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Oxidative stress-induced DNA damage by particulate air pollution

Lotte Risom, Peter Møller, Steffen Loft *

Institute of Public Health, University of Copenhagen, Øster Farimagsgade 5, 1014 Copenhagen K, Denmark

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Abstract

Exposure to ambient air particulate matter (PM) is associated with pulmonary and cardiovascular diseases and cancer. The mechanisms of PM-induced health effects are believed to involve inflammation and oxidative stress. The oxidative stress mediated by PM may arise from direct generation of reactive oxygen species from the surface of particles, soluble compounds such as transition metals or organic compounds, altered function of mitochondria or NADPH-oxidase, and activation of inflammatory cells capable of generating ROS and reactive nitrogen species. Resulting oxidative DNA damage may be implicated in cancer risk and may serve as marker for oxidative stress relevant for other ailments caused by particulate air pollution.

There is overwhelming evidence from animal experimental models, cell culture experiments, and cell free systems that exposure to diesel exhaust and diesel exhaust particles causes oxidative DNA damage. Similarly, various preparations of ambient air PM induce oxidative DNA damage in in vitro systems, whereas in vivo studies are scarce. Studies with various model/surrogate particle preparations, such as carbon black, suggest that the surface area is the most important determinant of effect for ultrafine particles (diameter less than 100 nm), whereas chemical composition may be more important for larger particles.

The knowledge concerning mechanisms of action of PM has prompted the use of markers of oxidative stress and DNA damage for human biomonitoring in relation to ambient air. By means of personal monitoring and biomarkers a few studies have attempted to characterize individual exposure, explore mechanisms and identify significant sources to size fractions of ambient air PM with respect to relevant biological effects. In these studies guanine oxidation in DNA has been correlated with exposure to $PM_{2.5}$ and ultrafine particles outdoor and indoor.

Oxidative stress-induced DNA damage appears to an important mechanism of action of urban particulate air pollution. Related biomarkers and personal monitoring may be useful tools for risk characterization.

Keywords: Oxidative DNA damage; Diesel exhaust particles; Air pollution; Toxicology; Biomonitoring; DNA repair

Abbreviations: DEP, diesel exhaust particles; FPG, formamidopyrimidine glycosyles; HO-1, heme oxygenase 1; MTH1, mut T homologue 1; NEIL, nei (or endonuclease VIII)-like; NQO1, NADPH quinone oxidoreductase; Nrf2, nuclear response erythroid-2 factor 2; Ogg1, oxoguanine glycosylase; PAH, polyaromatic hydrocarbons; PM, particulate matter; ROS, reactive oxygen species; SB, strand breaks; SRM, standard reference material; TSP, total suspended particulates; UFP, ultrafine particles; 8-OxodG, 8-oxo-7,8-dihydro-2'-deoxyguanosine

* Corresponding author. Tel.: +45 3 5327649; fax: +45 3 5327610.
E-mail address: s.loft@pubhealth.ku.dk (S. Loft).

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1. Introduction

Epidemiological studies have associated ambient air particulate matter (PM) with pulmonary and cardiovascular diseases and cancer [1,2]. Particles are usually defined by their size, e.g. as PM_{10} and $PM_{2.5}$, which are mass of particles with aerodynamic diameter less than 10 and 2.5 μm, respectively. Recently, however, interest has also focused on the ultrafine particles (UFP) fraction with a diameter less than 100 nm, which are abundant in numbers but contribute little to particle mass. From a mechanistic point of view, UFP are considered important with respect to health effects because of their very high alveolar deposition fraction, large surface area, chemical composition, ability to induce inflammation, and potential to translocate to the circulation [3–9]. Vehicle emissions, in particular related to diesel engines, are a major source of ambient UFP, which penetrate indoor [10,11]. Heavy duty vehicles are almost exclusively diesel powered; there is increasing number of private cars and other light duty vehicles that are diesel powered.

The mechanisms of PM-induced health effects are believed to involve inflammation and oxidative stress [3–5,12–15]. Particularly for cancer, bulky DNA adducts from polyaromatic hydrocarbons (PAH) are also thought to be important [16]. Oxidative stress has been implicated in many diseases, including cardiovascular disease, macular degeneration, pancreatitis, and cancer [17]. The term "Oxidative Stress" has broadly been defined as an unbalance in favour of pro-oxidants and disfavour of antioxidants, potentially leading to damage of biomolecules [18]. Oxidative stress is generated by a large variety of mechanisms, including mitochondrial respiration, ischemia/reperfusion, inflammation, and metabolism of foreign compounds. DNA is considered to be an important target for reactive oxygen species (ROS) generated as a consequence to air pollution exposure. Oxidative DNA damage may be implicated in cancer risk and may serve as marker for oxidative stress relevant for other ailments caused by air pollution. The present review will focus on experimental work on the mechanisms of induction of oxidative stress and DNA damage by ambient air PM as well as the related human biomonitoring.

2. Mechanisms of oxidative stress induced by air pollution particles

The production of ROS has been argued to play an important role in the primary cytotoxic effects of respirable diesel exhaust particles (DEP) and urban street PM [12]. Increased formation of hydroxyl radicals has been detected by non-invasive electron spin resonance spectroscopy in cell free experiments, cell culture experiments, and in mice lungs following intratracheal instillation of DEP [19–21]. The oxidative stress mediated by PM may arise from mixed sources (Fig. 1), involving (i) direct generation of ROS from the surface of particles, (ii) soluble compounds such as transition metals or organic compounds, (iii) altered function of mitochondria or NADPH-oxidase [12,22,23] and (iv) activation of inflammatory cells capable of generating ROS and reactive nitrogen species [5,12,24]. Direct particle generation of ROS can occur through the presence of free radicals and oxidants on the particle surface [25]. Although this mechanism can explain the generation of DNA damage in cell free experiments, generation of particle-induced oxidative damaged DNA in intact cells can only be explained by this mechanism under the assumption that: (i) particles traverse to the nucleus; (ii) extracellular ROS initiate free radical chain reactions which ultimately reach the nucleus and damage DNA. It should be noted that ozone and NO_2 are usually present together with particles in ambient air and they are also oxidants with potential effects in terms of oxidative DNA damage. Similarly, volatile compounds, such as benzene, in urban air pollution can induce oxidative DNA damage [26–28].

