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REVIEW

8-Hydroxydeoxyguanosine as a biomarker of workplace exposures

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To date, the 8-hydroxydeoxyguanosine (8OHdG) DNA adduct has been used as a biomarker in 11 occupational health studies examining the potential for ten different workplace exposures to cause oxidative DNA damage. Exposures examined include asbestos, azo-dyes, benzene, chromium, coal dust, glassworks, rubber manufacturing, styrene, toluene and environmental tobacco smoke (ETS). Experimental designs that applied 8OHdG as biomarker varied dramatically among the studies. For example, one study detected increased urinary excretion in retired workers with a history of exposure to mining dusts, while a study of workers exposed to benzene showed that the pattern of urinary excretion of 8OHdG varied over a 24 h period following exposure. All but one study reported increased 8OHdG relative to controls, but in three cases the increases were not statistically significant. Only one study demonstrated a dose–response relationship between a chemical exposure (benzene) in the workplace and elevated 8OHdG. In most cases, exposure data were lacking and the elevated 8OHdG could only be considered to be associated with a generalized job category. Numerous animal and human studies have demonstrated an effect of tobacco smoke on 8OHdG, including a study of ETS in the workplace. In the majority of occupational studies, however, smoking was found not to be a confounding variable. 8OHdG levels tended to be higher in women than men as did the response to an occupational exposure and/or smoking. Two of three studies that stratified workers by age found it to be a confounder for the 8OHdG adduct, but the relationship between age and 8OHdG was non-linear. Only one study considered the impact of dietary supplements on 8OHdG levels in workers despite the fact that diet can have a marked effect on an individual's response to oxidative stress. It is premature to consider 8OHdG as biomarker that can be used for decision making or for regulatory purposes. Nonetheless, these studies demonstrate that with additional characterization of the role 8OHdG plays in the exposure–disease continuum it may well serve as a powerful biomonitoring tool in the future.

Keywords: 8-hydroxydeoxyguanosine, DNA, oxidative damage, biomarker

Introduction

Several recent studies have examined the association between oxidative DNA damage and adverse exposures in the workplace (table 1). These studies represent sentinel events in that in the coming years oxidative DNA damage and oxidative stress profiles may well become common endpoints for assessing the adversity of workplace exposures.

Despite the apparent consensus that oxidative damage is an adverse biological response (Ames 1989, Beckman and Ames 1997, Kasai 1997), it is unclear and certainly debatable as to how adverse or how critical it is to the disease process. Several factors contribute to this uncertainty. First, free radical biology is extremely complex and many aspects of it are not well understood (Cheesman 1996). Therefore, linking measurable endpoints with disease often requires

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Table 1. Lymphocyte or urinary 8OHdG in workers with occupational exposures.

Exposure	8OHdG Source (units)	Control 8OHdG (*N*)[e, f]	Exposed 8OHdG (*N*)[e, f]	%Difference E vs C [g]	*P* <	Reference
Asbestos	Urine (μmol mol^{-1} creatinine)	1.07 ± 0.41 (41)	1.40 ± 0.56 (30)	+ 31	0.01	Tagesson, *et al.* (1993)
Asbestos [a]	Lymphocytes (8OHdG /10^{5}dG)	1.0 ± 0.17 (7)	1.03 ± 0.2 (6)	+ 3	NS [h]	Hanaoka, *et al.* (1993)
Asbestos [b]	Leucocytes (8OHdG /10^5 dG)	1.78 ± 1.46 (19)	2.39 ± 1.33 (20)	+ 34	0.05	Takahashi, *et al.* (1997)
Azo-dyes	Urine (μmol mol^{-1} creatinine)	1.07 ± 0.41(41)	1.92 ± 0.85 (30)	+ 79	0.0002	Tagesson, *et al.* (1993)
Benzene	Urine (μmol mol^{-1} creatinine)	—	Range 0.5–3.0 (65)	r = + 0.340	0.01	Lagorio, *et al.* (1994)
Benzene	Lymphocytes (8OHdG / 10^5 dG)	3.74 ± 1.62 (30)	29.89 ± 3.28 (28)	+ 700	0.05	Liu, *et al.* (1996)
Benzene [c]	Urine (μmol mol^{-1} creatinine)	0.72 (30)	0.99 (30)	+ 37	0.002	Nilsson, *et al.* (1996)
Chromium	Lymphocytes (8OHdG/dG)	0.041 ± 0.006(6)	0.037 ± 0.007 (9)	–10	NS	Gao, *et al.* (1994)
Coal dust [d]	Lymphocytes (8OHdG/10^5 dG)	1.67 ± 0.27 (24)	2.96 + 0.34 (30)	+ 77	0.01	Schins, *et al.* (1995)
Glass work	Urine (nmol l^{-1})	11.8 ± 5.7 (49)	13.4 ± 6.1 (181)	+ 14	0.087	Tagesson, *et al.* (1996))
Rubber work	Urine (μmol mol^{-1} creatinine)	1.07 ± 0.41 (41)	1.48 ± 0.57 (28)	+ 38	0.002	Tagesson, *et al.* (1993
Styrene	WBC (8OHdG/10^5 dG)	1.52 + 0.45 (67)	2.23 + 0.54 (17)	+ 47	0.001	Marczynski, *et al.* (1997)
Toluene	Lymphocytes (8OHdG/10^{5}dG)	3.38 ± 1.62 (30)	4.09 ± 1.94 (9)	+ 21	NS	Liu, *et al.* (1996
ETS	Whole blood (pg ug^{-1})	17.2 ± 2.3 (27)	28.0 ± 3.6 (29)	+ 63	0.05	Howard, *et al.* (1998)

