Available online at www.sciencedirect.com
ScienceDirect
Mutation Research 613 (2006) 103–122
Reviews in Mutation Research
www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

Toxicological assessment of ambient and traffic-related particulate matter: A review of recent studies

Theo M.C.M. de Kok [a,*], Hermen A.L. Driece [a], Janneke G.F. Hogervorst [b], Jacob J. Briedé [a]

[a] *Department of Health Risk Analysis and Toxicology, Maastricht University, P.O. Box 616, Maastricht, The Netherlands*
[b] *Department of Epidemiology, Maastricht University, P.O. Box 616, Maastricht, The Netherlands*

Received 5 April 2006; received in revised form 17 July 2006; accepted 17 July 2006
Available online 1 September 2006

Abstract

Particulate air pollution (PM) is an important environmental health risk factor for many different diseases. This is indicated by numerous epidemiological studies on associations between PM exposure and occurrence of acute respiratory infections, lung cancer and chronic respiratory and cardiovascular diseases. The biological mechanisms behind these associations are not fully understood, but the results of *in vitro* toxicological research have shown that PM induces several types of adverse cellular effects, including cytotoxicity, mutagenicity, DNA damage and stimulation of proinflammatory cytokine production. Because traffic is an important source of PM emission, it seems obvious that traffic intensity has an important impact on both quantitative and qualitative aspects of ambient PM, including its chemical, physical and toxicological characteristics. In this review, the results are summarized of the most recent studies investigating physical and chemical characteristics of ambient and traffic-related PM in relation to its toxicological activity. This evaluation shows that, in general, the smaller PM size fractions ($<PM_{10}$) have the highest toxicity, contain higher concentrations of extractable organic matter (comprising a wide spectrum of chemical substances), and possess a relatively high radical-generating capacity. Also, associations between chemical characteristics and PM toxicity tend to be stronger for the smaller PM size fractions. Most importantly, traffic intensity does not always explain local differences in PM toxicity, and these differences are not necessarily related to PM mass concentrations. This implies that PM regulatory strategies should take PM-size fractions smaller than PM_{10} into account. Therefore, future research should aim at establishing the relationship between toxicity of these smaller fractions in relation to their specific sources.

Keywords: Ambient particulates; PM_{10}; Traffic; Genotoxicity; Mutagenicity; Cytotoxicity; Radical formation; PAH

Contents

* Corresponding author. Tel.: +31 43 3881091; fax: +31 43 3884146.
E-mail address: t.dekok@grat.unimaas.nl (T.M.C.M. de Kok).

1383-5742/$ – see front matter
doi:10.1016/j.mrrev.2006.07.001

1. Particulate air pollution and traffic

Epidemiological studies have demonstrated that exposure to urban particulate matter (PM) is associated with several adverse health effects. Long-term exposure to high concentrations of PM increases the risk of lung cancer, respiratory diseases and arteriosclerosis, whereas short-term exposure peaks can cause exacerbation of several forms of respiratory diseases, including bronchitis and asthma, as well as changes in heart rate variability [1–3]. The results of epidemiological studies on the relationship between human health and exposure to fine particulates have recently been reviewed and will not be further discussed here [4,5].

The total of ambient particulate air pollution, also indicated as total suspended particulates (TSP), can be subdivided into different size fractions. The larger particles, $>30\,\mu m$, remain suspended for only a relatively short period of time as compared to the smaller size fractions PM_{10} and $PM_{2.5}$, which are PM fractions with an aerodynamic diameter of less than 10 and 2.5 μm, respectively. Particularly $PM_{2.5}$ and the even smaller ultrafine particulates ($<0.1\,\mu m$) penetrate deeply into the alveoli and are thus more likely to induce adverse health effects than larger particles, such as PM_{10} and TSP [6,7]. Furthermore, it is generally acknowledged that specific chemicals present in PM, such as metals or polycyclic aromatic hydrocarbons (PAHs) and their derivatives, determine to a large extent the toxic potency of PM.

Particulate air pollution originates from both anthropogenic sources and natural sources. It contains biological material, organic compounds, hydrocarbons, acid aerosols and metals attached to a carbonaceous core. Traffic intensity is one of the most important determinants of ambient anthropogenic PM concentrations, but its contribution varies between PM size fractions. Based on studies in Copenhagen, the contribution of traffic to fine ($PM_{2.5}$) and coarse particles (PM_{10}–$PM_{2.5}$) has been estimated to be 13%, and 32% respectively [8]. Other studies provide estimates of up to 50% of $PM_{2.5}$ mass concentrations originating from traffic emissions and re-suspension of road dust [9–12]. Comparison of PM levels in several European cities demonstrated a positive correlation between ambient PM_{10} concentrations on the one hand and population and traffic density on the other hand [13]. Recently, Ariola et al. published the first long term sampling data on urban PM1 concentrations (50% of PM10) and elemental composition [14]. Unfortunately, these data were not linked to parameters of traffic intensity. All motorised vehicles operating on fossil fuel combustion emit PM, but diesel engines emit considerably more particulates per vehicle kilometre than gasoline engines. As the latter are far more prevalent, gasoline vehicles may also make a substantial contribution to the overall PM exposure, even if only

a relatively low percentage of engines emits an excess of particulates as a result of poor functioning. Nevertheless, only little is known on the chemical composition and toxicity of gasoline emissions. Virtually all of the particles emitted by fossil fuel-driven engines are smaller than 2.5 μm in diameter, but most of the total mass of these particles consists of particles that are smaller than 0.5 μm [15,16]. Traffic contributes to increased levels of coarse PM through wear of brake linings and tires, or by resuspension of road dust [17,18].

Since traffic is a major source of $PM_{2.5}$ and ultrafine particulates, traffic intensity probably contributes significantly to PM-related health effects. Traffic intensity is also known to influence the physicochemical characteristics of ambient PM, particularly with regard to concentrations of PAH, metals and radical generating capacity [19–27]. PAHs and PAH-derivatives are formed during combustion in fossil fuel-driven engines, whereas metals (including transition metals) originate from car catalysts [26].

Since no threshold for PM-induced adverse health effects has been established, there is a general consensus that ambient PM levels should be reduced as much as possible. Although an overall reduction of ambient concentrations of PM is likely to result in reduction of PM-associated health risks, emission reduction strategies that take chemical and toxicological PM characteristics into account are probably more effective and efficient. In order to develop such environmental health policies, profound knowledge of the relationships between emission sources, physicochemical characteristics and toxicity of PM is needed. Over the last decades, a great effort has been made to study the adverse health effects of PM using many different research designs and approaches, including large epidemiological studies and animal experiments. In this review, we summarise, compare and evaluate the results from studies that used *in vitro* toxicological assays to establish mutagenicity, cytotoxicity and DNA-reactivity in relation to the chemical composition of PM and specific PM size fractions. We specifically selected those studies that examined the chemical composition and toxicity of ambient PM in relation to traffic intensity at the sample sites, or that compared PM characteristics between samples from areas with low and high levels of urbanisation.

2. Methodological approach

In Pubmed, we searched for publications on ambient PM, PM_{10}, $PM_{2.5}$, particulates or fine dust in combinations with the search terms traffic, diesel, mutagenicity, cytotoxicity, DNA-reactivity, adducts, oxidative damage, radical formation, metals, elemental composition, PAH, nitro-PAH, quinones and semi-quinones. As we aimed to review the literature on ambient PM samples specifically, results from studies evaluating toxicity and chemical composition of diesel exhaust particles (DEP), or other PM generated at standardised or stationary conditions are not included in this review.

3. Chemical characteristics and radical generating capacity of PM

Ambient PM contains biological material, organic compounds, hydrocarbons, acid aerosols and metals absorbed or attached to a carbonaceous core. The TSP and PM_{10} fractions consist primarily of crustal materials, sea salt and biological factors (including bacteria and pollen) and are generated by mechanical processes rather than combustion. On the other hand, $PM_{2.5}$ and ultra fine particulates are predominantly produced by combustion processes and consist primarily of metals, hydrocarbons and secondary particles formed by chemical reactions with gaseous compounds in the atmosphere [6]. The mechanisms of secondary organic aerosol formation from reactions of n-alkanes with OH radicals in the presence of NOx are complex and the types of products formed depend on several combustion conditions and meteorologic or atmospheric features [28,29]. Studies on atmospheric transformation processes in smog chambers have demonstrated that such reactions occurring in the atmosphere can have a major influence on the overall genotoxic burden of ambient air. The smallest particles tend to be those formed by combustion processes and by gas-to-particle conversions. As a result, their composition is complex and generally includes sulfates, nitrates and organics, particularly polar oxidized organics [30]. Claxton et al. [31] recently reviewed different classes of ambient air pollutants, including hydrocarbons, organometallic compounds, oxygen-, nitrogen-, sulphur and halogen-containing compounds. Among the hydrocarbons originating from combustion processes, there are several known carcinogenic PAH, such as benzo(*a*)anthracene, benzo(*k*)fluoranthene, benzo(*a*)-pyrene, benzo(*b*)fluoranthene, indeno(1,2,3-*cd*)pyrene and dibenzo(*ah*)anthracene. Furthermore, several directly mutagenic mono- and di-nitro PAH derivatives have been identified in extracts of primary combustion-generated particles collected from diesel soot, automobile exhaust, coal fly ash, wood smoke as well as in polluted ambient air [30,32,33]. A number of specific

nitro PAH, such as 1-nitropyrene, 3-nitrofluoranthene and several dinitropyrenes, have a very strong direct mutagenic potential [34], and particularly the levels of 1-nitropyrene are inversely correlated with particle size [35]. It has been suggested that sub-micron ambient particles may have a relatively high PAH content because soot from combustion sources consists primarily of fine particles with high PAH content, and also because smaller particles have a relatively high surface area for PAH adsorption [36,37]. Significantly higher PAH levels were indeed found in ultrafine particles (<0.1 μm) as compared to fine (<2.5 μm) and coarse (2.5–10 μm) PM by Li et al. [38]. This study showed no differences in PAH content of fine and coarse PM sampled at an urban site (Los Angeles) as compared to PM sampled 45 km downwind. However, the PAH content of ultrafine particles at the urban site was significantly higher than at the downwind site. In one of our previous studies, we reported that the $PM_{2.5}$ fraction contains higher levels of total PAHs and carcinogenic PAHs as compared to PM_{10} and TSP [39]. Other studies have clearly demonstrated the importance of diesel emissions as well as differences between urban/industrial and rural sites in the total PAH content of PM [3,40–43]. However, these studies did not observe a clear relationship between the PAH content of PM and local differences in traffic intensity.

Transition metals, such as cobalt (Co), cupper (Cu), iron (Fe), manganese (Mn), nickel (Ni), vanadium (V) and titanium (Ti), contribute to the oxidative capacity of PM [44,45]. Therefore, the proportion of PM originating from traffic emissions is likely to influence the toxicity of PM in terms of radical generating capacity and thereby also its ability to induce cell damage in the lung.