2.1. Oxidative stress mediated by transition metals

On their surface, particles may contain soluble transition metals such as iron, copper, chromium and vanadium that can generate ROS through Fenton type reactions and act as catalysts by Harber–Weiss reactions [17]:

$$^{\bullet}O_2^- + H_2O_2 \xrightarrow[Fe]{} {}^{\bullet}OH + OH^- + O_2$$

(Net result of the iron catalyzed Harber Weiss or Fenton reaction)

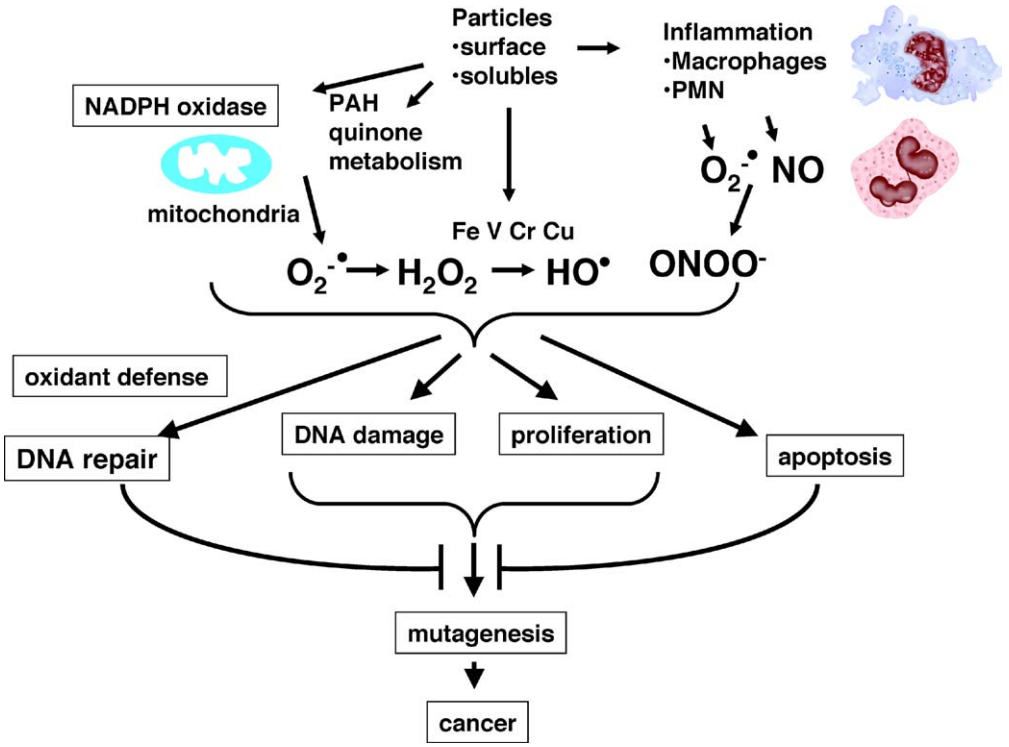

Fig. 1. Possible mechanisms for induction of oxidative stress and DNA damage by air pollution particles and the roles in carcinogenesis.

Ferrous iron (Fe^{2+}) reduces hydrogen peroxide (H_2O_2) with the formation of hydroxyl radical and oxidation of ferrous iron to ferric iron (Fe^{3+}). This reaction can recycle by reductants such as superoxide anions, glutathione and ascorbic acid by reducing Fe^{3+} to Fe^{2+}. The hydroxyl radical ($^\bullet$OH) is extremely reactive (reaction rate constant usually above of 10^8 M^{-1} s^{-1}), which implicates that it attacks any biological molecules at diffusion distance [17].

Several studies have shown that iron and other transition metals leaching from particles or by their presence on particle surfaces play a role in the generation of ROS in biological systems [21,29]. It was recently suggested that DEP contain surface functional groups with the capacity to complex host iron, whereby iron accumulates and oxidative stress is induced [21,29]. This is in accordance with in vitro studies demonstrating that DEP generate superoxide anions [20,30], which can lead to hydrogen peroxide and hydroxyl radicals without any biochemical or biological activation.

2.2. *Oxidative stress induced by the organic fraction*

A large number of substituted and un-substituted PAH and other organic molecules, absorbed on the surface of carbonaceous particles, can be extracted in various organic solvents. There is evidence that the organic fraction of DEP induces oxidative stress [12]. Particle bound benzo[*a*]pyrene has been shown to be bioavailable and also to exert non-oxidative genotoxicity via the formation of bulky DNA adducts [31]. Orally administered benzo[*a*]pyrene can induce oxidative DNA damage in systemic targets, including lung and kidney [32]. In cell-free experiments, ambient UFP generated ROS in a manner proportional to the content of PAH [22]. The organic fraction of urban air particles contains quinone radicals that may undergo redox-cycling producing hydroxyl radicals [20]. However, in cells it is unresolved how much this genuine ROS-producing capacity of PM contributes to the overall generation of ROS, especially considering that a

large proportion of ROS in metabolically competent cells arise from metabolism. For example, organic compounds of DEP undergo metabolic activation in the lung and liver of exposed animals, causing induced expression of cytochrome P450 enzymes (CYP1A1) that generates ROS and reactive PAH-quinones [33]. In airway epithelial cells, organic compounds adsorbed on particle surfaces elicit inflammation through CYP1A1-mediated ROS generation, which activate transcription factors and release of cytokines [34].

2.3. Oxidative stress and inflammation

Oxidative stress caused by activation of the inflammatory system, encompassing alveolar macrophages and neutrophils, probably is a very important contributor describing detrimental effects of particles in multicellular organisms [12,35]. Neutrophils and alveolar macrophages play a key role in the defense reactions in the lungs against foreign compounds and infectious agents. Alveolar macrophages are capable of phagocytosis of poorly soluble particles and participate in the initiation of inflammatory responses in the lung. Activation of the inflammatory system is commonly investigated by upregulation of proinflammatory mediators at the level of mRNA or protein expression level by immunoassays.