[a] Retired dockyard workers with asbestosis.
[b] Working and retired asbestos-factory workers with asbestosis.
[c] Pre- and post-shift (late evening) comparison.
[d] Retired miners with a history of coal dust exposure.
[e] 8OHdG was determined by HPLC-EC in all studies.
[f] Values are mean±SD or SE as presented by authors except as noted.
[g] Percentage difference in 8OHdG (Exposed–Control/Control ×100) except where noted.
[h] P value not reported, *et al.* NS = no significant difference between exposed and control.

considerable conjecture. Second, as oxygen breathing creatures, humans maintain a balance between the continual production of highly reactive oxygen radicals and antioxidant defences (Loft *et al.* 1994). The essential elements of an adequate defence have not been defined. As a result, cross-sectional intervention studies, including energy restriction and antioxidant supplements, have produced mixed results in terms of influencing oxidative DNA damage (Loft *et al.* 1992, Prieme *et al.* 1997) or cancer (ATBC 1994). Third, despite the evidence from experimental studies that support the notion of oxidative DNA damage as an important mutagenic (Taddei *et al.* 1997) and apparently carcinogenic factor (Collins *et al.* 1996), proof of a causal relationship between cancer and a chemical exposure in humans that causes oxidative DNA damage is still lacking. Fourth, although severe oxidative stress may be acutely toxic, it is, for the most part, considered to contribute to chronic degenerative diseases. Therefore, teasing out the role of a specific workplace exposure that is often far removed in time from an adverse outcome from all the other possible factors that can contribute to degenerative disease can be nearly impossible. There is a need for a demonstration that the rate of oxidative DNA damage is an independent risk factor for cancer.

Nonetheless, if an occupational exposure consistently induces an oxidative burden on an individual and this burden results in an increase in DNA damage, then oxidative DNA damage may serve as a biomarker. Furthermore, if the appearance of oxidative DNA damage correlates with dose, then it has the potential to be used as a marker of biologically effective dose. If the effect is considered to be on the pathway to cancer, then the biomarker can be used to determine if a worker maybe be at increased risk of developing cancer as a result of a workplace exposure. Although it may be several years or more before satisfactory answers can be provided for these questions, there is a sound rationale for investigating the contribution of workplace exposures to an individual's oxidative-stress status. In the following article, an overview is provided of the subject and issues are presented that are germane to investigations of worker populations that may be subjected to exposures that increase oxidative DNA damage.

Source of oxidative DNA damage

Any process that leads to the generation of reactive oxygen species can result in oxidative DNA damage (reviewed by Breen and Murphy 1995). Reactive oxygen molecules arise in cells as intermediates or byproducts of aerobic metabolism, from the normal metabolism of endogenous compounds, or from the metabolism of exogenous compounds from the environment. Cells have a host of defence mechanisms that include antioxidant vitamins and enzymes that bind or react with these highly reactive oxygen species. However, a fraction of generated reactive oxygen species escapes antioxidant defences and reacts with cellular lipids, proteins, and nucleic acids in DNA. When the generation of reactive oxygen species exceeds antioxidant capability a condition of oxidative stress ensues (Joenje 1989) and can result in oxidative damage, including an increase in above background levels of oxidative DNA damage. The reactive oxygen species that are formed include the superoxide radical ($O_2^{\cdot -}$), singlet oxygen (O_2^1), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($^{\cdot}OH$). Among these reactive intermediates only the hydroxyl radical and the superoxide radical are capable of directly damaging DNA (Joenje 1989). It is believed that singlet oxygen causes