3.1. PM-related formation of reactive oxygen species

After inhalation, PM deposited in the lung may stimulate the formation of reactive oxygen species (ROS), such as hydroxyl and superoxide anion radicals. These ROS can be either directly derived from PM or endogenously produced by chemical components of PM, such as transition metals [46] and quinone structures that undergo redox cycling [47]. Although antioxidants, like ascorbate, urate and glutathione, are present in epithelial lining fluid (ELF) and may act as a first line of oxidant defence, particularly ascorbate may actually enhance particle-derived hydroxyl radical formation. This may occur either when ROS are generated via quinoid redox cycling [47] or by action of transition metals present in PM [48]. Valavanidis et al. demonstrated that redox-active transition metals, redox cycling quinoids and PAHs act synergistically to produce ROS and that particularly ferrous ions in PM play an important role in the generation of hydroxyl radicals [49]. ROS can damage membrane lipids, proteins and DNA, which can result in cell death via either necrotic or apoptotic processes. Furthermore, enhanced ROS formation in the lung is likely to be involved in the activation of transcription factors and the induction of cytokines and chemotactic factors [50]. Via these mechanisms, continuous exposure of the lung to PM-induced ROS formation can cause pulmonary inflammation and eventually cause and/or aggravate impairment of lung development and lung diseases like COPD, cystic fibrosis and asthma [51–53].

3.2. Assessment of the radical generating capacity of PM

Oxygen radicals cannot be detected directly because of their short half-lifes, and therefore several alternative methods have been developed. In one type of methods, a molecular probe reacts with the radical species and forms a stable product that can be analysed with a variety of analytical methods, *e.g.* spectrophotometric analysis of thiobarbituric-acid reactive substances (TBARS) [54] or the dithiothreitol (DTT) assay [38]. Other methods are based on biological indicators to assess the formation of ROS by PM, such as the induction of strand breaks in ϕX174 RF plasmid DNA [47,55] or the formation of oxidised DNA-bases like 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) [56]. It should be noted that these indirect measurements do not always correlate well with more direct free radical measurements, indicating that such data should be interpreted as indicators of DNA-reactivity rather than as measurements of radical generating capacity [39]. A third type of methods, is based on the detection of free radicals by electron spin/paramagnetic resonance (ESR/EPR) spectroscopy in combination with spin trapping compounds. Spin trapping agents react in a more or less selective manner with a specific type of radical, which offers the advantage that the PM-derived oxygen radical species can be identified. In Table 1 an overview is presented of ESR/EPR spectroscopy techniques that have been used for the assessment of PM-derived radical formation. The spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) is frequently used to detect PM-related hydroxyl ($OH^{\bullet}$) and superoxide radical anion (O_2^{-}) formation. Although both hydroxyl and superoxide anion radicals produce the same DMPO signal,

Table 1
ESR/EPR methods for detection of PM-derived radicals and associated toxicological effects

Study	PM fraction	Extraction solvent	Solvent	Spin trap	Radical type	Toxicological effect
Dellinger et al. [47]	$PM_{2.5}$	None	None	None	Semiquinone	DNA strand breaks Meyloid leukemia/lung epithelial
Shi et al. [58]	TSP, PM_{10}, PM_1	Water	PBS	DMPO	Hydroxyl	None investigated
Shi et al. [61]	$PM_{10-2.5}$, $PM_{<2.5}$	Water	PBS	DMPO	Hydroxyl	8-OxodG DNA/Lung epithelial
Schaumann et al. [62]	$PM_{2.5}$	Water	PBS	DMPO	Hydroxyl	BAL: increased monocytes
Baulig et al. [60]	$PM_{2.5}$	Water	PBS	DMPO	Hydroxyl	DCF fluorescence Lung epithelial cells (not correlated)
Salonen et al. [63]	PM_{10}	Water	PBS	DMPO	Hydroxyl	8-OxodG (not correlated)
Valavanidis et al. [49]	TSP	None Water DMSO	None Carbonate buffer/DMSO	None DMPO	Semiquinone, hydroxyl and superoxide anion	None investigated
De Kok et al. [39]	TSP, PM_{10}, $PM_{2.5}$	None	None	DMPO	Hydroxyl and superoxide anion	Mutagenicity (TA98) (not correlated), DNA adducts, 8-oxodG
Briedé et al. [57]	TSP, PM_{10}, $PM_{2.5}$	None	None	DMPO	Hydroxyl and superoxide anion	None investigated
Valavanidis et al. [59]	TSP, PM_{10}, $PM_{2.5}$	Water	Aqueous H_2O_2 solution	MNP	dG radical species	8-oxodG

the specific oxygen species can be identified by using the enzymes superoxide dismutase and catalase [57]. Using coated model particles, it has clearly been shown that H_2O_2-induced oxygen radical formation is catalysed by several transition metals [58]. Furthermore, PM suspensions were shown to produce in presence of H_2O_2 radical species of dG, which were spin-trapped by 2-methyl-2-nitroso-propane (MNP) [59]. Also, stable forms of semiquinone radical intermediates that are involved in ROS formation through quinoid redox cycling have be detected directly by ESR without the use of spintrapping agents [47,49].

3.3. Traffic intensity and radical generating capacity of PM

Only a relatively small number of studies have investigated the relationship between ambient PM size fractions, traffic intensity and radical formation as assessed with ESR/EPR spectroscopy. Two of these studies showed the highest ROS generating capacity of PM in the smallest size fractions that were investigated (PM_1 or $PM_{2.5}$) [39,58]. The ROS generating capacity of kerbside $PM_{2.5}$ was higher than that of $PM_{2.5}$ from an urban background location that was not in close proximity of a heavy traffic road [60]. This suggests that traffic-related PM is likely to possess a relatively high capacity to generate radicals compared to PM from other sources. Another study, in which samples were taken at various locations within one city, no correlations were found between ROS generating capacity and traffic intensity [39]. This study also showed a positive correlation between PAH concentrations and radical generating capacity of PM_{10} and $PM_{2.5}$. No correlations were found between radical formation and metal or transition metal concentrations or the interaction between PAHs and metal concentrations. This suggests that the radical generating capacity of PM is predominantly determined by the presence of PAH or the concentration of components that correlate strongly with PAH content.

It should be noted that the application of different analytical pre-treatment methods in the different studies may affect the extent of radical formation. In most studies, organic or polar solvents were applied to extract PM from the filters [49,58,60–63]. A recent study showed that the semiquinone radical species involved in quinoid recycling cannot be extracted from diesel exhaust particles, which implies that the ROS generating capacity of PM-containing extracts may not reflect the actual exposure to ROS after inhalation of PM [64]. Although the radical generating capacity established in

PM extracts may correlate well with *in vitro* biological activity of these extracts, such as mutagenicity and DNA reactivity, as will be discussed in this paper, measurement of radical formation without extraction is likely to yield a better estimate of exposure.

3.4. Conclusions

In summary, only a limited number of studies have quantified the radical generating capacity of ambient and traffic-related PM. The results from these studies indicate that smaller PM size fractions show the highest levels of radical formation and that the PAH concentration seems to be a more important determinant of the radical generating capacity than the total metal or transition metal level. Although it seems likely that the relatively small PM size fractions originating from traffic emissions contribute significantly to the radical generating capacity of ambient PM, this remains to be established. Further standardisation of methods is needed in order to enable comparison of results from different studies in the future.

4. Mutagenicity

4.1. Mutagenicity of PM

The Ames mutagenicity test is a short-term *in vitro* assay that has frequently been used to establish the mutagenicity of ambient and indoor PM [31,65,66]. Short-term mutagenicity assays in general can detect the genotoxic effect of either single chemical and physical agents or heterogeneous mixtures, such as ambient PM. As some PAH and other organic molecules require metabolic activation in order to exert their mutagenic activity, metabolising enzymes from rat liver microsomes or S9, are often added in order to evaluate the overall mutagenic capacity of PM. Other compounds that are formed during combustion processes, such as nitro-PAH originating from diesel engines [67], are direct-acting mutagens and do not require metabolic activation. Due to its high sensitivity and specificity, the *Salmonella typhimurium* tester strain TA98 has frequently been used to characterise organic extracts of airborne particles. Using a microsuspension procedure and preincubation with concentrated extracts before plating, a considerable enhancement of the sensitivity of the standard plate assay has been achieved [68,69]. The enhanced sensitivity of a factor 5–10 compared to the standard plate assay has the advantage that the mutagenic potential of fractionated organic extracts of ambient air particulate matter can be assessed.

Mutagenicity data from different studies are not always easy to compare because several methods for PM extraction have been used. Dichloromethane, toluene, acetone and *n*-hexane, are frequently used to obtain organic extracts of PM and also various extraction methods have been applied, including soxhlet extraction, ultrasonication or brushing of PM from the filter into a solvent. It has been demonstrated that different extraction procedures applied to the same PM samples result in differences in mutagenic activities [70] and the impact of using different single or multiple solvent systems has previously been reviewed by Claxton et al. [31]. In some studies, the extracts are subdivided into fractions with different polarity or pH prior to resuspension in DMSO, methanol, or KOH/hexane. Additionally, several strains of *Salmonella typhimurium* have been used, which differ in their sensitivity for specific mutagenic compounds. As a result, studies using different bacterial strains cannot always be compared directly with each other. It has been demonstrated that, depending on the strain used, the effect of exogenous activation systems can be different in the microsuspension assay, as compared to the standard plate assay. These findings indicate that the study protocols should be carefully selected based upon the specific purpose of the study, and taking the characteristics of the mutagens that are expected to be present into account [71].