There is strong evidence from cell culture experiments and animal models that exposure to particles is associated with inflammation. The inflammatory response of UFP has mainly derived from investigations of model particle compounds, whereas fine and coarse size particle studies encompass also ambient collected particle preparations. Although cell culture experiments can mimic specific mechanisms of inflammation, animal experimental models are required to fully understand the ability of particles to cause inflammation. Components in particles that elicit inflammation are poorly investigated, although recent research points to the contribution of compositional elements and particle size. More extensive pulmonary inflammation is observed following intratracheal instillation of UFP as compared to the same mass of fine particles [36,37]. On the other hand, coarse ambient particles (PM_{10}) are associated with pulmonary inflammation following intratracheal instillation in rats, whereas the same mass of fine particles ($PM_{2.5}$) did not elicit inflammation [5]. For practical purposes, these results imply that small (ultrafine) particles cause inflammation by surface-mediated effects, whereas large (coarse) authentic particles cause inflammation through presence of endotoxins. DEP and ultrafine carbon black particles elicited a similar extent of inflammation after inhalation in mice suggesting that the chemical composition is not so important for the response [38]. That study also showed that tumour necrosis factor is not required for the inflammatory response or oxidative damage induced by DEP and carbon black [38]. Humans exposed to $PM_{2.5}$ ambient particles from two different areas in Germany caused lung inflammation in a manner dependent on the metal content of particles [39]. Mimicking real-life exposures it has been reported that DEP exposure by intratracheal instillation interact with ozone causing increased inflammation and injury of the lungs of rats as compared to rats only exposed to DEP [40]. Rats pre-treated with endotoxin by inhalation have greater inflammatory response of combined ozone and ultrafine carbon particle exposure, and this response seems more pronounced in rats at old age [41]. Moreover, ozone is a strong inducer of inflammation per se in animal studies with inhalation and DNA strand breaks [38] although without guanine oxidation are also found [42]. This underscores the importance of holistic perspectives when data from animal experimental models are extrapolated to human exposures and when interpreting biomonitoring studies, where many air pollutants are present.

2.4. Oxidative stress-induced DNA damage and repair

Excessive generation of ROS that overwhelms the antioxidant defense system can oxidize cellular biomolecules. Free radicals generate a large number of oxidative modifications in DNA, including SB and base oxidations [43–46]. Among oxidative DNA damage products, 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is probably the most studied oxidation product due to its relative ease of measurement and premutagenic potential [47]. In DNA, 8-oxodG may be formed by oxidation of guanine or incorporated during replication or repair as oxidized nucleotides (8-oxodGTP).

Oxidative DNA damage is repaired by a number of different enzymes. This implies that the level of

oxidative DNA damage in intact cells or animal experimental models exposed to particles must be interpreted as a steady-state level. DNA base lesions mainly are repaired by base excision repair enzymes; the main guardian against damage due to cellular metabolism, ROS, methylation, deamination, and hydroxylation. OGG1 is the base excision repair enzyme that is involved in removal of 8-oxodG [48–50]; it is expressed in all tissues examined although the resulting enzyme activity is low in some cells, e.g. testicular cells [49,51]. Pioneer reports showed accumulation of 8-oxodG in tissues of *ogg1*−/− mouse, indicating that OGG1 is the major repair enzyme for 8-oxodG base [52,53]. The *MTH1* gene encodes an 8-oxodGTPase that hydrolyzes 8-oxodGTP; the importance of the *MTH1* gene is revealed by the large number of tumours in *MTH1*-deficient mice [54]. Other repair pathways for 8-oxodG include nucleotide excision repair processes, mismatch repair, and NEIL proteins [46].

2.5. DEP and oxidative stress response genes

At low level of oxidative stress defined by minimal changes in the cellular reduced glutathione/glutathione disulphide ratios, cells mount antioxidant and cytoprotection responses by, e.g. heme oxygenase (HO-1) and superoxide dismutase expression [55]. HO-1 is highly responsive to oxidative stress and with its potent antioxidant properties it is a crucial part of the generalized response that protects cells and tissues against oxidative stress [56]. The mechanisms by which HO-1 confers protection against oxidant stress is still unclear, however a protective role of HO-1 could be explained by the increased production of bilirubin exhibiting potent cellular antioxidant property as an effective scavenger of oxygen radicals. Also, the induction of ferritin as a result of iron removal from the degradation of heme by HO-1 may serve to restrict iron from participation in the Fenton reaction and thereby reducing the oxidant burden of the cell. Thus, HO-1 may be an important part of the pulmonary defense against DEP-induced oxidative stress.

In vitro studies showed that DEP, and organic extracts of DEP, increased *HO-1* expression in macrophages [57]. This was confirmed in vivo where carbon monoxide levels in expired air was a sensitive marker for discerning effects in the lung after DEP inhalation [58]. The induction of *HO-1* by DEP is thought to occur through the antioxidant responsive element and *Nrf2* is a key transcription factor [59,60]. Moreover, *HO-1* expression was upregulated after single and repeated doses of DEP by inhalation [61]. Elevated expression of *HO-1* in macrophages upon DEP exposure was recently confirmed by cDNA microarray gene profiling analysis of DEP exposure, together with increased transcription of three other antioxidative enzymes (TDPX-2, GST-P, and NAD(P)H dehydrogenase) [62]. This suggests that alveolar macrophages play a role in the pulmonary defense by induction of antioxidative enzymes. Also manganese superoxide dismutase, Jun Kinase, and interleukin 8 were induced in a macrophage cell line by DEP exposure [57]. Jun Kinase is part of the intracellular signaling cascade and activation may thus lead to transcriptional activation of cytokines, e.g. interleukin 8.

3. Experimental evidence of oxidative stress and DNA damage induced by air pollution particles

Effects of ambient air particles can be characterised by experimental toxicology applying in vitro cell assays and in vivo studies with exposure by inhalation or instillation. Probably because of concern relating to occupational exposure, DEP are the best studied in this respect with an IARC group 2A carcinogen classification [16] and may serve as paradigm. Diesel exhaust is a complex mixture of different chemicals and can therefore not be analyzed based on toxicity of individual compounds. The initial research on the health effect risks associated with inhalation of DEP focused on chemical characterization of the particle-pollutant complex and the identified compounds were tested for individual toxic effects, including mutagenicity and carcinogenicity. In the late 1970s, DEP and organic extracts of DEP were identified as mutagenic agents in the Ames assay [63]. Since then, various toxicological effects of DEP have been analyzed in a number in vitro cell assays and in vivo animal inhalation experiments.