oxidation of DNA via dismutation to hydrogen peroxide, which forms the hydroxyl radical in the presence of a transition metal such as iron. Copper is a biologically important transition metal that binds to DNA at the N7 of guanine and will produce high levels of oxidative DNA damage in the presence of hydrogen peroxide via Fenton-type reactions (Kennedy *et al.* 1997). Ionizing radiation not only damages DNA directly, but also ionizes water in cells and leads to formation of the hydroxyl radical which goes on to react with DNA and is believed to be the basis for radiation-induced cancer (Kehrer 1993). Nitric oxide is an example of a biological mediator that can form peroxynitrite, which will produce increased oxidative DNA damage and strand breaks (Kennedy *et al.* 1997). An increasing number of organic and inorganic compounds have been found to cause oxidative DNA damage. These include compounds such as benzene (Kolochana *et al.* 1993), potassium bromate (Kasai *et al.* 1987), 2-nitropropane, paraquat, or hydroquinone (Suzuki *et al.* 1995). Fiala *et al.* (1989) have described the possible metabolic steps for 2-nitropropane induced oxidative DNA damage. In an aqueous solution, 2-nitropropane exists in equilibrium with its nitronate anion. Oxidation of this anion produces superoxide, which is dismutated to hydrogen peroxide. In the presence of transition metals, hydroxyl radicals are then formed and are responsible for oxidation of DNA. In the case of benzene, peroxidative metabolism or autoxidation of the phenolic-benzene metabolites produces active oxygen species that are capable of causing oxidative DNA damage (Kolachana *et al.* 1993).

Consequence of oxidative DNA damage

It can be inferred from the above discussion that with so many varied sources of reactive oxidants that the steady-state of oxidative DNA damage would be quite large. Beckman and Ames (1997) have estimated that there may be as many as 1.5×10^5 oxidative DNA adducts per human cell. The majority of these are efficiently repaired. Oxidative DNA damage is removed by nucleoside and base excision pathways that include endonuclease III and Fapy glycolase which remove oxidized pyrimidines and purines (Beckman and Ames 1997). However, in cases where there is a significant increase in hits arising from exposure to chemically induced oxidative stress, repair mechanisms may not be adequate. The consequence is that damaged DNA is copied during replication resulting in increased risk of mutation and possibly cancer. Feig *et al.* (1994) have identified four pathways by which oxidative DNA damage can lead to mutation. The first is through chemical modification of nucleotide moieties in DNA that result in alterations in coding specificity. The second is damage-mediated exacerbation of polymerase-specific hot spot mutations. A third mechanism is damage-induced conformational change in the DNA template that prevents accurate replication of DNA polymerases, and the fourth mechanism involves induction of a polymerase conformational change that is error prone. Each of these mechanisms results in a specific type of damage-induced substitution that constitute the variety of mutations that oxygen free radicals produce.

8OHdG

Oxidative damage in DNA includes a number of specifically oxidized purines and pyrimidines. Breen and Murphy (1995) have identified 13 oxidized bases that are produced by radiation. These include 5-hydroxyuracil; thymine glycol;

4,6-diamino-5-fromidopyrimide; 2,6-diamino-4-hydroxy-5-formamidopyrimidine; and 8-hydroxyadenine. One of the most, if not the most, abundant oxidative DNA adduct is 8-hydroxydeoxyguanosine (8OHdG) (Breen and Murphy 1995, Wang *et al.* 1995). It has been estimated that 8OHdG represents about 5 % of all oxidative adducts (Beckman and Ames 1997). The prevalence and relative ease of measurement of 8OHdG has made it the most popular oxidative adduct to be used in molecular epidemiology studies of increased oxidative DNA damage from occupational exposures (Tagesson *et al.* 1993, Lagorio *et al.* 1994, , Schins *et al.* 1995, Liu *et al.* 1996, Loft and Poulsen 1997). In addition to being abundant, 8OHdG is highly mutagenic resulting in predominately G-T transversions which are frequently found in tumour relevant genes (Kuchino *et al.* 1987, Shibutani *et al.* 1991, Guyton and Kensler 1993), it results from a wide array of treatments that cause oxidative damage, and it has been implicated in carcinogenesis (Park *et al.* 1992). 8OHdG can be measured in hydrolysed DNA from lymphocytes where it represents the steady state between damage and repair. The repair process results in the excised 8OHdG adduct being excreted in the urine. The average daily 8OHdG excretion in humans is approximately 200–300 pmol kg^{-1}, which corresponds to 140–200 oxidized guanine bases $cell^{-1}$ day^{-1} (Loft *et al.* 1993). Assuming a steady state such that the rate of repair will be equal to the rate of damage, the excision product of repair excreted in the urine can serve as a biomarker of oxidative DNA damage (Collins *et al.* 1996).