Table 2 gives a summary of recent studies in which the mutagenicity of ambient and traffic-related PM was investigated. The examined PM fraction, the extraction method, the extraction solvent, the solvent used in the test, the bacterial strains and the effect of metabolic activation by rat liver S9 are specified for each study. The majority of studies that have used TA98 and TA100, showed an increase in mutagenicity of PM in the Ames test upon addition of S9 [6,67–69,72–77]. Three studies showed that the S9-mediated mutagenicity was higher in PM samples taken during winter episodes than in samples taken during summer [72,75,77]. Two studies showed reduced mutagenicity after metabolic activation, using *Salmonella* strain TA98 and DMSO as solvent [39,78]. Although the study of Bronzetti et al. showed some day-to-day variations in the influence of S9 on the mutagenic activity in both strain TA98 and TA100, which suggests that the composition of PM can vary considerably, metabolic activation generally resulted in increased mutagenicity [73]. In studies using the YG1041 strain, S9 reduced the mutagenicity of PM [67,68,72]. As a result of the relatively high activity of nitroreductase and *O*-acetyltransferase in this specific strain, these bacteria are more sensitive to the mutagenic effect of nitro-PAH. The highest direct

Table 2
Studies on mutagenicity of traffic-related PM

Study	Investigated PM size fraction	Extraction	Resuspension solvent	*Salmonella* strain	Effect S9
Sato et al. [77]	TSP	Methylene chloride (son.)	Methylene chloride	TA98 TA98NR TA100 TA98/1,8-DNP6	No effect No S9 used ↑ No S9 used
Bronzetti et al. [73]	TSP	DCM, methanol	DMSO (extracts mixed together)	TA98, TA100	↑ (Although day-to-day variation)
Kuo et al. [83]	TSP	Acetone (shak.)	Acetone	TA98	Not reported
Delgado-Rodriguez et al. [74]	TSP, PM_{10}	Methanol (sox.)	DMSO	TA98	↑
De Martinis et al. [69]	PM_{10}	DCM (sox.) (fract.) Acetone (sox.) (fract.)	DCM Acetone	TA98	↑ ↓
Cerná et al. [68]	PM_{10}	DCM (sox.) (fract.)	DMSO	TA98 YG1041	↑ ↓
Nielsen et al. [76]	TSP	DCM, acetone (ultras.)	DMSO	TA98 TA98NR	↑ No S9 used
Cerná et al. [67]	PM_{10}	DCM (sox.)	DMSO	TA98 YG1041	↑ ↓
Müller et al. [84]	TSP	*n*-Hexane (sox.)	DMSO	TA98, TA100	Only tests with S9 were performed
Buschini et al. [70]	TSP, PM_{10}, $PM_{2.5}$	Acetone, toluene (sox.)	DMSO	TA98, TA100	Variable, depending on strain, sample and extraction solvent
Massolo et al. [43]	$PM_{10–3}$, $PM_{3–0.49}$, $PM_{<0.49}$	Hexane (AES)	DMSO	TA98	Unknown
Wu et al. [27]	TSP, $PM_{2.5}$, $PM_{2.5–10}$	Not specified	Not specified	TA98 TA100	↑ ↑ (although seasonal variation)
Vinitket-Kumnuen et al. [7]	PM_{10}, $PM_{2.5}$	DCM (son.)	DMSO	TA100	↑
Zhao et al. [78]	TSP	DCM (son.)	DMSO	TA98 TA100	↓ No S9 used
Binkova et al. [72]	PM_{10}	DCM (unknown)	DMSO	TA98 YG1041	↑ ↓
Ducatti et al. [82]	TSP	Cyclohexane (son.) (fract.) DCM (son.) (fract.)	Cyclohexane/DCM	TA98, TA98NR, TA98/1,8-DNP6	No S9 used
Du Four et al. [75]	PM_{10}	Acetone	DMSO	TA98	↑
De Kok et al. [39]	TSP, PM_{10}, $PM_{2.5}$	DCM (sox.)	DMSO	TA98	↓

DMSO, dimethyl sulfoxide; DCM, dichloromethane; sox., soxhlet extraction; (ultra)son., (ultra)sonication; fract., fractionation according to pH and polarity; shak., use of a shaker; AES, accelerated extraction system (14 mPa, 150 °C).

mutagenicity in this strain was indeed found in the polar subfractions, which are most likely to contain nitro-PAH and nitro-PAH derivatives [67].

4.2. Mutagenicity of PAH and nitro-PAH in PM

Nitro-PAH are formed in combustion processes of organic chemicals and through atmospheric reactions of PAH with nitrogen oxides. They are among the most potent directly acting bacterial mutagens [67]. Diesel engines emit at least ten times more nitro-PAH as compared to gasoline engines [40]. Therefore, the combination of high traffic intensity and a high percentage of diesel engines and heavy duty vehicles is likely to have an important impact on the concentration of nitro-PAH in particulate air pollution and thereby also to enhance the direct mutagenicity of PM [40].

In order to determine the influence of nitro-PAH on PM mutagenicity, two approaches have been used. One approach was to divide PM extracts into more and less polar fractions, and the other approach was to use specific bacterial strains. Wanatabe et al. developed a series of YGs strains with a higher activity of nitroreductase (pYG216) and of *O*-acetyltransferase (pYG219) [79,80]. As a result, these strains, such as YG1041, are more sensitive to nitro-PAH induced mutagenicity. In contrast, NR (*e.g.* TA98NR) and 1,8-DNP_6 strains bear a deficiency in, respectively, nitroreducase and *O*-acetyltransferase, enabling the detection of mono- and dinitroarenes, respectively [41,81] These strains are relatively insensitive to the mutagenic effect of nitro-PAH [82]. The difference in mutagenicity between bacterial strains of the TA98 type on one hand and TA98NR or TA98/1,8-DNP_6 strains on the other hand is therefore indicative of the mutagenic effect of nitro-PAH. It should be emphasized that strain selection can bias the interpretation of the results. For example, if only a YG strain is used, without other strains, the results will always tend to indicate that nitro-PAH containing fractions are the most mutagenic. This may, however, be a misinterpretation because other classes of compounds are less responsive in these strains and their effects are therefore underestimated in the assessment [31].

In Table 3, an overview is presented of studies on the relationship between concentrations of nitro-PAH and carcinogenic PAH and mutagenicity of specific PM size fractions. In total, six studies focussed on the assessment of mutagenicity of nitro-PAH by using specific nitro-PAH sensitive or insensitive bacterial strains and found a significantly positive correlation in PM_{10} [67,68,72] and in TSP [76,77,82]. Two of these studies observed also a positive correlation between mutagenicity and the concentration of carcinogenic PAH in PM_{10} [68,72]. Additionally, two out of four studies reported a relatively high direct mutagenicity in PM_{10} [75] or different PM fractions [39], and at the same time found correlations between mutagenicity and levels of carcinogenic PAH. Du Four et al. [75] were able to predict direct mutagenicity based on concentrations of 16 indirect-acting PAHs (using calculation models), thereby indicating that concentrations of these PAH are strongly correlated with concentrations of direct-acting mutagens like oxy- and nitro-PAHs. In the studies of Muller et al. [84], Kuo et al. [83], and Massolo et al. [43] only positive correlations between S9-mediated, but not direct mutagenicity, and levels of carcinogenic PAH were observed. As already shown in Table 2, several studies observed a decreased mutagenicity upon the administration of S9 as metabolising system to either nitro-PAH sensitive [67,68] or normal *Salmonella* strains [39,69,78], which indicates the relatively high importance of direct-acting mutagens. It has repeatedly been shown that direct-acting mutagenicity is more pronounced in PM sampled during summer periods than during the winter [68,78].

4.3. Traffic intensity and mutagenicity of PM

In only a limited number of studies the relationship between traffic intensity and mutagenicity was investigated [39,73,82,85]. Ducatti and Vargas demonstrated a positive effect of automotive vehicle density on mutagenicity of PM [82]. Bronzetti et al. observed higher mutagenicity of TSP in an area with high traffic intensity as compared to that of TSP from an area with low traffic intensity. In this study, it was also observed that the concentrations of heavy metals and chemicals like benzene, toluene, nitrogen dioxide and carbon oxide correlated positively to traffic intensity [73].

Vargas et al. evaluated the mutagenicity of TSP, using the bacterial strains TA98NR and TA98/1,8-DNP6, various extraction solvents, in several fractions of TSP that varied in polarity of the solvent used to extract the samples. They observed a positive relationship between mutagenicity and PAH concentration, as well as nitro-PAH concentration [85]. Also, the analysis of specific traffic-related volatile organic components in PM confirmed the positive association of these compounds with traffic intensity. On the other hand, we investigated the relationship between traffic intensity and mutagenicity, as well as the relationship between mutagenicity and several physical and chemical parameters of PM, but found no clear correlations between traffic intensity and mutagenicity per

Table 3
Studies on the relationship between PM-mutagenicity and levels of nitro-PAH and carcinogenic PAH

Study	PM size fractions investigated	Nitro-PAH mutagenicity as indicated by specific bacterial strains			Studies indicating direct mutagenicity of nitro-PAH (*TA98*)	Positive correlation between mutagenicity and carcinogenic PAH concentrations (*strain*)	
		YG1041	TA98NR	TA98/1,8-DNP6		Direct	S9-mediated
Sato et al. [77]	TSP		TSP	TSP			
Kuo et al. [83]	TSP						TSP
Nielsen et al. [76]	TSP		TSP (not sign.)				
De Martinis et al. [69]	PM_{10}				PM_{10}[f]		
Cerná et al. [68]	PM_{10}	PM_{10}				PM_{10} (TA98, YG1041)[e]	PM_{10} (TA98, YG1041)[e]
Cerná et al. [67]	PM_{10}	PM_{10}			PM_{10}[d]		
Müller et al. [84]	TSP						TSP
Massolo et al. [43]	PM_{10-3}, $PM_{3-0.49}$, $PM_{<0.49}$						PM_{10-3}, $PM_{3-0.49}$, $PM_{<0.49}$
Zhao et al. [78]	TSP				TSP, organic acid fraction[a,b]		
Binkova et al. [72]	PM_{10}	PM_{10}[a]				PM_{10} (YG1041)	PM_{10} (TA98, YG1041)
Ducatti et al. [82]	TSP		TSP	TSP			
Du Four et al. [75]	PM_{10}				PM_{10}	PM_{10}[c]	
De Kok et al. [39]	TSP, PM_{10}, $PM_{2.5}$				TSP, PM_{10}, $PM_{2.5}$[a]	TSP	TSP, $PM_{2.5}$

The PM size fractions for which the correlation is found are indicated.
[a] Reduced mutagenicity after addition of S9.
[b] Particularly in PM sampled during the summer season.
[c] Based op PLS model analysis (partial least-squares).
[d] High direct mutagenicity in slightly polar subfractions containing nitro-PAHs.
[e] Only in plate incorporation assay, not in microsuspension assay.
[f] Reduced mutagenicity of acetone (not DCM) extract after addition of S9; highest mutagenicity in fractions within the mid-range of polarity.

microgram of PM [39]. It should be emphasized that the mutagenicity of ambient PM is likely to be caused by a combination of several hundreds of compounds from different chemical classes, and that the carcinogenic PAHs, which have been studied most extensively, do not account for the major part of the mutagenic activity [31].

Overall, this implies that the mutagenicity of outdoor PM increases as a result of traffic-related PM emissions, although a relationship between traffic intensity and the mutagenic potential of the PM itself has not been established unequivocally.