3.1. Sources of diesel exhaust particles

Various studies of the health effects related to diesel emission have used a broad array of particle preparations. Many authentic urban preparations are too small

to allow large-scale toxicity testing in experimental models, whilst these can be characterized in cell free experiments and cell cultures. Although new technology of concentrated ambient particulates is being introduced, it has not yet received widespread use in genetic toxicology. Whereas most of the studies of the DNA damaging effect of diesel preparations from Japan have employed diesel exhaust or DEP from light-duty passenger cars and vans, other researchers have used several types of standardized reference materials (SRM) from the National Institute of Standards and Technology. Urban air particle preparations from Ottawa, Canada (EHC93) are characterized well and have been investigated in both in vitro and in vivo experiments of lung toxicity, including inflammatory responses [64]. However, to the best of our knowledge genotoxic effects in terms of oxidative DNA damage have not been investigated for EHC93 preparations.

The SRM1650 preparation is particulate matter collected from a heavy-duty diesel engine, operating under a variety of conditions in order be representative of any particulate emission obtained from heavy-duty diesel engines. The material was produced in 1983 and issued in 1985 (it was later re-analyzed and re-bottled as SRM1650a preparation). The SRM 1650a preparation is no longer available from the National Institute of Standards and Technology, i.e. it has been replaced by SRM2975, which is PM collected from a diesel-powered industrial forklift. In addition to samples representative of DEP, standardized urban PM are available for research. The SRM1648 and SRM1649 (later re-analyzed and rebottled as SRM1649a) are urban PM preparations that in the late 1970s were been collected over a period of 12 months in St. Louis and Washington DC, respectively.

Critically, it could be argued the toxicity of most of the authentic PM preparations has not been characterized sufficiently because of limited material. This gives reports on the toxicity of authentic particle preparation somewhat an anecdotal appearance because the results are not easy to reproduce by independent researchers. On the other hand, the preparations from the National Institute of Standards and Technology and Japan are available, but have not been extensively analyzed in several laboratories. Only very recently has the SRM 2975 and the Japanese DEP preparations been compared in the same laboratory trial; this showed that the two preparations differed in respect to in vitro mutagenecity [65] and pulmonary toxicity following intratracheal instillation [66]. Clearly these data outline the need to characterize authentic PM throughout and compare the effect with suitable reference material.

3.2. In vitro studies of oxidative DNA damage induced by air pollution particles

In vitro studies of DEP have yielded relevant information of the mechanisms related to the primary genotoxicity of particles suspended in aqueous or organic solutions. Secondary genotoxicity caused by migrating inflammatory effects are omitted in cell free systems and in cell culture experiments, unless the target cells are co-cultured with inflammatory cells. Oxidative DNA base damage and SB have been extensively investigated in cell free systems and cell culture experiments (Table 1) [13,15,19,20,67–85]. The generation of SB in cell free systems has been assessed as relaxation of supercoiled bacteriophage and plasmid DNA in agarose gel electrophoresis. DEP generation of 8-oxodG in calf thymus DNA has been investigated by HPLC-EC or immunoassays. There is overwhelming evidence for genotoxic effects in cell free experimental settings (Table 1). It appears that DEP generate SB per se in bacteriophage and plasmid DNA, although high doses of DEP and extended incubation periods are the norm in the relaxation assays. There is a tendency that DNA base oxidations in terms of 8-oxodG are generated in the presence of H_2O_2 at lower doses of DEP, whereas high DEP doses are less likely to require H_2O_2 to generate oxidative DNA damage. It is likely that the strand breaking effect and DNA base oxidations are partly a result of metal-catalyzed reactions. The connection between cell free experiments and whole animal experiments is uncertain because of the high doses of DEP and the non-physiological nature of the experiments. It is likely that cell and nuclear membranes as well as the large number metal-sequestering proteins and antioxidants in body fluids and intracellularly will influence metal-catalyzed reactions.

In cell culture experiments, DEP cause SB and 8-oxodG in short-term (few hours) and long-term (24–48 h) incubations. The majority of studies have investigated DEP genotoxicity in A549 lung epithelial cells, which is an immortalized cell line from human lung carcinoma with properties of type II alveolar cells.

Table 1
Oxidative DNA damage induced by particulate matter in vitro

Particle type (solvent)	Concentration (μg/ml)	Time (h)	Endpoint (assay)	Cell or DNA type	Effect	Reference
Cell free experiments						
TSP, PM_{10}, $PM_{2.5}$ from Cardiff, UK (aqua)	50–400	6	SB (supercoil relaxation)	Bactriophage DNA (φX174-RF)	PM_{10} more potent than TSP and $PM_{2.5}$ (without H_2O_2)	[77]
$PM_{2.5}$ urban particles (aqua)	183	1	SB (supercoil relaxation)	Bacteriophage DNA (NX174)	Increased SB (without H_2O_2)	[20]
SRM1648, SRM1649 (aqua)	100–500	0.5	SB (supercoil relaxation)	Bactriophage DNA (φX174-RFI)	Only SB when ascorbate is added	[78]
PM10 urban particles (Edinburgh, UK) (aqua)	3.7 or 7.5 μg/assay	8	SB (supercoil relaxation)	Bactriophage DNA (φX174-RFI)	Increased SB at the lowest dose	[80]
PM10 urban particles (Edinburgh, UK) (aqua)	3.7 μg/assay	8	SB (supercoil relaxation)	Bactriophage DNA (φX174-RFI)	Increased SB	[81]
Diesel exhaust particles	500 μg/ml	2	SB (supercoil relaxation)	Plasmid DNA (pUC)	Increased SB (ascorbate required)	[82]
Urban particles (Leeds, UK) (aqua)	1000	5	SB (supercoil relaxation)	Plasmid (pBR322)	Increased SB (small size particles more potent than large particles)	[85]
SRM1649	1000	2	8-OxodG (HPLC-EC)	Calf thymus DNA	Increased level of 8-oxodG (without H_2O_2)	[13]
Fine urban particles (aqua)	160	1	8-OxodG (dot-blot)	Calf thymus DNA	8-OxodG increased (H_2O_2 required)	[19]
$PM_{2.5}$ and PM_{10} urban particles (aqua)	12.5–200	1.5	8-OxodG (IHC)	Calf thymus DNA	Increased 8-oxodG, and PM_{10} more potent than $PM_{2.5}$ fraction (with H_2O_2)	[75]
Diesel exhaust particles	10000	0.5–3	8-OxodG (HPLC-EC)	Calf thymus DNA	Increased level of 8-oxodG (H_2O_2 required)	[76]
Diesel exhaust particles from light-duty engine (aqua)	Not specified	0.25–2	8-OxodG (HPLC-EC)	Calf thymus DNA	Dose-dependent 8-oxodG increase	[79]
Cell culture experiments						
$PM_{2.5}$ urban particles (aqua)	33	3	SB (comet)	Myeloid leukaemia (K562) Lung epithelia cells (IB3-1)	SB increased in both cell types. Probably no difference between particle samples	[20]
SRM1650 (aqua)	10–500	3, 5, 24	SB (comet)	Lung epithelial cell (A549)	SB increased 100 and 500 μg/ml after 2, 5, 24	[67]
SRM1650 (aqua)	0.016–1.6	48	SB (comet)	Lung epithelial cell (A549) Monocyte (THP-1)	Increased SB at 0.016 μg/ml (THP-1) and 0.16 μg/ml (A549)	[69]