Methods for measuring 8OHdG

Several methods are available for measuring 8OHdG in human biological samples and considerable discussion has been given as to which methods are most effective (Collins *et al.* 1996, 1997b, Beckman and Ames 1997, Cadet *et al.* 1997). The most commonly used method and the method used in the occupational studies reviewed here is high performance liquid chromatography with electrochemical detection (HPLC-EC) (Tagesson *et al.* 1995). The level of detection for HPLC-EC is in the order of 50 fmole of oxidized base. This corresponds to one oxidized base per 10^6 normal guanine bases (dG) and 8OHdG measured by HPLC-EC is frequently expressed as the ratio of 8OHdG/10^5 dG. Another method showing considerable promise is gas-chromatography coupled with mass spectroscopy (GC–MS), which has the advantage of being able to assess several oxidative DNA adducts at once (Cadet *et al.* 1997). On average the method is as sensitive as HPLC, but there is a concern regarding artifactual oxidation of DNA. In general, 8OHdG measured by GC–MS gives values ten-fold greater than those obtained with HPLC-EC. As with HPLC, hydrolysed DNA is used with GC–MS, but the DNA also must be derivatized before analysis. Both the hydrolysis and derivatization steps can contribute to oxidation of dG and apparently this is responsible for high 8OHdG values (Beckman and Ames 1997). Cadet *et al.* (1997) have outlined the necessary procedures to reduce the overestimation of 8OHdG using GC–MS.

One approach that avoids auto-oxidation of DNA is to measure 8OHdG *in situ*. Two recent studies have used monoclonal antibodies to estimate changes in 8OHdG content of rat kidney slices (Toyikuni *et al.* 1997); and in rat liver sections, cultured hepatocytes, and human buccal cells (Yarborough *et al.* 1996). The use of the antibodies *in situ* is an extension of an ELISA-based assay for 8OHdG (Degan *et al.* 1991, Yin *et al.* 1995). The problem with immunohistochemical or ELISA

methods is that they are semi-quantitative and there is cross-reactivity with other types of DNA damage. However, these disadvantages are outweighed by their ability to detect localized changes in 8OHdG *in situ*, the need for only a small sample, and the absence of need to extract or hydrolyse DNA, which avoids auto-oxidation. Both immunohistochemical and ELISA methods have been used to study changes in 8OHdG in tobacco smokers (Yarborough *et al.* 1996); they have yet to be applied to studies of occupational exposures. Avidin, which has a high affinity for biotin and is widely used in immunoassays, has recently been found to also have a high affinity for 8OHdG (Struthers *et al.* 1998). If avidin proves to have adequate specificity for 8OHdG, there is great potential for the development of simple inexpensive methods for direct detection of 8OHdG.

8OHdG has also been detected by using single cell gel electrophoresis referred to descriptively as the comet assay. Collins *et al.* (1993, 1996) modified the comet assay by including Fapy glycosylase in the assay, which converted oxidized pyrimidines to DNA strand breaks. As with immunohistochemical techniques, it is not necessary to extract or hydrolyse DNA, thereby eliminating the problem of auto-oxidation. Additional methods for measuring oxidative DNA damage include alkaline elution and nick translation ^{32}P-postlabelling (Collins *et al.* 1996, 1997b). Although not used for measurement of of 8OHdG, an ultra sensitive method used to measure the oxidative DNA adduct, thymine glycol has been described (Le *et al.* 1998). This method couples immunochemical recognition with electrophoresis and flourescent detection, which improves detection limits by 4–5 orders of magnitude and should be modifiable for measurement of 8OHdG. However, these latter techniques have not been used for biological monitoring, and in the studies presented here only HPLC-EC has been employed.

Occupational exposure studies

Asbestos

The earliest investigation of oxidative DNA damage resulting from occupational exposures was conducted by Tagesson *et al.* (1993). They examined urinary excretion of 8OHdG in workers exposed to asbestos, rubber processing, or azo-dyes. Asbestos exposures were mainly to chrysotile at levels that did not exceed 1 mg m^{-3}. Jobs with asbestos involved making fabrics, ropes, and asbestos packings. Some workers experienced higher exposures during particular operations, but were equipped during these procedures with respiratory masks or dust-proof helmets. Urine samples were collected randomly both from the first half of the work shift on Monday and the last half of the work shift on Friday. Urinary excretion of 8OHdG from 30 asbestos-exposed workers was significantly greater than excretion from 41 controls. The Friday and Monday values of asbestos-exposed workers were not significantly different.

Asbestos can generate free radicals in cell-free systems containing oxygen, and can augment the release of reactive oxygen intermediates from neutrophils and alveolar macrophages (Kamp *et al.* 1992). Furthermore, asbestos fibres are capable of producing hydroxyl radicals that hydroxylate deoxyguanosine residues from calf-thymus DNA (Leanderson *et al.* 1988). Therefore, there is a mechanistic rationale for asbestos exposures to increased oxidative DNA damage in workers. Because of this, Hanaoka *et al.* (1993) expected 8OHdG levels to be high in