4.4. Conclusions

Many different methods have been used to establish the mutagenicity of ambient and traffic-related PM. In general, the mutagenicity of PM has been found to be higher upon metabolic activation by S9. This was particularly the case in mutagenicity tests on *Salmonella* strain TA98. Studies on bacterial strains that are especially sensitive for directly mutagenic effects of nitro-PAH, and using procedures to extract and fractionate nitro-PAH, have indicated that these compounds are important particle-bound contributors to the mutagenicity in bacteria. This underlines the great influence of diesel engine emissions and heavy-duty traffic on the genotoxic potential of ambient PM. Only a limited number of studies have examined the relationship between traffic intensity and PM-induced mutagenicity, and most of these studies have found a positive association. The limited data that are available on the mutagenicity of various PM size fractions indicate that smaller particles possess the highest mutagenic potential, but additional data are needed to substantiate these findings. Although evaluation of the mutagenic activity of ambient PM allows us to characterise the air quality in urban and industrial areas and provides information on potential health risks of exposure, further studies on fractionated PM samples and chemical identification of the mutagenic compounds are needed. Only when such data are available from studies using well-standardised extraction procedures and methodologies, the mutagenic risks of exposure to traffic-related PM can be reliably assessed.

5. Cytotoxicity

5.1. Cytotoxicity of different PM size fractions

Several different methods have been used to establish the cytotoxicity of PM. The most important distinctions between these tests are the use of various cell types and the time of incubation, varying from 4 to 72 h. Also, different PM fractions and extraction procedures have been used. These differences and variables should be taken into account when results from different studies are compared.

Table 4 shows the results of the most recent studies on the cytotoxicity of PM. Three out of seven studies showed positive dose–response curves of cytotoxicity induced by increasing amounts of PM, for various PM size fractions [39,84,86]. Hsiao et al. [86] and Tong et al. [87] showed that $PM_{2.5}$ had the highest cytotoxicity and also the study of Massolo et al. showed the highest cytotoxicity for the smallest PM fraction ($PM_{<0.49}$) [43]. Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT)-test in Human Embryonic Lung cells and different PM size fractions, we found the highest cytotoxicity for $PM_{2.5}$, although cytotoxicity was not observed for all $PM_{2.5}$ samples in the concentration range that was tested (unpublished results from our earlier study [39]). These data also confirm the tendency that smaller PM size fractions are more cytotoxic than larger ones.

5.2. Cytotoxicity and physico-chemical charateristics of PM

Some studies on cytotoxicity showed a positive correlation between total PAH and carcinogenic PAH content of TSP and cytotoxicity [84], PM_{10} [72] or PM size fraction <0.95 μm [43], whereas others observed no such correlations [43,72,84,86,88]. The study of Massolo et al. showed higher cytotoxicity in the relatively smaller PM size fractions ($PM_{<1.5}$) [43]. Tong et al. found a positive relationship between the concentration of transition metals (chromium, manganese and iron) in and the cytotoxicity of PM. They suggested that soluble transition metals may be main determinants of PM-induced cytotoxicity [87]. In addition, we found a positive correlation between the cytotoxicity of TSP and the sum of the concentrations of transition metals (unpublished results).

5.3. Conclusions

Data on cytotoxicity of PM are limited, but generally indicate that smaller PM size fractions are generally more cytotoxic than larger ones. Positive associations between cytotoxicity and concentrations of PAH, metals and transition metals have been reported. The effect of metabolic activation by S9 on cytotoxicity has not been reported. Additional research is needed to establish the

Table 4
Studies on the cytotoxicity of traffic-related PM

Study	Investigated PM size fraction	PM size fraction showing cytotoxicity	Extraction solvent (method)	Resuspension solvent	Cell system	Incubation period (h)	Correlations with carcinogenic PAH content	Correlations with metal content
Hsiao et al. [86]	PM_{10}, $PM_{2.5}$	PM_{10}, $PM_{2.5}$	DCM (ultrason.)	DMSO	F2408 (fibroblasts)	4	–	–
Müller et al. [84]	TSP	TSP	*n*-Hexane (sox.)	DMSO/aqueous	Tetrahymena pyriformis	24	Positive	–
Alfaro-Moreno et al. [88]	PM_{10}	PM_{10}	None (dry ultra-son.)*	Not reported	Balb-c, J774A.1, A549	72	–	–
Tong et al. [87]	$PM_{>2.5}$, $PM_{2.5}$	$PM_{>2.5}$, $PM_{2.5}$	Acidification with fuming sulfuric acid	Distilled water	Chinese Hamster Lung cells	4	None	Fe, Cr, Mn
Massolo et al. [43]	PM_{10-3}, $PM_{3-0.49}$, $PM_{<0.49}$	PM_{10-3}, $PM_{3-0.49}$, $PM_{<0.49}$	Hexane (AES)	DMSO	Tetrahymena pyriformis	24	Positive	–
Binkova et al. [72]	PM_{10}	PM_{10}	DCM (method not specified)	DMSO	Chick embryotoxicity screening test (CHEST)	72	Positive	–
De Kok et al. [39]	TSP, PM_{10}, $PM_{2.5}$	TSP, PM_{10}, $PM_{2.5}$	DCM (sox.)	DMSO	Human Embryonic Lung cells	48	None	Sum of transition metals (only TSP)

DMSO, dimethyl sulfoxide; DCM, dichloromethane; sox., soxhlet extraction; (ultra)son., (ultra)sonication; fract., fractionation according to pH and polarity; shak., use of a shaker; AES, accelerated extraction system (14 mPa, 150 °C); –, not investigated.
* Dry sonication and recovery of particles from filter by smoothly sweeping them into an endotoxin-free flask with a brush.

relationship between traffic emissions, chemical composition and cytotoxicity of PM.

6. DNA-reactivity

6.1. DNA-reactivity of different PM size fractions

Generally, two types of DNA-reactivity have been studied in order to characterise PM genotoxicity, which either focus on the analysis of DNA-adduct formation or on oxidative DNA damage. The latter can be measured either as induction of DNA strand breaks in for instance the Comet assay or as the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG). The summary presented in Table 5, shows that ten out of eighteen studies demonstrated positive dose–response curves of PM-induced DNA-reactivity [39,61,70,78,86,88–94]. Although most studies did not compare DNA-reactivity of different PM size fractions, four out of six studies showed that the $PM_{2.5}$ or smaller size fractions exerted the highest DNA-reactivity [39,70,86,95], It is remarkable that surrogate Epithelial Lining Fluid (ELF) showed significant amelioration of DNA damage in the study of Greenwell et al. [89], whereas the presence of vitamin C in *in vitro* systems at concentrations as occurring in ELF is known to stimulate radical formation as a result of enhanced redox cycling of PAH [57]. Pre-treatment of HepG2 cells with vitamin C resulted in considerable reduction of DNA breaks and oxidative DNA lesions induced by urban PM_{10} samples from the Czech Republic [94].

Two studies investigated the formation of DNA-adducts in both the presence and absence of S9 [39,72], whereas one of these also investigated the effect of metabolic activation on the induction of oxidative DNA damage [39]. Both studies reported higher DNA-reactivity upon metabolic activation of PM.

6.2. Chemical composition and DNA-reactivity of PM

Several studies investigated both DNA-reactivity in relation to the PAH content of PM. One found a positive correlation between the direct as well as the S9-mediated formation of DNA-adducts on the one hand and the interaction of transition metal content and concentration of total or carcinogenic PAH in $PM_{2.5}$ on the other hand [39]. In this study, also positive correlations were found between direct and indirect DNA-reactivity (formation of adducts as well as breakages) and PAH content and the total level of transition metals, and the radical generating capacity of

Table 5
Studies on DNA-reactivity of traffic-related PM

Study	Investigated PM size fraction	PM size fraction showing DNA-reactivity	Extraction solvent and (method)	Resuspension solvent	DNA-reactivity indicator (method)	(Cell) System	Incubation time (h)	Dose–effect-relation
Gilmore et al. [97]		PM_{10}	Water (son.)	Water	Breakages	ϕX174 RF1 supercoiled phage DNA	8	–
Donaldson et al. [22]		PM_{10}	Water (son.)	Water	Breakages	ϕX174 RF1 supercoiled phage DNA	8	–
Dellinger et al. [96]	$PM_{2.5}$	$PM_{2.5}$	PBS (nutator and vortex)	PBS	Breakages (electrophoresis/ Comet)	ϕX174 supercoiled phage DNA	1	–
						Human K562 myeloid leukaemia cells	1–6	time-response curve
Hsiao et al. [86]	PM_{10}, $PM_{2.5}$	PM_{10}, $PM_{2.5}$	DCM (ultrason.)	DMSO	Breakages (Comet)	Rat embryo fibroblasts	72	–
Dellinger et al. [47]	$PM_{2.5}$	$PM_{2.5}$	PBS (nutator and vortex)	PBS	Breakages (electrophoresis/ Comet-Assay)	ϕX174 supercoiled phage DNA	1	–
							3	–
Buschini et al. [70]	TSP, PM_{10}, $PM_{2.5}$	TSP, PM_{10}, $PM_{2.5}$	Acetone, toluene (sox.)	DMSO	Breakages (Comet-assay)	Leukocytes	1	+
Zhao et al. [78]	TSP	TSP	DCM (son.)	DMSO	Breakages (DNA-repair (unscheduled DNA synthesis)	Rat hepatocytes	3	+
					Chromosomal aberrations (micronuclei assay)	Kun-ming mice bone-marrow cells	6	+
						Human K562 meyeloid leukemia cells or IB3-1 human lung epithelial cells		
Alfaro-Moreno et al. [88]	PM_{10}	PM_{10}	Not used (dry ultrason.)	Unknown	Breakages (Comet)	Balb-c cells	72	+
Greenwell et al. [89]	TSP, PM_{10}, $PM_{2.5}$	PM_{10} more than TSP and $PM_{2.5}$	Water (vortex)	Water	Breakages	ϕX174 supercoiled phage DNA	6	+
Knaapen et al. [98]	TSP	TSP	Water (shaking, sonication)	Water	Oxidative DNA damage (8-oxodG) Comet	Calf thymus DNA (immuno-staining)	1	–
							3	–
Shi et al. [61]	$PM_{10–2.5}$, $PM_{2.5}$	$PM_{10–2.5}$, $PM_{2.5}$	Ultrapure water (agitation)	Ultrapure water	Oxidative DNA damage (8-oxodG)	Calf thymus DNA (immuno-staining)	1,5	+
						A549 (immunocytochemistry) Lung epithelial cells (A549)		–
Uphadyay et al. [90]	TSP	TSP	Water	Water	Breakages (FADU)	Lung epithelial cells (A549)	24	+
Binkova et al. [72]	PM_{10}	PM_{10}	DCM (unknown)	DMSO	Adducts (postlabelling)	Calf thymus DNA +/−S9	4	–
Gabelova et al. [92]	PM_{10}	PM_{10}	DCM	DMSO	Breakages (Comet)	HepG2 cells	2	+
Brits et al. [93]	PM_{10}	PM_{10}	Water or tetrahydrofurane/ hexane (20/80)	DMSO	Breakages (Comet)	Lymphocytes	24	+
					Micronucleus test		44	+
Lazarova et al. [94]	PM_{10}	PM_{10}	DCM	DMSO	Breakages (Comet ± ENDOIII/FPG)	HepG2 cells	24	+
						Caco-2 cells	24	+