Table 1 (*Continued*)

Particle type (solvent)	Concentration (μg/ml)	Time (h)	Endpoint (assay)	Cell or DNA type	Effect	Reference
SRM1648 (aqua)	0.016–1.6	48	SB (comet)	Lung epithelial cell (A549) Monocyte (THP-1)	Increased SB at 0.016 μg/ml (THP-1) and 0.16 μg/ml (A549)	[69]
Urban particles (Dusseldorf, Germany)	10–100	24	SB (FADU)	Lung epithelial cell (A549)	Increased SB at 10 μg/ml	[15]
Urban particles (Leeds, UK) (aqua)	25	24	SB (comet)	Lung epithelial cell (A549)	Increased SB (small size particles most potent)	[85]
SRM 1649 (aqua)	0.1–100 $\mu g/cm^2$	24	SB (comet)	Fibroblast (MUF)	Increased SB at 10 $\mu g/cm^2$	[83]
PM_{10} subway or street particles (aqua)	5–40 $\mu g/cm^2$ (comet) or 10 $\mu g/cm^2$ (8-oxodG)	4	SB (comet) and 8-oxodG (HPLC-EC)	Lung epithelial cell (A549)	Increased SB at 10 (subway) and 20 (street) $\mu g/cm^2$. Increased 8-oxodG by subway, but not street particles	[84]
SRM1649	4×10^5	2	8-OxodG (HPLC-EC)	Airway epithelial cell (BEAS)	Increased level of 8-oxodG	[13]
Fine urban particles (aqua)	40, 200	3	SB (comet)	Lung epithelial cell (A549)	SB increased at 200 μg/ml	[19]
$PM_{2.5}$ and PM_{10} urban particles (aqua)	50	2	8-OxodG (IHC)	Lung epithelial cell (A549)	Increased 8-oxodG in both preparations	[75]
PM_{10} urban particles (not specified)	5–20	72	SB (comet)	Balb-c mouse fibroblast	SB increased at 5 μg/ml in two samples. Not increased in one sample	[68]
PM_{10} urban particles (dichloromethane)	5–150	2	SB (comet)	Hepatoma (HepG2)	SB increased at 5 μg/ml	[71]
PM_{10} urban particles (dicholomethane)	1–50	24	SB ± ENDOIII/FPG (comet)	Hepatoma (HepG2) Colon carcinoma (Caco-2)	SB (±ENDOIII/FPG sites) increased at 1 μg/ml	[73]
PM_{10} urban particles (aqua or tetrahydrofurane)	5–20 m^3 air equivalents/ml	24	SB (comet)	Leukocytes (±S9 mix)	SB increased at 10 (particle) or 20 (extracts) m^3 air equivalents/ml	[72]
$PM_{2.5}$ urban particles (dichloromethane)	0.8–2.1[a]	72	SB (comet)	Rat 6 rodent fibroblasts	Increased SB and difference in samples collected in different seasons	[70]
TSP, PM10, PM2.5 (acetone or toluene)	1–3 m^3 air equivalents/ml	1	SB (comet)	Leukocytes	Increased SB for all size fractions and extraction procedures	[74]

[a] Concentration differs between samples that are tested 1/4 and 1/8 of the LC_{50} concentrations in cytotoxicity assays.

These properties include the ability to engulf particles and fibers by endocytosis [86]. Also fibroblasts, freshly isolated leukocytes, and cells with metabolic capabilities (HepG2 and Caco-2) have been investigated as target cells. In general, aqueous and organic DEP suspensions (or particle free compounds extracted in water and organic solvents) induce DNA damage in all of the investigated cell types, and there appears to be no requirement for metabolic activation (Table 1). This suggests that both the particle and constituents of particles possess the ability to cause oxidative DNA damage. Moreover, it is striking that a majority of the studies find effects at the lowest dose tested. Although this may be the result of inadequate testing at the low-dose concentrations, it implies that even low doses of bio-persistent particles are hazardous for cells.

3.3. *In vivo studies of DEP and model compounds*

During the last decade many toxicological studies have investigated the mechanisms of DEP-induced adverse health effects. The experimental protocols have included several animal species and different modes of administration, including instillation and inhalation. Several of these studies have reported that diesel exhaust is a pulmonary carcinogen in animals chronically exposed by inhalation and with a dose-related increase in lung tumours [87–89].

The aggregated data from rodent studies of pulmonary exposure to DEP indicate increased levels oxidative DNA damage in lung tissue as summarized in Table 2 [38,61,67,76,79,90–94]. The large majority of the studies have found increased 8-oxodG levels after exposure to DEP (Table 2). One of the largest studies investigated both the dose–response effect and time course of effect following intratracheal instillation of DEP to ICR male mice; this revealed a bell-shaped induction of 8-oxodG in lung tissue with peak effect 12 h after the instillation and 0.2–0.3 mg having the highest effect [79]. Other time course studies have indicated that the level of 8-oxodG was highest 2 days after the instillation [79,90,92,95]. Accumulation of 8-oxodG as a marker of oxidative damage is considered an important factor in enhancing the mutation rate leading to lung cancer. Indeed, 8-oxodG levels were associated with tumour development [76] and formation of 8-oxodG in lung DNA from mice treated with DEP showed a dose dependent increase [96]. Rats exposed to carbon black showed the same type of tumours as the diesel-exposed rats [88]. Studies of intratracheal instillation of poorly soluble particles such as quarts, carbon black, and titanium dioxide have shown increased mutation frequency in type II lung epithelial cells depending on neutrophilic inflammatory responses and production of oxidants by macrophages and neutrophils [97]. The mechanism of carcinogenicity has been considered to be merely associated with inflammatory properties of particles and oxidative stress rather than the absorbed organic compounds [12,98].