dockyard workers with 19–24 years of asbestos exposure and suffering from asbestosis. However, they found no significant difference between controls and the asbestosis patients. They (Hanaoka *et al.* 1993) concluded that 8OHdG in peripheral blood cells was not a sensitive biomarker of oxidative DNA damage due to past asbestos exposures. Takahashi *et al.* (1997) also examined workers with a history of asbestos exposure who were suffering from asbestosis. They found that 8OHdG in DNA from peripheral leucocytes was significantly elevated in the asbestosis population. Takahashi *et al.* (1997) attributed their contrasting results to two important differences. First, the asbestosis population from the Takahashi *et al.* (1997) study was a homogenous group of active and retired workers with defined asbestos exposure employed by a large-scale asbestos plant in China. This contrasted with the asbestosis outpatients in the Hanaoka *et al.* (1993) study who were retired for at least 5 years from work that involved undefined exposures to asbestos. Second, the control population in the Hanaoka *et al.* (1993) study were outpatients with various diseases other than asbestosis, whereas controls in the Takahashi *et al.* (1997) study were disease-free. It is still uncertain as to whether a fibrotic disease such as asbestosis can lead to elevated 8OHdG in peripheral blood cells. Takahashi *et al.* (1996) speculated that this would be possible if some sort of signalling mechanism occurred between pulmonary cells and peripheral blood cells in the presence of asbestos fibres. Studies in rats intratracheally instilled with silica do not support this as silica increase 8OHdG levels in broncho-alveolar lavage, but not in peripheral blood cells (Yamano *et al.* 1995).

Azo-dyes

Tagesson *et al.* (1993) examined urinary excretion of 8OHdG in 30 workers exposed to azo-dyes. Workers were exposed to the dyes when they loaded the raw material into mixing vats and when they were involved in the drying of dyes during the manufacturing. The workers had contact with mono and polyazo dyes such as C.I. Direct Black, C.I. Acid Black, and C.I. Mord. The raw materials for these dyes were amine- and nitro-derivatives of phenylene- and naphthalene sulphonic acids. Conditions in the factory were unsatisfactory because of insufficient protective measures and only occasional use of personal respirators. Urine samples from azo-dye workers were collected. On Monday morning, 8OHdG averaged 2.15 ± 1.07 μmol mol^{-1} creatinine and on Friday afternoon the value was 1.70 ± 0.88. Friday and Monday values were not significantly different, but both were significantly greater than the control value of 1.07 ± 0.41. The control group consisted of unexposed officer workers from the same city in Poland. Although some azo-dyes have been found to be mutagenic and carcinogenic (IARC 1982) it is not apparent how they would contribute to oxidative DNA damage in workers. Furthermore, the authors indicated that the dye workers were exposed to complex mixtures, therefore, the question remains open as to what was responsible for the increased urinary 8OHdG found in these workers.

Benzene

Benzene and its metabolite phenol were first demonstrated to cause oxidative DNA damage in HL60 cells and in bone marrow of C57/BL6 mice (Kolachana *et al.* 1993). In the bone marrow of mice, 200 mg kg^{-1} benzene produced a five-fold

increase in 8OHdG. Based on the potential for benzene to cause oxidative damage, 65 filling station attendants in Rome, Italy, exposed to benzene in gasoline were evaluated for increased 8OHdG excretion in urine (Lagorio *et al.* 1994). The yearly average exposure to benzene was estimated and this was positively correlated with urinary excretion of 8OHdG. Excretion of 8OHdG was not significantly correlated with age, length of employment, cigarette smoking intensity (among smokers) or with exposure to toluene and xylene. In disagreement with other studies, this study did not detect a significant increase in 8OHdG in smokers relative to non-smokers. In addition to benzene, filling station attendants are exposed to other constituents of gasoline that could cause oxidative stress. They are also exposed to combustion engine exhaust, which is considered a possible human carcinogen by IARC (1994) and increases oxidative DNA damage in humans (Suzuki *et al.* 1995). Therefore, this study only demonstrated an association between increased oxidative DNA damage and an occupation that included a history of benzene exposure.

A weakness of the Lagorio *et al.* (1994) study was that benzene exposures were estimated and not measured. The estimates were yearly exposures and 8OHdG increases probably reflect recent exposures. Recognizing this, Nilsson *et al.* (1996) conducted a study of 33 benzene-exposed workers and examined 8OHdG in urine and DNA single strand breaks in lymphocytes before and after a work shift. Furthermore, the exposure to benzene was measured in the breathing zone of workers on the days that urine and blood samples were taken. Statistically significant differences in urinary 8OHdG were not seen between control and benzene-exposed workers. In fact post-shift levels of 8OHdG were higher in controls. Nilsson *et al.* (1996) reported that although 8OHdG in controls did not differ statistically from exposed workers, 8OHdG in late-evening urine samples from exposed workers were significantly elevated over pre-shift values. Unfortunately, a comparable late-evening measurement was not made in the control population. Therefore, it is not possible to determine if this elevated late-evening 8OHdG is an exposure-related effect. Nonetheless, using regression analysis, the authors (Nilsson *et al.* 1996) state that there was a significant correlation between benzene exposure and the increase in 8OHdG during the shift. However, they did not provide the results of this analysis. Therefore, their claim of a dose–response relationship is not well founded.