De Kok et al. [39]	TSP, PM_{10}, $PM_{2.5}$	TSP, PM_{10}, $PM_{2.5}$	DCM (sox.)	DMSO	Oxidative DNA damage (8-oxodG; HPLC-ECD) DNA-Adducts (postlabeling)	Salmon testis DNA +/− S9	4	−
Healey et al. [95]	Size fractionated PM_{10}	Most damage induced by smaller fractions	Water (vortex or brushing)	PBS	Breakages (electrophoresis/ Comet)	Plasmid (pBR322)	5	−
						Lung epithelial cells (A549)	24	−
Karlsson et al. [91]	PM_{10}	PM_{10}	Water	Water	Breakages (Comet) Oxidative DNA damage (8-oxodG HPLC-ECD)	Lung epithelial cells (A549)	4	+

DMSO, dimethyl sulfoxide; DCM, dichloromethane; PBS, phosphate buffered saline; sox., soxhlet extraction; (ultra)son., (ultra)sonication; fract., fractionation according to pH and polarity; shak., use of a shaker; AES, accelerated extraction system (14 mPa, 150 °C); FADU, fluorimetric analysis of DNA unwinding.

various PM size fractions [39]. Two other studies showed a positive relationship between the concentration of carcinogenic PAH and DNA-adduct formation in the PM_{10} size fraction [72] or total PAH levels and genotoxicity as assessed by the comet-assay and the micronucleus test [93]. However, the authors of the latter study indicate that the chemical analyses were not predictive of the extent of genotoxic activity of the samples. Although PM_{10} samples collected in three European cities during the winter showed both higher total PAH levels and higher genotoxic potential as compared to samples collected in the summer, no correlations between PAH levels and genotoxicity have been calculated in this specific study [92].

A relationship between DNA-breakages and the radical generating capacity of $PM_{2.5}$, as established by ESR spectroscopy, was reported in two different studies. Dellinger et al. measured high concentrations of semiquinone radicals in the $PM_{2.5}$ size fraction, which can result in the formation of several types of oxygen free radicals that are known to induce DNA strand breaks [47,96]. We found a positive correlation between radical generating capacity of various PM size fractions and both direct and indirect DNA-reactivity (formation of adducts as well as breakages) [39].

Other studies found indications for a role of PM-bound iron or other transition metals in DNA-reactivity in supercoiled plasmid DNA via formation of hydroxyl radicals and the induction of 8-oxodG [22,90,97,98]. Based on the observation that in human epithelial lung cells, the DNA damaging capacity (comet assay) of the particle suspension was stronger than that of the corresponding particle-free filtrates, Knaapen et al. concluded that apart from ROS generation, a direct 'particle' effect contributes to the genotoxic potential of ambient PM [98]. Healey et al. reported that both the organic and inorganic components of several size-fractionated samples of urban PM contributed to the induction of DNA damage, but that the majority (approximately 75%) was induced by the organic extract [95].

6.3. Relationship between traffic intensity and DNA-reactivity of PM

Most studies on DNA-reactivity of ambient PM, do not provide detailed information on traffic intensity at sampling locations or make comparisons between high and low traffic sample sites. The limited data available show no indications of a relationship between traffic intensity and DNA-adduct formation or the induction of oxidative DNA damage [39]. Furthermore, PM from

locations with a relatively high intensity of heavy-duty vehicles did not induce higher levels of DNA-adducts than that from locations with a lower intensity of heavy-duty vehicles. This seems to be in agreement with the findings from another study, in which no differences in PAH–DNA-adduct levels were found in human bronchial epithelial cells when exposed to either diesel or gasoline PM extracts [99], despite the fact that the concentration of PAHs was higher in the diesel PM extracts. Although Karlsson et al. did not study genotoxicity of PM in relation to motorised traffic intensity, their finding that subway particles are more genotoxic than particles sampled in a busy urban street (Stockholm) is quite striking, particularly in view of the high number of people exposed on a daily basis to relatively high PM concentrations in subway systems [91]. Analysis of the elemental composition showed that iron (Fe_3O_4) was a dominating metal in the subway PM. As the addition of a metal chelator resulted in the reduced induction of oxidative DNA damage, it appears that indeed the presence redox active metals contribute to the genotoxicity of this specific type of PM.

6.4. Conclusions

Most research on DNA-reactivity of PM has been focussed on DNA-breakages without metabolic activation. Generally, the smaller PM fractions (*i.e.* $PM_{2.5}$) showed the highest DNA-reactivity. Positive relationships were found between radical formation (superoxides, hydroxyperoxide, hydroxyl radicals, semiquinone radicals) and the induction of DNA-breakages. Furthermore, DNA-reactivity correlated positively with concentrations of total PAH, carcinogenic PAH, transition metals, and with the interaction between PAH and transition metals. These findings support the hypothesis that the radical generating capacity of PM is influenced by its chemical composition and that radical formation by PM is directly related to the induction of DNA strand breaks.

The formation of DNA-adducts upon PM exposure and the effect of metabolic activation on DNA-reactivity have not been studied extensively. The results of the few studies so far showed higher DNA-reactivity (both DNA-adduct formation and DNA-breakages) after metabolic activation. The relationship between traffic intensity and DNA-reactivity of PM has not been studied in great detail either, and the limited data available show no clear relationship between the two.

7. Chemical composition and toxicological effects of specific PM size fractions

7.1. Chemical composition of specific PM size fractions

Information on particle size distribution is essential to understand the potential health effects of PM exposure. Although there is no strict relation between size distribution and sources of emission, it has been demonstrated that different PM size fractions originate from different types of PM sources and that the chemical speciation and bioavailability of PM components also depend on the source of emission. In order to evaluate differences in chemical composition and toxicological characteristics of specific PM size fractions, we summarised the results of relevant studies in Table 6. This overview shows that the concentration of PAH is generally higher in the smaller PM size fractions [38,39,43]. This is likely to be the consequence of the fact that PAH are formed during combustion processes, which are known to contribute particularly to ambient concentrations of fine and ultrafine particulates. Furthermore, smaller particles have a relatively large surface for PAH adsorption [36,37]. With regard to traffic, PAH emission profiles vary among engine types. Petrol engines emit the greatest amount of high molecular weight PAH, such as benzo[*a*]pyrene and dibenzo[*a*,*h*]anthracene, whereas diesel engines are the principal source of low molecular weight PAHs [100–102]. With regard to the metal content, Table 6 shows that no particular PM size fraction has consistently higher total or transition metal content, although smaller particles seem to contain more transition metals than larger ones. There are no studies that indicate a relationship between traffic intensity and metal content of any specific PM size fraction.

As shown in Table 6, particles smaller than $PM_{2.5}$ tend to possess a higher radical generating capacity than larger ones [38,61], although differences found between $PM_{2.5}$, PM_{10} and TSP were not always consistent. The radical generating capacity of the PM_1 fraction was found to correlate positively with the levels of the transition metals Fe and Cu [61], whereas for the larger fractions no correlations were found with any type of metal. For both $PM_{2.5}$ and PM_{10}, positive correlations between radical generating capacity and PAH content were found [39]. Overall, these data suggest that radical formation in PM fractions larger than PM_1 is predominantly determined by PAH content, whereas the level of transition metals or the interaction between

transition metals and PAH is of more importance in PM size fractions smaller than PM_1. As the number of studies that assessed and compared radical formation in various PM size fractions and its relation with chemical composition is limited, more of these studies are needed to substantiate these conclusions.

7.2. Toxicological effects of specific PM size fractions

The studies summarised in Table 6 show quite consistently higher mutagenicity, DNA reactivity and cytotoxicity in the smaller PM size fractions. Furthermore, the relationships between toxicological effects and chemical composition tend to be stronger in the smaller size fractions. Hsiao et al. investigated $PM_{10–2.5}$ and $PM_{2.5}$ and observed the highest cytotoxic effects in $PM_{2.5}$, which correlated positively with the concentrations of solvent-extractable organic compounds, alkanes and PAH [86]. Tong et al. analysed the cytotoxicity of $PM_{>2.5}$ and $PM_{2.5}$ and found that transition metals, in particular iron, were more easily dissolved from $PM_{2.5}$ than from the $PM_{>2.5}$ fraction, which suggests that soluble transition metals may be the main determinants of PM-cytotoxicity [87]. Massolo et al. observed stronger positive correlations between PAH, including nitro-PAH and carcinogenic PAH, and the mutagenicity and cytotoxicity of the smaller PM fractions ($PM_{<0.95}$) than was the case in larger PM size fractions [43]. Several studies showed that the $PM_{2.5}$ or smaller size fractions exerted the highest DNA-reactivity [39,70,86,95], whereas the study of Shi et al. showed a higher induction of oxidative DNA-damage by the course fraction ($PM_{10–2.5}$) than by $PM_{2.5}$ [61]. In this study DNA-reactivity correlated positively with the radical generating capacity of PM. Samples from Cardiff, UK, showed a higher DNA breaking potency for PM_{10} as compared to both TSP and $PM_{2.5}$ [89].

In addition to the *in vitro* toxicological characteristics of PM reviewed here, animal studies have shown that ultrafine particles produce serious adverse health effects. However, the toxicological and epidemiological data on ultrafine particles are even less complete than for fine and coarse particulates.

7.3. Conclusions

In summary, these data strongly indicate that the highest concentrations of PAH and transition metals are found in the smaller PM size fractions. Concomitantly, these fractions possess the highest radical generating capacity, mutagenic and cytotoxic potential and are the most DNA-reactive. Moreover, correlations between toxicological potential and chemical composition are generally stronger in the smaller size fractions. This implies that particles smaller than PM_{10} are likely to present a greater health hazard than PM_{10} and TSP.