A large proportion of inhaled particles is caught in the airways and transported by the mucociliary clearance to the oral cavity and swallowed. Food stuff, e.g. plant material may be polluted with DEP during growth and transport. Accordingly, gastrointestinal exposure to DEP may be important. Rats orally exposed to DEP had higher levels of oxidative stress biomarkers, SB and bulky adducts in the colon epithelium, liver, and lung; the importance of this finding is underscored by the low DEP exposure used in the experiment, i.e. the doses were only slightly higher than relevant human exposures in ambient air [99,100]. A recent study reported unaltered 8-oxodG in embryos 4 h and 2 days after oral exposure of DEP [101]. In the same study there was increased frequency of DNA deletions in the mouse fetus; due to the lack of 8-oxodG lesions the authors consider that DEP particles do not cross the placenta and the deletions arise from transplacental transport of released constituents of DEP. In recent years, few studies have investigated the role of dietary constituents on the tumourigenesis and DNA damaging potential of DEP. High dietary intake of fat significantly enhanced the formation of 8-oxodG in mice lungs compared with DEP and no fat intake [79,96]. There is some evidence that sucrose may possesses either co-carcinogenic or tumour promoter effects in colon carcinogenesis induced by genotoxic carcinogens, e.g. food carcinogens [102,103]. Dietary intake of sucrose, above 10% sucrose by weight, is associated with increased mutation frequency in the colon of Big Blue rats, whereas no mutations were observed in the liver [104]. However, no interaction between a diet with high sucrose and DEP was found on DNA damage in colon and liver of rats [105].

As well as containing co-carcinogens the diet may also process antioxidant activities. The study with

Table 2
Lung effects in terms of oxidative DNA damage in rodents after diesel-exposure

Animal (route)	Type of DE/DEP	Dose of DEP	Total dose (mg DEP/kg bodyweight)	Duration	Results	Reference
Rat (I)	DE (light-duty engine)	1 or 6 mg/m^3	35 or 210[a]	4 weeks	Increased MF, 8-oxodG and DNA adduct levels	[90]
Rat (I)	DE (light-duty engine)	3.5 mg/m^3	36 (1st month)[b]	1, 3, 6, 9 and 12 months	Correlation in time vs. deposition Effect in 8-oxodG, DNA adducts	[76]
Mouse *Nrf2*−/−(I)	DE (light-duty engine)	3 mg/m^3	34[c]	4 weeks	Increased 8-oxodG	[91]
Mouse (n)	SRM1650	20 or 80 mg/m^3	2.5 or 10[d]	1.5 h and 4× 1.5 h	Increased 8-oxodG after single high dose	[61]
Mouse (I)	SRM1650 or SRM2975	20 or 80 mg/m^3	2.5 or 10[d]	4× 1.5 h	Increased SB in BAL cells	[67,38]
Mouse (i.t.)	DEP (light-duty engine)	0.1–0.6 mg and 0.3 mg	3–19[e]	12 h (for 0.1–0.6 mg) or 3, 6, 12 h and 1, 2, 3, 5 and 7 days after treatment (0.3 mg)	Increased 8-oxodG at 0.2 and 0.3 mg (12 h), increased 8-oxodG, peak 2 days after treatment at 0.3 mg, high fat enhancing effect	[79]
Rat (i.t.)	DEP (light-duty engine)	2 or 4 mg	17 or 33[f]	2 or 8 h and 1, 2, 5 or 7 days after treatment	Increased 8-oxodG peak 2 h after treatment, decreased 8-oxodG repair activity, increased mRNA levels after 7 days	[92]
Guinea pig (i.t.)	DEP (SRM 1650)	0.7 and 2.1 mg	1.4 and 4	5 days (after treatment)	Increased SB and 8-oxodG. No DNA adduct effect	[95]
Mouse (i.t.)	DEP (light duty) or airborne particles	1, 2.5, 5 mg (DEP) or 10, 25, 50 mg (airborne particles)	>33.3 (DEP) or >333 (airborne particles)[g]	24 h after a single dose	Increased 8-oxodG after DEP (2.5 mg), no effect of airborne particles	[94]

Point-line indicates exposure by inhalation (I) assuming 100% deposition or intracheal instillation (i.t.).

[a] Assuming 5-week male F344 rats inhale 0.20 m^3/day and weight 80 g.

[b] Based on weight of 3-month female Fisher rat being 180 g (and a rat inhale 0.2 m^3/day). Deposition fraction = 100%.

[c] Assuming mice inhale 0.040 m^3/day and 7-month mice weight 50 g.

[d] Inhalation in mice 0.040 m^3/day.

[e] Assuming the weight of 6-week male ICR mice is 32 g.

[f] Assuming the weight of 7-week female Fisher 344 rat is 120 g.

[g] Assuming weight of 6–7-week male DYY mice is 30 g.

dietary high fat did also investigate the protective effect of β-carotene but found insignificant results in this respect [96]. Lately, rosmarinic acid, with antioxidative activities, has been shown to inhibit DEP-induced inflammation and ROS, and is suggested to be supplemented in the diet for beneficial health effects [106].

DNA repair may modify DNA damage induced by particles, so that upregulation of the involved enzymes may alleviate effects of repeated exposures (Fig. 1 and Table 3). Indeed, a single dose of DEP increased the level of 8-oxodG in the lungs of mice, whereas there was no increase after the same dose given in four daily fractions, possibly due to upregulation of *OGG1* as indicated by increased mRNA levels [61]. In that study there was an inverse correlation between 8-oxodG and *OGG1* mRNA. Similarly, after 4 weeks oral exposure to DEP 8-oxodG levels were unchanged in colon mucosa and liver where *OGG1* expression was increased, whereas formamidopyrimidine DNA glycosylase (FPG) sensitive sites increased in the lungs where *OGG1* expression was unchanged (Table 3) [99,100]. In a study with repeated inhalation exposure to DEP 8-oxodG levels were only increased in *nrf2*−/−mice, whereas no change was observed in wild type mice [91]. Although *OGG1* is considered a housekeeping gene it has both *Nrf2* and *NFYA* recognition sites in the promoter region indicating possible regulation [107,108]. Future studies with *ogg1*−/− and other DNA repair knockout mice may further reveal the importance of their regulation with respect to DNA damage induced by air pollution particles. Inhibition of DNA repair enzymes by metals such as nickel may also modify effects of PM [109,110].