Subsequent to the reports that excretion of 8OHdG in urine was increased in benzene-exposed workers, a study examining 8OHdG in lymphocytes from 117 paint workers in an auto factory was reported (Liu *et al.* 1996). This study monitored air benzene, toluene and xylene concentrations with personal samplers. In addition to air samples, urine was collected at the end of the workshift and urinary *trans,trans* muconic acid (TTMA) (a metabolite of benzene) was used as a measure of internal dose. Benzene-exposed workers were placed in a low, medium or high exposure group depending on TTMA excretion, which correlated with benzene air concentrations. Micronuclei in lymphocytes were significantly increased in all three groups of benzene-exposed workers. 8OHdG levels were increased in all three groups relative to controls, but were significantly elevated only in the medium and high exposure groups. In contrast, toluene exposures were inversely correlated with 8OHdG levels. This is consistent with findings in the study of filling station attendants. Interestingly, toluene can inhibit the metabolism of benzene (Nakajima *et al.* 1996) and high toluene exposures may have protected

against the oxidative damage caused by benzene and prevent the significant increase in 8OHdG at low benzene exposures. The investigation by Liu *et al.* (1996) represents an excellent example of the use of biomarkers of exposure (TTMA) and biomarkers of effective exposure (micronuclei and 8OHdG). Furthermore, the biomarker of exposure was demonstrated to be correlated with the exposure assessment made with personal monitors, and the two biomarkers of effective exposure demonstrated that DNA damage was increased in the low exposure group relative to controls. This is significant since the low exposure group was exposed to a benzene concentration of 0.75 ppm, which is less than the U.S. Occupational Safety and Health Administration (OSHA) permissible exposure limit of 1 ppm.

Coal workers with silica exposure

Occupational exposure to mineral dusts can lead to the development of fibrotic lung disease, and reactive oxygen species are thought to be involved in the biological effects of these dusts. Therefore, Schins *et al.* (1995) investigated the possibility that coal workers who had been chronically exposed to silica-containing dust would exhibit increased levels of oxidative DNA damage. They assessed oxidative damage in lymphocytes from 38 retired miners. Eight of the miners had coal-workers pneumoconiosis. The 8OHdG in miners with pneumoconiosis (2.61 ± 0.44) was not different from miners without pneumoconiosis (2.96 ± 1.86). However, the 8OHdG in miners was significantly greater than 8OHdG in controls. The 24 controls were defined as age-matched and non-dust-exposed workers. 8OHdG in miners was not associated with cumulative years of exposure to dust, nor was it found to be related to smoking habits. It has been shown that acute silica exposure in rats increases 8OHdG (Yamano *et al.* 1995). It is not clear how chronic exposure to dusts can continue to induce an oxidative stress long after exposures have ceased. The authors speculated that miners chronically exposed to dust were experiencing an increased level of oxidative stress as a result of their chronic exposure which resulted in elevated 8OHdG levels in peripheral lymphocytes. They support this conclusion by noting that their previous work showed that antioxidant factors in the blood were modified by chronic silica exposure or by the presence of silicotic lesions (reviewed in Schins *et al.* 1995).

Glass workers

Swedish glass workers have an increased risk of dying from cancer of the stomach, colon, and lung and from cardiovascular disease (reviewed in Tagesson *et al.* 1996). They also experience increased risk for prostate and pharynx cancers, and for cerebrovascular disease. In addition to being exposed to hydrofluoric and sulphuric acids, they are also exposed to a number of metals including, antimony, lead, cadmium, chromium and nickel. These compounds have the capacity to promote the generation of reactive oxygen species in humans. This may lead to an oxidative stress with subsequent oxidative DNA damage. Tagesson *et al.* (1996) hypothesized that some of the compounds that increase cancer incidence in glass workers could do so through a mechanism involving reactive oxygen species. To test this hypothesis, they examined 8OHdG in urine of 199 glass workers and also measured a urinary marker of lipid peroxidation, malondialdehyde (MDA). The

control population consisted of 144 workers that performed clerical, warehouse or other service work and were considered unexposed. They found that glass work *per se* did not significantly increase urinary 8OHdG or MDA. However, in male glass workers who smoked urinary excretion of MDA was significantly increased and in females urinary MDA and 8OHdG were significantly increased. Smoking, alone, did not significantly increase either endpoint in this study. The authors concluded that the increased 8OHdG excretion in workers who smoked may be associated with a higher risk of developing free radical-dependent degenerative diseases including cancer. The author's data and views on the contribution of smoking to the outcome of this study are discussed further in the section on smoking.

