eur Respir J 15:716–24.

Swanson KJ, Madden MC, Ghio AJ. (2007). Biodiesel exhaust: the need for health effects research. Environ Health Perspect 115:496–9.

Taylor K, Foster ML, Law JM, et al. (2013). Assessment of geographical variation in the respiratory toxicity of desert dust particles. Inhal Toxicol 25:405–16.

Thorpe A, Harrison RM. (2008). Sources and properties of non-exhaust particulate matter from road traffic: a review. Sci Total Environ 400: 270–82.

Tomao E, Tomei G, Rosati MV, et al. (2009). Luteinizing hormone (LH) levels in male workers exposed to urban stressors. Sci Total Environ 407:4591–5.

Tomei G, Tomao E, Ciarrocca M, et al. (2009). Follicle-stimulating hormone levels in male workers exposed to urban chemical, physical, and psychosocial stressors. Toxicol Ind Health 25: 395–402.

Tomei G, Ciarrocca M, Fiore P, et al. (2008). Exposure to urban stressor and effects on free testosterone in female workers. Sci Total Environ 392:198–202.

Upadhyay S, Stoeger T, Harder V, et al. (2008). Exposure to ultrafine carbon particles at levels below detectable pulmonary inflammation affects cardiovascular performance in spontaneously hypertensive rats. Part Fibre Toxicol 5:19.

Urch B, Brook JR, Wasserstein D, et al. (2004). Relative contributions of PM2.5 chemical constituents to acute arterial vasoconstriction in humans. Inhal Toxicol 16:345–52.

Urch B, Silverman F, Corey P, et al. (2005). Acute blood pressure responses in healthy adults during controlled air pollution exposures. Environ Health Perspect 113:1052–5.

Verma V, Polidori A, Schauer JJ, et al. (2009). Physicochemical and toxicological profiles of particulate matter in Los Angeles during the October 2007 Southern California wildfires. Environ Sci Technol 43: 954–60.

Wegesser TC, Last JA. (2008). Lung response to coarse PM: bioassay in mice. Toxicol Appl Pharmacol 230:159–66.

Wegesser TC, Pinkerton KE, Last JA. (2009). California wildfires of 2008: coarse and fine particulate matter toxicity. Environ Health Perspect 117:893–7.

Wilfong ER, Lyles M, Rietcheck RL, et al. (2011). The acute and long-term effects of Middle East sand particles on the rat airway following a single intratracheal instillation. J Toxicol Environ Health A 74: 1351–65.

WHO Regional Office for Europe. (2006). Air quality guidelines global update 2005: particulate matter, ozone, nitrogen dioxide and sulfur dioxide. Copenhagen: WHO Regional Office for Europe. Available from: http://www.euro.who.int/__data/assets/pdf_file/0005/78638/E90038.pdf. [Last accessed: 6 April 2013].

WHO Regional Office for Europe. (2012). Health Effects of Black Carbon. Copenhagen: WHO Regional Office for Europe. Available from: http://www.euro.who.int/__data/assets/pdf_file/0004/162535/e96541.pdf. [Last accessed: 28 June 2013].

WHO Regional Office for Europe. (2013). Review of evidence on health aspects of air pollution – REVIHAAP Project, Technical Report. Copenhagen: WHO Regional Office for Europe. Available from: http://www.euro.who.int/__data/assets/pdf_file/0004/193108/REVIHAAP-Final-technical-report.pdf [Last accessed: 28 Oct 2013].

Zuurbier M, Hoek G, Oldenwening M, et al. (2011a). Respiratory effects of commuters' exposure to air pollution in traffic. Epidemiology 22: 219–27.

Zuurbier M, Hoek G, Oldenwening M, et al. (2011b). In-traffic air pollution exposure and CC16, blood coagulation, and inflammation markers in healthy adults. Environ Health Perspect 119:1384–9.