4. Biomonitoring and oxidative stress in relation to air pollution

4.1. Air pollution exposure and biomarkers of oxidative DNA damage

The knowledge concerning mechanisms of action of PM has prompted the use of markers of oxidative stress and DNA damage for human biomonitoring in relation to ambient air [111]. A limited number of studies have been published so far. Ideally, external dose should be carefully assessed in order to provide optimum possibilities to describe relationships with biomarkers of oxidative stress. This can be achieved by the use of personal monitors and/or efforts to achieve large exposure gradients.

The urinary excretion of 8-oxodG has been investigated in 57 non-smoking bus drivers on a workday and on a day off work. On the workday, 8-oxodG excretion was significantly higher in bus drivers from central areas compared with bus drivers from suburban/rural areas of Copenhagen [112]. Another study found increased levels of 8-oxodG by an immunohistochemical method in nasal biopsies from 87 children living in Mexico City as compared with 12 controls from less-polluted coastal towns [113]. In addition, SB in the nasal biopsies was also increased in the children from Mexico City and the level increased two-fold in young men who moved to Mexico City [114]. It should be noted that exposure to ozone may very well be more important than particles in these and several other studies finding enhanced DNA damage in epithelial cells from the upper airways in subjects from Mexico city

Table 3
Interaction between expression of *OGG1* and guanine oxidation measured as 8-oxodG or FPG sensitive sites in experimental animals with repeated exposure to DEP

Species	Exposure route	Total dose[a] (mg/kg body weight)	Organ	8-OxodG/FPG sites	*OGG1* mRNA	Strand breaks	Reference
Mouse	Inhalation	4 or 15	Lung	No change	Increase	Increase	[61]
Mouse *nrf2*+/+	Inhalation	34	Lung	No change	Not determined	Not determined	[91]
Mouse *nrf2*−/−	Inhalation	34	Lung	Increase	Not determined	Not determined	[91]
Rat	Oral	0.024, 0.096, 0.24, 0.96, 2.4 or 9.6	Colon	No change	Increase	Increase	[99]
Rat	Oral	0.024, 0.096, 0.24, 0.96, 2.4 or 9.6	Liver	No change	Increase	Increase	[99]
Rat	Oral	0.024, 0.096, 0.24, 0.96, 2.4 or 9.6	Lung	Increase	No change	Increase	[100]

[a] See Table 2 for assumptions.

[115–118]. Similarly, benzene may appear concomitantly with particles and ozone and induce oxidative DNA damage in white blood cells [28]. In contrast to these studies, a Greek study did not find differences in SB in lymphocytes between 40 subjects from urban areas (central Athens) and 40 subjects from rural areas with half of the subjects of each groups being smokers [119]. There was no difference with respect to SB induced by hydrogen peroxide. Smokers, however, had significantly increased both base line level of SB and hydrogen peroxide-induced SB. In addition, a large study from the Czech republic did not find any difference in the level of SB in mononuclear blood cells of inhabitants of a highly polluted area (Teplice) compared with a low polluted area [120].

A recent study was the first to relate measured personal particle exposure to oxidative DNA damage in non-occupationally exposed subjects [121]. Personal exposure to $PM_{2.5}$ and black smoke was measured four times in 50 students living and studying in Copenhagen. 8-OxodG was measured in lymphocyte DNA and in 24-h urine by HPLC, and SB and FPG sensitive sites were measured in lymphocytes by the comet assay. The 8-oxodG concentrations in lymphocyte DNA was found to be significantly associated with personal $PM_{2.5}$ exposure, whereas the levels $PM_{2.5}$ and other pollutants measured at urban background monitoring stations showed no association with the DNA damage [121]. There was no association between the comet assay or urinary excretion of 8-oxodG and any measure of exposure to particles. Further analysis of the collected samples from the study has shown that the 8-oxodG level was significantly associated with the content of soluble vanadium and chromium in $PM_{2.5}$ material independent of the mass of the material [122]. Vanadium may be a marker of UFP in Copenhagen air and chromium appears to be more related to the fine fraction with brake pads as a potentially important source. In contrast, bulky adducts to DNA were not associated with personal or ambient $PM_{2.5}$ levels in the Copenhagen study [121] and similar lack of association with personal or ambient $PM_{2.5}$ levels was found in a Greek study comparing subjects from Athens and a rural surrounding [123].

In the Copenhagen study on $PM_{2.5}$ the effect of exposure to particles was also assessed on protein oxidation and lipid peroxidation in plasma [124]. There was a significant relationship between personal black smoke exposure and plasma 2-amino-adipic semialdehyde, which is an oxidation product of lysine in plasma proteins and reflects changes in damage over a few days up to several weeks [125,126]. Similarly, lipid peroxidation in terms of plasma malondialdehyde was significantly related to personal $PM_{2.5}$ exposure in women [124]. These results are in agreement with an earlier study of Copenhagen bus drivers, which found 2-amino-adipic semialdehyde and malondialdehyde levels to be significantly higher in bus drivers from central Copenhagen compared with both bus drivers from rural/suburban areas and Copenhagen postal workers [127]. This suggests that inhaled particles can cause protein and lipid oxidation in peripheral blood, which may be important in the pathogenesis of atherosclerosis.

Very recent developments in equipment now also allow study of exposure to UFP, which from a mechanistic point of view may be more relevant than measuring $PM_{2.5}$ or PM_{10} by mass or black smoke by reflectance of material collected on filters. In a study in Cotonou, Benin, four groups each of 30 subjects were compared [128]. The comparison of rural residents, suburban residents, residents along streets with dense traffic and taxi drivers created a strong exposure gradient in relation to UFP because most vehicle transport in Cotonou is based on two-stroke engines and gasoline of poor quality. There was a clear dose–response relationship between level of UFP and FPG sensitive sites and SB in lymphocytes based on comparison of the groups. Similarly, significant associations between the level of FPG sensitive sites in lymphocyte DNA and personal exposure to UFP measured by portable monitors have been found in a yet unpublished study (available online 31 May 2005 from Environmental Health Perspectives doi:10.1289/ehp.762 via http://dx.doi.org).

The studies suggest that even at low exposure concentrations PM may induce systemic oxidative stress with effects on DNA as well as other biomolecules [121,124]. At high levels of exposure as in the study from Benin described above a categorization of exposure by group is sufficient to demonstrate such associations, whereas at lower levels of exposure, such as in Copenhagen, personal monitoring of exposure was required to show associations and there was no significant relationship between urban background particle concentration and DNA damage in lymphocytes. This implies that, compared with urban background parti-

cle concentration, personal exposure is more directly related to the component(s) inducing oxidative stress.