Chromium

Chromium(VI) is able to induce DNA strand breaks in human lymphocytes *in vitro* and in rat lymphocytes *in vivo*. Chromium(VI) or chromium(V) treatment of isolated DNA leads to the formation of 8OHdG and the mechanism may involve the production of hydroxyl radicals (Gao *et al*. 1994). Gao *et al*. (1994) followed up this observation with an evaluation of 8OHdG in lymphocytes from ten workers exposed to chromium and ten unexposed controls. Chromium-exposed workers had significant elevation of chromium in blood, plasma and urine. The elevated chromium was attributed to a working environment containing air levels of 0.1 mg m^{-3} of chromium. 8OHdG levels in chromium-exposed workers were not significantly different from values obtained from controls. The authors attribute this lack of effect to the low chromium exposure levels. The authors also note, but do not provide data, that although smoking alone did not increase 8OHdG, the combination of smoking and chromium exposure resulted in a small increase in 8OHdG.

Rubber industry workers

Urinary excretion of 8OHdG was compared in workers manufacturing rubber boots and employees of a public health institute (Tagesson *et al*. 1993). The rubber workers attached soles to boots with glue that consisted of naphtha, rubber, carbon black, accelerators and softeners. The footwear section was separated from the varnish and vulcanization sections of the plant. In the rubber plant, urine was collected on Monday morning and on Friday afternoon. 8OHdG excretions were comparable for these two time points and averaged 1.48 ± 0.57 μmol mol^{-1} creatinine, which were significantly greater than the value of controls (1.07 ± 0.41). Because rubber workers were exposed to several potential carcinogens, the causative agent responsible for the 8OHdG increase was not identified.

Styrene

Styrene is considered to be a probable human carcinogen (IARC 1994) and, therefore, is a potential carcinogenic hazard to occupationally-exposed workers. Marczynski *et al*. (1997) examined whether styrene exposure increased 8OHdG in white blood cells of boat builders under workplace conditions. The 8OHdG/dG ratios from 17 styrene-exposed workers was significantly elevated above the mean value from 67 age-matched non-styrene-exposed controls. The authors

(Marczynski *et al.* 1997) examined the effect of age, smoking habits and years of exposure on 8OHdG. They found that workers who had been occupationally-exposed to styrene for more than 10 years showed higher 8-OHdG/10^5 dG ratios in comparison to workers with less than 6 years of occupational styrene-exposure. However, the difference was not statistically significant. 8OHdG levels were not affected by age or smoking habits of exposed workers or controls. Although the authors grouped the workers by years of exposure, they did not provide any information on the nature or extent of the exposure. The workers were defined only as boat builders. Therefore, the increased 8OHdG can not be defined as a styrene-specific effect, and must be regarded as an effect in a specific working population.

Toluene

In their study of benzene-exposed workers, Liu *et al.* (1996) also examined the association between toluene exposure and blood lymphocyte 8OHdG. Workers were grouped into five exposure groups. These included a control (0 mg m^{-3}) group and groups exposed to 90, 288, 566 and 937 mg m^{-3} toluene. The OSHA Threshold Limit Value (TLV) is 766 mg m^{-3}, but the National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) is half that at 375 mg m^{-3}. Regardless of the high exposures, no effect of toluene on 8OHdG was found. In fact there was a statistically significant negative correlation between toluene exposure and 8OHdG. The toluene-exposed workers were also exposed to benzene, which was monitored in workers by measuring the benzene urinary metabolite TTMA. Interestingly the higher the toluene exposure the lower the urinary TTMA. This latter finding is consistent with toluene inhibiting the metabolism of benzene (Plappert *et al.* 1994). Liu *et al.* (1996) postulated that in workers exposed to low benzene and high toluene, the absence of a significant effect of benzene exposure on 8OHdG could be attributed to the inhibition of benzene metabolism by toluene.

Environmental tobacco smoke (ETS)

ETS exposure in non-smokers is linked to a number of diseases; especially lung cancer. Recognizing ETS as a contaminant in a tourism-based workplace in Reno, Nevada, USA, Howard *et al.* (1998) determined if ETS caused oxidative DNA damage in these workers. Study subjects were non-smokers that did not live with smokers and had not taken antioxidant supplements for 6 weeks prior to the study. Self-reported exposures averaged 6.6 hours per day and cotinine (metabolite of nicotine) levels in the exposed population were 65 % greater than controls. Fifty-seven percent of the study subjects were women, but gender was not evaluated as a confounder. Blood 8OHdG levels were 63 % greater in workers exposed to ETS than in controls. Alcohol consumption was 33 % higher in the ETS-exposed group. This was not a significant difference and the authors did not consider it to be a confounding factor. However, Nakajima *et al.* (1996) found alcohol intake to be the only factor among smoking, exercise, stress or nutrition to be associated with leucocyte 8OHdG. Nonetheless, the results of Howard *et al.* (1998) add to the growing body of evidence that ETS is a risk factor for disease. The following section further discusses the link between smoking and 8OHdG.