8. Final remarks and conclusions

Epidemiological studies have provided ample evidence of a positive association between PM exposure and the induction of serious adverse health effects. As no threshold for PM induced health effects has been established up to now, a certain level of impact of PM on human health probably has to be accepted. Current policies in the EU might lead to reductions of the emissions of PM in general, but current EU standards are only defined for PM_{10} (annual average of 40 $\mu g/m^3$; daily average of 50 $\mu g/m^3$, permitting 35 exceedances per year). Whether a reduction in ambient PM_{10} concentrations will lead to a proportional reduction of health effects is, however, uncertain. There is considerable heterogeneity in PM characteristics as a result of local and seasonal differences in PM sources, composition and toxicity. Changes in the composition of this complex mixture are likely to modify its health impact. In order to define the most cost-effective policies, the most health-relevant PM fractions will have to be identified, based on their physicochemical and toxicological characteristics, and linked to their source of origin. Because traffic is known to contribute importantly to PM exposure, traffic intensity must be an important determinant of the qualitative characteristics of PM in the atmosphere. In this review, we therefore aimed to summarise the results from those studies that characterised ambient PM toxicity in relation to chemical composition and traffic intensity. This inventory clearly shows that our knowledge on PM characteristics is quite fragmentary and that most studies have focussed on a limited number of characteristics only. Inconsistencies between studies may well be the result of the use of different methods. Nevertheless, this review shows that in general, smaller size fractions have the highest toxicological effects, particularly mutagenicity, cytotoxicity and DNA-reactivity. Furthermore, this review demonstrates that smaller PM fractions generally contain higher concentrations of PAH, semiquinones, metals and transition metals and have a higher radical generating capacity. Several of the reviewed studies also demonstrated stronger relationships between chemical composition (nitro-PAH, solvent-extractable organic compounds,

Table 6
Studies in which the chemical composition and toxicity of specific PM size fractions were compared

Study	PAH content	Metal content	RGC	Mutagenicity	DNA reactivity	Cytotoxicity
Delgado-Rodriguez et al. [74]	–	–	–	$PM_{10} > TSP$	–	–
Hsiao et al. [86]	–	–	–	–	DNA breakage (Comet) $PM_{2.5} > PM_{2.5–10}$	$PM_{2.5} > PM_{2.5–10}$
Buschini et al. [70]	–	–	–	$PM_{2.5} > PM_{10}$, TSP	Breakages (Comet) Inconsistent, but seasonal variation	–
Massolo et al. [43]	$PM_{0.49} > 5$ different larger size fractions[b]	–	–	$PM_{<0.49} > PM_{3–0.49}$	–	$PM_{<0.49} > PM_{3–0.49}$
Tong et al. [87]	–	Soluble transition metals; $PM_{2.5} > PM_{>2.5}$	–	–	–	–
Greenwell et al. [89]	–	–	–	–	DNA breakage (ϕX174) $PM_{10} >$ TSP and $PM_{2.5}$	–
Wu et al. [27]	–	–	–	$PM_{2.5} > PM_{2.5–10}$[a]		–
Fang et al. [103]	–	Fe: $PM_{2.5} > PM_{2.5–10}$, TSP; Mg, Cu and Mn: $TSP > PM_{2.5}$, $PM_{2.5–10}$; Pb, Zn, Cr and Ni: $PM_{2.5} > TSP$, $PM_{2.5–10}$	–	–	–	–
Li et al. [38]	$PM_{<0.1} > PM_{2.5}$, $PM_{2.5–10}$	$PM_{2.5–10} > PM_{2.5}$, $PM_{<0.1}$	DTT, GSH depletion and HO-1 induction: $PM_{<0.1} > PM_{2.5}$, $PM_{2.5–10}$	–	–	–
Shi et al. [58]	–	Fe and Cu: correlation with RGC (PM_1)	ESR: $PM_1 > PM_{2.5}$, PM_{10}	–	–	–
Shi et al. [61]	–	Cu: correlation with oxidative DNA damage	ESR: $PM_{10–2.5} > PM_{2.5}$	–	Oxidative DNA damage (8-oxo-dG) $PM_{10–2.5} > PM_{2.5}$	–
De Kok et al. [39]	$PM_{2.5} > PM_{10}$, TSP	$PM_{2.5} \cong PM_{10} \cong TSP$	ESR: $PM_{2.5} \cong PM_{10} \cong TSP$	$PM_{2.5} > PM_{10}$, TSP Without S9	Adducts (postlabeling) $PM_{2.5} > PM_{10}$, TSP Without S9; 8-oxodG adducts $PM_{2.5} > PM_{10}$ $PM_{2.5} >$ TSP With S9	$TSP \cong PM_{10}$
Healey et al. [95]	–	–	–	–	DNA breakage pBR3224/Comet) Most damage induced by smallest out of 9 size-fractionated PM_{10} samples	–

RGC: radical generating capacity; DTT, dithiothreitol-assay; HO-1, haem oxygenase-1; –, not assessed.
[a] Tendency, not statistically evaluated.
[b] $PM_{0.95–0.49}$; $PM_{1.5–0.49}$; $PM_{3–1.5}$; $PM_{7.2–3}$; $PM_{10–7.2}$.

alkanes, transition metals and radical generating capacity) and toxicological effects (mutagenicity, cytotoxicity and DNA-reactivity) for smaller PM size fractions than for coarse fractions.

In those studies that characterised traffic intensity, differences in PM toxicity did not systematically depend on traffic intensity. This implies that traffic intensity is not always the most important variable for explaining local differences in PM toxicity. As it has been shown that differences in PM toxicity are not necessarily related to PM mass concentrations, monitoring PM mass concentrations without taking physicochemical and toxicological characteristics into account, may easily overlook PM 'hot spots' with increased toxicological risks. As the current data do not provide sufficient evidence to demonstrate what chemical composition or toxicological mechanism is primarily responsible for PM induced health effects, additional research is needed to link PM characteristics to increased human health risks.

There is also emerging evidence for a relationship between ultrafine particulates and health risks. It is known that diesel engine emissions contribute greatly to the ultrafine PM fraction, which consists of aggregates of soluble sulphates, hydrocarbons and metals with a carbon core, and can deposit with high efficiency in the lungs. However, at this moment, the available data from toxicological and epidemiological studies is insufficient for regulating ultrafine particle concentrations.

When assessing human health risks associated with PM exposures, it should be kept in mind that traffic emissions also contribute to increased air pollution with volatile organic compounds, NO_x and SO_2. Although these gaseous compounds do not affect the toxicological characteristics of ambient PM directly, they are quite likely to influence cellular susceptibility to cytotoxic and genotoxic damage upon PM exposure. For example, partial depletion of antioxidative systems as a result of exposure to NOx or reactive aldehydes (*e.g.* PAN) in both lung epithelial cells and lining fluid, may result in increased cytotoxicity and oxidative DNA damage due to PM-mediated radical formation. However, when interpreting data from epidemiological studies on PM-related health effects that do not simultaneously take exposure to volatile traffic emissions into account, potential health risks should be attributed to the complex chemical mixture of air pollution as a whole rather than to the PM fraction.

All together, these findings indicate that in addition to the current European standards for PM_{10}, indicators for smaller sized (in the range between 1 and 2.5 μm) or source-related PM should also be considered for regulation. In order to identify appropriate PM emission abatement measures, further research is needed to more convincingly establish the relationship between toxicity of these smaller fractions and specific PM sources.

References

[1] L. Chiaverini, Asthma, particulates, and diesel exhaust, Med. Health RI 85 (2002) 140–142.

[2] J.M. Samet, F. Dominici, F.C. Curriero, I. Coursac, S.L. Zeger, Fine particulate air pollution and mortality in 20 U.S. cities, 1987–1994, N. Engl. J. Med. 343 (2000) 1742–1749.

[3] M. Sorensen, H. Autrup, P. Moller, O. Hertel, S.S. Jensen, P. Vinzents, L.E. Knudsen, S. Loft, Linking exposure to environmental pollutants with biological effects, Mutat. Res. 544 (2003) 255–271.

[4] A.D. Kappos, P. Bruckmann, T. Eikmann, N. Englert, U. Heinrich, P. Hoppe, E. Koch, G.H. Krause, W.G. Kreyling, K. Rauchfuss, P. Rombout, V. Schulz-Klemp, W.R. Thiel, H.E. Wichmann, Health effects of particles in ambient air, Int. J. Hyg. Environ. Health 207 (2004) 399–407.

[5] N. Englert, Fine particles and human health—a review of epidemiological studies, Toxicol. Lett. 149 (2004) 235–242.

[6] G.L. Squadrito, R. Cueto, B. Dellinger, W.A. Pryor, Quinoid redox cycling as a mechanism for sustained free radical generation by inhaled airborne particulate matter, Free Radic. Biol. Med. 31 (2001) 1132–1138.

[7] U. Vinitketkumnuen, K. Kalayanamitra, T. Chewonarin, R. Kamens, Particulate matter, PM 10 & PM 2.5 levels, and airborne mutagenicity in Chiang Mai, Thailand, Mutat. Res. 519 (2002) 121–131.

[8] F. Palmgren, P. Wahlin, S. Loft, The Pollution of Air with Particles in Copenhagen, Ministry of the Environment, Copenhagen, 2003 (in Danish).

[9] P. Bagnoli, S. Carrozzino, B. Pisani, F. Righini, Chemical characterization of the PM10 fraction of airborne particulate matter in the urban atmosphere, J. Environ. Pathol. Toxicol. Oncol. 16 (1997) 219–225.

[10] K.S. Chen, C.F. Lin, Y.M. Chou, Determination of source contributions to ambient PM2.5 in Kaohsiung, Taiwan, using a receptor model, J. Air Waste Manage. Assoc. 51 (2001) 489–498.

[11] H. Visser, E. Buringh, P.B. Breugel, Composition and Origin of Airborne Particulate Matter in the Netherlands, National Institute of Public Health and the Environment, Bilthoven, 2001 .

[12] M. Cackovic, K. Sega, V. Vadic, I. Beslic, Z. Soljic, Seasonal distributions of acid components in PM2.5 fraction of airborne particles in Zagreb air, Bull. Environ. Contam. Toxicol. 67 (2001) 704–711.

[13] WHO, Air Quality Guidelines for Europe, WHO Regional Office for Europe, Copenhagen, 2000.

[14] V. Ariola, A. D'Alessandro, F. Lucarelli, G. Marcazzan, F. Mazzei, S. Nava, I. Garcia-Orellana, P. Prati, G. Valli, R. Vecchi, A. Zucchiatti, Elemental characterization of PM10, PM2.5 and PM1 in the town of Genoa (Italy), Chemosphere 62 (2006) 226–232.

[15] USEPA Compilation of Air Pollutant Emission Factors. USEPA report AP-42, US Environmental Protection Agency, Washington, DC, 1995.