4.2. Genetic susceptibility and oxidative stress related to air pollution

Genetic susceptibility related to functional polymorphisms in genes coding for xenobiotic metabolism, antioxidant and DNA repair enzymes may be expected to modify the levels of oxidative DNA damage caused by exposure to air pollution. Only few studies have addressed the issue with respect to ambient air particles and so far no modifying effects of metabolism enzymes were found on the association between 8-oxodG levels and personal exposure to $PM_{2.5}$ [124]. However, other air pollutants may be present together with particles at levels capable of inducing oxidative DNA damage. Thus, ozone is a well-known oxidant and benzene metabolites can redox cycle with generation of oxidative DNA damage as shown in a number of experimental studies [26,27]. For benzene exposure in urban air the polymorphic NADPH quinone oxidoreductase (NQO1) appears to be effect-modifying [28,129]. NQO1 catalyzes two-electron reduction of quinones and reduce the risk of their redox cycling. A modifying effect on oxidative DNA damage due to benzene exposure is thus expected. This has also been shown in two studies in Copenhagen, Denmark, and Cotonou, Benin, showing associations between oxidative DNA base damage in terms of 8-oxodG or SB and benzene exposure assessed by urinary excretion of the metabolite phenylmercapturic acid in subjects with *NQO1* variant genotypes, whereas there was no significant association in subjects homozygous for the wild type [28,128]. This is consistent with apparently increased susceptibility to benzene toxicity and cancer risk in human subjects with variant *NQO1* genotypes and in *nqo1*−/−mice [129–132]. If urban air particles are present together with high levels of benzene it may be difficult to determine exactly which of these are responsible for the effects on DNA damage levels. Indeed, in the study from Cotonou, Benin, there was a strong exposure gradient with respect to both UFP and benzene [128]. Nevertheless, *NQO1* appeared only to modify the effect of benzene on SB, whereas GSTP1 may have modified the effect of UFP on DNA base damage in terms of FPG sensitive sites in that study.

One study has found increased levels of 8-oxodG after biking in ozone rich ambient air in subjects with the *NQO1* wild type and *GSTM1* null genotype with unchanged levels in other haplotypes. Although ozone is an oxidant, these findings require a complicated mechanistic explanation if repeatable [133].

So far, DNA repair enzymes have not been studied directly in relation to oxidative DNA damage and air pollution. However, the *OGG1* ser326cys polymorphism appeared to modify the risk of lung cancer related to indoor exposure to smoky coal in China, suggesting an important role [134]. Moreover, the previously described experimental studies support a role for OGG1 and its regulation, which could cause differences in DNA damage after short term exposures to particles and long term exposures, where *OGG1* may be upregulated. Similarly, other defence genes, such as *HO-1* may be upregulated and modify effects. This issue needs study in relation to biomonitoring.

5. Conclusion

There is overwhelming evidence from animal experimental models, cell culture experiments, and cell free systems that exposure to diesel exhaust and DEP causes oxidative DNA damage, although dose–response relationships have not been investigated in detail. Especially doses realistic compared to human exposures are seldom investigated. Expressed as the total dose per bodyweight, the doses used in animal experimental models have been in the excess of 1.4 mg DEP/kg bodyweight (or even 10-fold higher in some studies). Assuming a 30% deposition fraction for inhalation of UFP, similar exposure has been used in the inhalation and instillation studies (Table 2). Urban concentrations of UFP typically are measured in the range of 1.7–3.6 $\mu g/m^3$ [135]. Assuming that humans inhale 16 m^3/day and weigh 65 kg, the lowest dose used in the animal experimental models corresponds to 1580 days (or 4.3 years) exposure at the highest ambient concentrations.

In cell culture experiments, justification of doses is rarely stated although this clearly is important for the interpretation of the results in terms of toxicological relevance. It is difficult to compare the potency of different preparations of particles in the cell culture experiments, because the assessment is hampered by a

lack of information of the total number of cells exposed. As an example of dose justification, we have observed elevated level of oxidative DNA damage in cell culture experiments at 25 μg/well (with surface area of wells being 9.6 cm^2 and confluent cell cultures). Assuming the total alveolar surface area of human lung is 75 m^2 and 3% of the cells are type II epithelia cells (presumed target cell for carcinogenesis), this dose corresponds to 1356 days (or 3.7 years) of exposure at the highest ambient particle concentration. Similar calculations for alveolar macrophages as target cells also have illustrated that the doses used in cell culture experiments clearly exceed what is considered realistic exposures in humans [136]. However, in further perspective, mathematical modeling of particle deposition in the airways indicate that cells located in the vicinity of the bifurcations in the bronchial receive 100-fold higher particle doses than the average dose for the whole airways [137].

It is intriguing that virtually similar results of dose justifications are obtained by the cell culture experiments and animal experimental models. It may be speculated that this represent the critical particle/cell ratio that exceeds the detection limit of DNA damage assays. On the contrary, it may then surprise that effects of air pollution can be detected by the same assays of DNA damage in leukocytes in biomonitoring studies. This may be because: (i) humans are more vulnerable than rodents to particle toxicity; (ii) the DNA damage observed in biomonitoring studies is due to synergism between particles and gaseous components, such as ozone, in air pollution; (iii) the effect observed in biomonitoring studies are because of oral (not pulmonary), exposure, i.e. low doses of DEP do not disturb the lung clearance which means that most particles are transported to the pharynx and swallowed; (iv) humans are mainly exposed to UFP which are the most toxic, whereas rodents have been exposed to particles in agglomerated form. The latter explanation implies that exposure characterization based on the mass of particles is invalid for dose–response relationships; surface area or particle number is more reliable exposure markers of particles for comparison between animal experimental models and human biomonitoring.

It appears that in order to demonstrate relationships between external dose of particles in ambient air and biologically effective dose in terms of oxidative DNA damage in human biomonitoring, relatively strong exposure gradients and/or individual exposure monitoring are required. Oxidative damage to DNA and possibly protein and lipid appears to be more sensitive than bulky DNA adducts as biomarker of exposure to ambient particulate air pollution, although the contribution of each of these mechanism to risk of cancer are as yet not determined. Modifying effect related to genetic susceptibility has mainly been demonstrated with respect to benzene exposure, whereas there is limited data on susceptibility genes influencing effects of ambient air particles on oxidative DNA damage. Similarly, possible modifying effects of altered expression of DNA repair and other defense genes have yet to be studied.

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