[16] TNO Particulate Mater Emissions (PM10, PM2.5 PM<0.1) in Europe in 1990 and 1993, TNO report TNO-MEP-R96-472, TNO, Zeist, the Netherlands, 1997.
[17] W. Birmili, A.G. Allen, F. Bary, R.M. Harrison, Trace metal concentrations and water solubility in size-fractionated atmospheric particles and influence of road traffic, Environ. Sci. Technol. 40 (2006) 1144–1153.
[18] I. Salma, W. Maenhaut, Changes in elemental composition and mass of atmospheric aerosol pollution between 1996 and 2002 in a Central European city, Environ. Pollut. (2006).
[19] B. Parsons, L.F. Salter, Air quality effects of traffic in a canyon-like street (Falmouth, U.K.), Environ. Monit. Assess. 82 (2003) 63–73.
[20] B. Bocca, F. Petrucci, A. Alimonti, S. Caroli, Traffic-related platinum and rhodium concentrations in the atmosphere of Rome, J. Environ. Monit. 5 (2003) 563–568.
[21] A. Vera Castellano, J. Lopez Cancio, P. Santana Aleman, J. Santana Rodriguez, Polycyclic aromatic hydrocarbons in ambient air particles in the city of Las Palmas de Gran Canaria, Environ. Int. 29 (2003) 475–480.
[22] K. Donaldson, D.M. Brown, C. Mitchell, M. Dineva, P.H. Beswick, P. Gilmour, W. MacNee, Free radical activity of PM10: iron-mediated generation of hydroxyl radicals, Environ. Health Perspect. 105 (Suppl. 5) (1997) 1285–1289.
[23] J.Y. Han, K. Takeshita, H. Utsumi, Noninvasive detection of hydroxyl radical generation in lung by diesel exhaust particles, Free Radic. Biol. Med. 30 (2001) 516–525.
[24] A. Hartwig, Role of DNA repair in particle- and fiber-induced lung injury, Inhal. Toxicol. 14 (2002) 91–100.
[25] S. Kawanishi, Y. Hiraku, M. Murata, S. Oikawa, The role of metals in site-specific DNA damage with reference to carcinogenesis, Free Radic. Biol. Med. 32 (2002) 822–832.
[26] S. Rauch, M. Lu, G.M. Morrison, Heterogeneity of platinum group metals in airborne particles, Environ. Sci. Technol. 35 (2001) 595–599.
[27] Y.S. Wu, G.C. Fang, P.P. Fu, C.J. Yang, The measurements of ambient particulates (TSP, PM2.5, PM2.5-10), chemical component concentration variation, and mutagenicity study during 1998–2001 in central Taiwan, J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 20 (2002) 45–59.
[28] Y.B. Lim, P.J. Ziemann, Products and mechanism of secondary organic aerosol formation from reactions of n-alkanes with OH radicals in the presence of NOx, Environ. Sci. Technol. 39 (2005) 9229–9236.
[29] Q. Zhang, C.O. Stanier, M.R. Canagaratna, J.T. Jayne, D.R. Worsnop, S.N. Pandis, J.L. Jimenez, Insights into the chemistry of new particle formation and growth events in Pittsburgh based on aerosol mass spectrometry, Environ. Sci. Technol. 38 (2004) 4797–4809.
[30] B.J. Finlayson-Pitts, J.N. Pitts Jr., Tropospheric air pollution: ozone, airborne toxics, polycyclic aromatic hydrocarbons, and particles, Science 276 (1997) 1045–1052.
[31] L.D. Claxton, P.P. Matthews, S.H. Warren, The genotoxicity of ambient outdoor air, a review: *Salmonella* mutagenicity, Mutat. Res. 567 (2004) 347–399.
[32] R. Westerholm, K.E. Egeback, Exhaust emissions from light- and heavy-duty vehicles: chemical composition, impact of exhaust after treatment, and fuel parameters, Environ. Health Perspect. 102 (Suppl. 4) (1994) 13–23.
[33] R. Atkinson, J. Arey, Atmospheric chemistry of gas-phase polycyclic aromatic hydrocarbons: formation of atmospheric mutagens, Environ. Health Perspect. 102 (Suppl. 4) (1994) 117–126.
[34] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Diesel and Gasoline Engine Exhausts and Some Nitroarenes. International Agency for Research on Cancer, IARC Monogr. Eval. Carcinog. Risks Hum. 46 (1989) 1-458.
[35] K. Hayakawa, Y. Kawaguchi, T. Murahashi, M. Miyazaki, Distributions of nitropyrenes and mutagenicity in airborne particulates collected with an Andersen sampler, Mutat. Res. 348 (1995) 57–61.
[36] K. Ravindra, A.K. Mittal, R. Van Grieken, Health risk assessment of urban suspended particulate matter with special reference to polycyclic aromatic hydrocarbons: a review, Rev. Environ. Health 16 (2001) 169–189.
[37] H.L. Sheu, W.J. Lee, S.J. Lin, G.C. Fang, H.C. Chang, W.C. You, Particle-bound PAH content in ambient air, Environ. Pollut. 96 (1997) 369–382.
[38] N. Li, C. Sioutas, A. Cho, D. Schmitz, C. Misra, J. Sempf, M. Wang, T. Oberley, J. Froines, A. Nel, Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage, Environ. Health Perspect. 111 (2003) 455–460.
[39] T.M. de Kok, J.G. Hogervorst, J.J. Briede, M.H. van Herwijnen, L.M. Maas, E.J. Moonen, H.A. Driece, J.C. Kleinjans, Genotoxicity and physicochemical characteristics of traffic-related ambient particulate matter, Environ. Mol. Mutagen. 46 (2005) 71–80.
[40] P. Boffetta, N. Jourenkova, P. Gustavsson, Cancer risk from occupational and environmental exposure to polycyclic aromatic hydrocarbons, Cancer Causes Control 8 (1997) 444–472.
[41] H.S. Rosenkranz, Mutagenic nitroarenes, diesel emissions, particulate-induced mutations and cancer: an essay on cancer-causation by a moving target, Mutat. Res. 367 (1996) 65–72.
[42] H. Yamazaki, N. Hatanaka, R. Kizu, K. Hayakawa, N. Shimada, F.P. Guengerich, M. Nakajima, T. Yokoi, Bioactivation of diesel exhaust particle extracts and their major nitrated polycyclic aromatic hydrocarbon components, 1-nitropyrene and dinitropyrenes, by human cytochromes P450 1A1, 1A2, and 1B1, Mutat. Res. 472 (2000) 129–138.
[43] L. Massolo, A. Muller, M. Tueros, M. Rehwagen, U. Franck, A. Ronco, O. Herbarth, Assessment of mutagenicity and toxicity of different-size fractions of air particulates from La Plata, Argentina, and Leipzig, Germany, Environ. Toxicol. 17 (2002) 219–231.
[44] A.K. Prahalad, J.M. Soukup, J. Inmon, R. Willis, A.J. Ghio, S. Becker, J.E. Gallagher, Ambient air particles: effects on cellular oxidant radical generation in relation to particulate elemental chemistry, Toxicol. Appl. Pharmacol. 158 (1999) 81–91.
[45] R.W. Clarke, B. Coull, U. Reinisch, P. Catalano, C.R. Killingsworth, P. Koutrakis, I. Kavouras, G.G. Murthy, J. Lawrence, E. Lovett, J.M. Wolfson, R.L. Verrier, J.J. Godleski, Inhaled concentrated ambient particles are associated with hematologic and bronchoalveolar lavage changes in canines, Environ. Health Perspect. 108 (2000) 1179–1187.
[46] B. Fubini, A. Hubbard, Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis, Free Radic. Biol. Med. 34 (2003) 1507–1516.
[47] B. Dellinger, W.A. Pryor, R. Cueto, G.L. Squadrito, V. Hegde, W.A. Deutsch, Role of free radicals in the toxicity of airborne fine particulate matter, Chem. Res. Toxicol. 14 (2001) 1371–1377.

[48] I. Fenoglio, G. Martra, S. Coluccia, B. Fubini, Possible role of ascorbic acid in the oxidative damage induced by inhaled crystalline silica particles, Chem. Res. Toxicol. 13 (2000) 971–975.
[49] A. Valavanidis, K. Fiotakis, E. Bakeas, T. Vlahogianni, Electron paramagnetic resonance study of the generation of reactive oxygen species catalysed by transition metals and quinoid redox cycling by inhalable ambient particulate matter, Redox Rep. 10 (2005) 37–51.
[50] N. Li, S. Kim, M. Wang, J. Froines, C. Sioutas, A. Nel, Use of a stratified oxidative stress model to study the biological effects of ambient concentrated and diesel exhaust particulate matter, Inhal. Toxicol. 14 (2002) 459–486.
[51] R.K. Brown, H. Wyatt, J.F. Price, F.J. Kelly, Pulmonary dysfunction in cystic fibrosis is associated with oxidative stress, Eur. Respir. J. 9 (1996) 334–339.
[52] L.M. Heunks, P.N. Dekhuijzen, Respiratory muscle function and free radicals: from cell to COPD, Thorax 55 (2000) 704–716.
[53] M. Kato, M. Nakano, A. Morikawa, H. Kimura, M. Shigeta, T. Kuroume, Ability of polymorphonuclear leukocytes to generate active oxygen species in children with bronchial asthma. Use of chemiluminescence probes with a Cypridina luciferin analog and luminol, Int. Arch. Allergy Appl. Immunol. 95 (1991) 17–22.
[54] A.J. Ghio, J. Stonehuerner, L.A. Dailey, J.D. Carter, Metals associated with both the water-soluble and insoluble fractions of an ambient air pollution particle catalyze an oxidative stress, Inhal. Toxicol. 11 (1999) 37–49.
[55] K. Donaldson, P.H. Beswick, P.S. Gilmour, Free radical activity associated with the surface of particles: a unifying factor in determining biological activity? Toxicol. Lett. 88 (1996) 293–298.
[56] A.K. Prahalad, J. Inmon, L.A. Dailey, M.C. Madden, A.J. Ghio, J.E. Gallagher, Air pollution particles mediated oxidative DNA base damage in a cell free system and in human airway epithelial cells in relation to particulate metal content and bioreactivity, Chem. Res. Toxicol. 14 (2001) 879–887.
[57] J.J. Briede, T.M. De Kok, J.G. Hogervorst, E.J. Moonen, C.L. Op Den Camp, J.C. Kleinjans, Development and application of an electron spin resonance spectrometry method for the determination of oxygen free radical formation by particulate matter, Environ. Sci. Technol. 39 (2005) 8420–8426.
[58] T. Shi, R.P. Schins, A.M. Knaapen, T. Kuhlbusch, M. Pitz, J. Heinrich, P.J. Borm, Hydroxyl radical generation by electron paramagnetic resonance as a new method to monitor ambient particulate matter composition, J. Environ. Monit. 5 (2003) 550–556.
[59] A. Valavanidis, T. Vlahoyianni, K. Fiotakis, Comparative study of the formation of oxidative damage marker 8-hydroxy-2′-deoxyguanosine (8-OHdG) adduct from the nucleoside 2′-deoxyguanosine by transition metals and suspensions of particulate matter in relation to metal content and redox reactivity, Free Radic. Res. 39 (2005) 1071–1081.
[60] A. Baulig, J.J. Poirault, P. Ausset, R. Schins, T. Shi, D. Baralle, P. Dorlhene, M. Meyer, R. Lefevre, A. Baeza-Squiban, F. Marano, Physicochemical characteristics and biological activities of seasonal atmospheric particulate matter sampling in two locations of Paris, Environ. Sci. Technol. 38 (2004) 5985–5992.
[61] T. Shi, A.M. Knaapen, J. Begerow, W. Birmili, P.J. Borm, R.P. Schins, Temporal variation of hydroxyl radical generation and 8-hydroxy-2′-deoxyguanosine formation by coarse and fine particulate matter, Occup. Environ. Med. 60 (2003) 315–321.
[62] F. Schaumann, P.J. Borm, A. Herbrich, J. Knoch, M. Pitz, R.P. Schins, B. Luettig, J.M. Hohlfeld, J. Heinrich, N. Krug, Metal-rich ambient particles (particulate matter 2.5) cause airway inflammation in healthy subjects, Am. J. Respir. Crit. Care Med. 170 (2004) 898–903.
[63] R.O. Salonen, A.I. Halinen, A.S. Pennanen, M.R. Hirvonen, M. Sillanpaa, R. Hillamo, T. Shi, P. Borm, E. Sandell, T. Koskentalo, P. Aarnio, Chemical and in vitro toxicologic characterization of wintertime and springtime urban-air particles with an aerodynamic diameter below 10 microm in Helsinki, Scand. J. Work Environ. Health 30 (Suppl. 2) (2004) 80–90.
[64] C.J. Pan, D.A. Schmitz, A.K. Cho, J. Froines, J.M. Fukuto, Inherent redox properties of diesel exhaust particles: Catalysis of the generation of reactive oxygen species by biological reductants, Toxicol. Sci. (2004).
[65] L.D. Claxton, R.S. Morin, T.J. Hughes, J. Lewtas, A genotoxic assessment of environmental tobacco smoke using bacterial bioassays, Mutat. Res. 222 (1989) 81–99.
[66] J. Lewtas, L. Claxton, J. Mumford, G. Lofroth, Bioassay of Complex Mixtures of Indoor Air Pollutants, IARC Science Publishers, 1993, pp. 85–95.
[67] M. Cerna, D. Pochmanova, A. Pastorkova, I. Benes, J. Lenicek, J. Topinka, B. Binkova, Genotoxicity of urban air pollutants in the Czech Republic. Part I. Bacterial mutagenic potencies of organic compounds adsorbed on PM10 particulates, Mutat. Res. 469 (2000) 71–82.
[68] M. Cerna, A. Pastorkova, V. Vrbikova, J. Smid, P. Rossner, Mutagenicity monitoring of airborne particulate matter (PM10) in the Czech Republic, Mutat. Res. 444 (1999) 373–386.
[69] B.S. De Martinis, N.Y. Kado, L.R. de Carvalho, R.A. Okamoto, L.A. Gundel, Genotoxicity of fractionated organic material in airborne particles from Sao Paulo, Brazil, Mutat. Res. 446 (1999) 83–94.
[70] A. Buschini, F. Cassoni, E. Anceschi, L. Pasini, P. Poli, C. Rossi, Urban airborne particulate: genotoxicity evaluation of different size fractions by mutagenesis tests on microorganisms and comet assay, Chemosphere 44 (2001) 1723–1736.
[71] L.D. Claxton, S. Warren, R. Zweidinger, J. Creason, A comparative assessment of Boise, Idaho, ambient air fine particle samples using the plate and microsuspension *Salmonella* mutagenicity assays, Sci. Total Environ. 275 (2001) 95–108.
[72] B. Binkova, M. Cerna, A. Pastorkova, R. Jelinek, I. Benes, J. Novak, R.J. Sram, Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer and winter seasons 2000–2001, Mutat. Res. 525 (2003) 43–59.
[73] G. Bronzetti, M. Cini, M. Paoli, G. Ciacchini, V. Giaconi, E. Morichetti, Mutagenicity and chemical analysis of airborne particulate matter collected in Pisa, J. Environ. Pathol. Toxicol. Oncol. 16 (1997) 147–156.
[74] A. Delgado-Rodriguez, R. Ortiz-Marttelo, R. Villalobos-Pietrini, S. Gomez-Arroyo, U. Graf, Genotoxicity of organic extracts of airborne particles in somatic cells of *Drosophila melanogaster*, Chemosphere 39 (1999) 33–43.
[75] V.A. Du Four, N. Van Larebeke, C.R. Janssen, Genotoxic and mutagenic activity of environmental air samples in Flanders, Belgium, Mutat. Res. 558 (2004) 155–167.
[76] T. Nielsen, A. Feilberg, M.L. Binderup, The variation of street air levels of PAH and other mutagenic PAC in relation to

regulations of traffic emissions and the impact of atmospheric processess, Environ. Sci. Pollut. Res. 6 (1999) 133–137.
[77] M.I. Sato, G.U. Valent, C.A. Coimbrao, M.C. Coelho, P. Sanchez Sanchez, C.D. Alonso, M.T. Martins, Mutagenicity of airborne particulate organic material from urban and industrial areas of Sao Paulo, Brazil, Mutat. Res. 335 (1995) 317–330.
[78] X. Zhao, Z. Wan, G. Chen, H. Zhu, S. Jiang, J. Yao, Genotoxic activity of extractable organic matter from urban airborne particles in Shanghai, China, Mutat. Res. 514 (2002) 177–192.
[79] M. Watanabe, M. Ishidate Jr., T. Nohmi, A sensitive method for the detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of *Salmonella typhimurium* strains TA98 and TA100, Mutat. Res. 216 (1989) 211–220.
[80] M. Watanabe, M. Ishidate Jr., T. Nohmi, Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated *O*-acetyltransferase levels, Mutat. Res. 234 (1990) 337–348.
[81] E.C. McCoy, E.J. Rosenkranz, L.A. Petrullo, H.S. Rosenkranz, Frameshift mutations: relative roles of simple intercalation and of adduct formation, Mutat. Res. 90 (1981) 21–30.
[82] A. Ducatti, V.M. Vargas, Mutagenic activity of airborne particulate matter as an indicative measure of atmospheric pollution, Mutat. Res. 540 (2003) 67–77.
[83] C.Y. Kuo, Y.W. Cheng, C.Y. Chen, H. Lee, Correlation between the amounts of polycyclic aromatic hydrocarbons and mutagenicity of airborne particulate samples from Taichung City, Taiwan, Environ. Res. 78 (1998) 43–49.
[84] A. Muller, P. Alzuet, O. Herbarth, A. Ronco, Assessment of toxicity and mutagenicity in air particulate matter from an urban industrial area in the coast of the Rio de la Plata, Environ. Toxicol. 16 (2001) 151–157.
[85] V.M. Vargas, Mutagenic activity as a parameter to assess ambient air quality for protection of the environment and human health, Mutat. Res. 544 (2003) 313–319.
[86] W.L. Hsiao, Z.Y. Mo, M. Fang, X.M. Shi, F. Wang, Cytotoxicity of PM(2.5) and PM(2.5–10) ambient air pollutants assessed by the MTT and the Comet assays, Mutat. Res. 471 (2000) 45–55.
[87] Y. Tong, X. Ni, Y. Zhang, F. Chen, G. Zhang, S. Ye, Study of the toxicological mechanism of acidified aerosols, Biol. Trace Elem. Res. 85 (2002) 149–156.
[88] E. Alfaro-Moreno, L. Martinez, C. Garcia-Cuellar, J.C. Bonner, J.C. Murray, I. Rosas, S.P. Rosales, A.R. Osornio-Vargas, Biologic effects induced in vitro by PM10 from three different zones of Mexico City, Environ. Health Perspect. 110 (2002) 715–720.
[89] L.L. Greenwell, T. Moreno, T.P. Jones, R.J. Richards, Particle-induced oxidative damage is ameliorated by pulmonary antioxidants, Free Radic. Biol. Med. 32 (2002) 898–905.
[90] D. Upadhyay, V. Panduri, A. Ghio, D.W. Kamp, Particulate matter induces alveolar epithelial cell DNA damage and apoptosis: role of free radicals and the mitochondria, Am. J. Respir. Cell. Mol. Biol. 29 (2003) 180–187.
[91] H.L. Karlsson, L. Nilsson, L. Moller, Subway particles are more genotoxic than street particles and induce oxidative stress in cultured human lung cells, Chem. Res. Toxicol. 18 (2005) 19–23.
[92] A. Gabelova, Z. Valovicova, E. Horvathova, D. Slamenova, B. Binkova, R.J. Sram, P.B. Farmer, Genotoxicity of environmental air pollution in three European cities: Prague, Kosice and Sofia, Mutat. Res. 563 (2004) 49–59.
[93] E. Brits, G. Schoeters, L. Verschaeve, Genotoxicity of PM10 and extracted organics collected in an industrial, urban and rural area in Flanders, Belgium, Environ. Res. 96 (2004) 109–118.
[94] M. Lazarova, D. Slamenova, Genotoxic effects of a complex mixture adsorbed onto ambient air particles on human cells in vitro; the effects of Vitamins E and C, Mutat. Res. 557 (2004) 167–175.
[95] K. Healey, J.J. Lingard, A.S. Tomlin, A. Hughes, K.L. White, C.P. Wild, M.N. Routledge, Genotoxicity of size-fractionated samples of urban particulate matter, Environ. Mol. Mutagen. 45 (2005) 380–387.
[96] B. Dellinger, W.A. Pryor, R. Cueto, G.L. Squadrito, W.A. Deutsch, The role of combustion-generated radicals in the toxicity of PM2.5, Proc. Combust. Inst. 28 (2000) 2675–2681.
[97] P.S. Gilmour, D.M. Brown, T.G. Lindsay, P.H. Beswick, W. MacNee, K. Donaldson, Adverse health effects of PM10 particles: involvement of iron in generation of hydroxyl radical, Occup. Environ. Med. 53 (1996) 817–822.
[98] A.M. Knaapen, T. Shi, P.J. Borm, R.P. Schins, Soluble metals as well as the insoluble particle fraction are involved in cellular DNA damage induced by particulate matter, Mol. Cell. Biochem. 234–235 (2002) 317–326.
[99] S.K. Pohjola, M. Lappi, M. Honkanen, L. Rantanen, K. Savela, DNA binding of polycyclic aromatic hydrocarbons in a human bronchial epithelial cell line treated with diesel and gasoline particulate extracts and benzo[*a*]pyrene, Mutagenesis 18 (2003) 429–438.
[100] T. Nielsen, Traffic contribution of polycyclic aromatic hydrocarbons in the center of a large city, Atmos. Environ. 30 (1996) 3481–3490.
[101] A.H. Miguel, On-road emissions of particulate polycyclic aromatic hydrocarbons and black smoke from gasoline and diesel vehicles, Atmos. Sci. Technol. 32 (1998) 450–455.
[102] W.F. Rogge, L.M. Hilderman, M.A. Mazurek, G.R. Cass, B.R.T. Simoneit, Sources of fine organic aerosol. 2: Noncatalyst and catalyst-equipped automobiles and heavy-duty diesel trucks, Environ. Sci. Technol. 27 (1993) 636–651.
[103] G.C. Fang, C.N. Chang, C.C. Chu, Y.S. Wu, P.P. Fu, I.L. Yang, M.H. Chen, Characterization of particulate, metallic elements of TSP, PM(2.5) and PM(2.5–10) aerosols at a farm sampling site in Taiwan, Taichung, Sci. Total Environ. 308 (2003) 157–166.