Confounding factors

Tobacco smoking (table 2)

Cigarette smoke contains a large number of compounds that promote oxidative damage directly (Church and Pryor 1985, Kodama *et al.* 1997, Pryor 1997) or indirectly as the result of reactive oxygen species generated by the increased activation of phagocytes following smoking (Chow 1993). *In vitro* studies have demonstrated the ability of cigarette-smoke condensates to produce the 8OHdG adduct in isolated DNA (Leanderson *et al.* 1988, Leanderson and Tagesson 1989, 1990). Numerous experimental studies in animals and humans have detected the capacity of cigarette smoke to increase the levels of 8OHdG (Kiyosawa *et al.* 1990, Loft *et al.* 1993, Fraga *et al.* 1996, Asami *et al.* 1996, 1997 Helen and Vijayammal 1997, Shen *et al.* 1997). Furthermore, Loft and Poulson (1997) included in their review six studies comparing 8OHdG in smokers and non-smokers and in all six comparisons smokers exhibited significantly elevated 8OHdG levels. Despite these findings, molecular epidemiological studies of workplace hazards have not always found a positive association between smoking and oxidative DNA damage. In a study of coal miners for example, 8OHdG levels in lymphocytes did not differ significantly between current smokers and non-smokers or between former smokers and never smokers (Schins *et al.* 1995). In fact, 8OHdG levels were 40 % less in smokers than in never smokers. Gao *et al.* (1994) also reported that smokers did not exhibit increased 8OHdG in lymphocytes. However, Gao *et al.* (1994) did report that the combination of smoking and chromium exposure increased 8OHdG levels, but no data were presented. Tagesson *et al.* (1993) found a small but non-significant increase in 8OHdG in urine of smokers. However, in workers exposed to rubber compounds or azo-dyes, smokers exhibited lower urinary 8OHdG levels than non-smokers. In another study by Tagesson *et al.* (1996), smoking also caused a small but non-significant increase in urinary 8OHdG excretion. Hanoaka *et al.* (1993), Liu *et al.* (1996), and Takahashi *et al.* (1997) stated that smoking status did not effect 8OHdG levels in their investigations and, therefore, they did not stratify workers by smoking status. These studies are not included in the 12 smoking and non-smoking comparisons shown in table 2. Six of the comparisons shown did not include statistical analyses of the difference between smokers and non-smokers because statistical comparisons were between groups exposed to occupational hazards and controls within smoking and non-smoking populations. In the present assessment, smokers were compared with non-smokers and were grouped either as control, occupationally-exposed or both. In five of these comparisons, 8OHdG levels were decreased in smokers. In four comparisons, 8OHdG levels were increased, but by less than 10 %, which was not statistically significant and could be classified as no change. The remaining three comparisons may constitute a real increase in 8OHdG, but only one was demonstrated to be statistically significant. Taken alone, studies of occupational hazards do not provide convincing evidence that smoking is a confounder. However, considering the overwhelming evidence for cigarette smoking to cause oxidative damage the reason for an absence of a clear effect of smoking in occupational studies is not evident.

Asami *et al.* (1996, 1997) identified two contributing factors to ambiguous results from studies assessing oxidative DNA damage in smokers. The first was that inter-individual variability increased in smokers compared with non-smokers. Most often smokers are viewed as a homogeneous group. In actuality, smoking

Table 2. Comparison of 8OHdG between smokers and non-smokers with comparable workplace exposures.

Exposure	8OHdG Source (units)	Non-smoker 8OHdG (*N*)[d, e]	Smoker 8OHdG (*N*)[d, e]	%Difference S vs NS[f]	*P* <	Reference
Control	Urine (μmol mol^{-1} creatinine)	1.01 ± 0.38 (21)	1.13 ± 0.45 (20)	+12	NT [h]	Tagesson, *et al.* (1993)
Asbestos	Urine (μmol mol^{-1} creatinine)	1.38 ± 0.58 (9)	1.41 ± 0.56 (21)	+ 2	NT	
Azo-dye	Urine (μmol mol^{-1} creatinine)	2.10 ± 0.75 (5)	1.88 ± 0.88 (25)	–10	NT	
Rubber work	Urine (μmol mol^{-1} creatinine)	1.60 ± 0.56 (16)	1.34 ± 0.55 (12)	–16	NT	
Control	Lymphocytes (8OHdG/10^{5}dG)	1.96 ± 0.44 (13)	1.31 ± 0.28 (11)	–33	NS [h]	Schins, *et al.* (1995)
Coal dust [a]	Lymphocytes (8OHdG/10^{5}dG)	3.01 ± 0.40 (20)	2.86 ± 0.65 (10)	–5	NS	
Control	WBC (8OHdG/10^5 dG)	1.51 ± 0.43 (46)	1.55 ± 0.47 (21)	+ 3	NS	Marczynski, *et al.* (1997)
Styrene	WBC (8OHdG/10^5 dG)	2.28 ± 0.61 (8)	2.18 ± 0.47 (9)	–4	NS	
Glass work, female[b]	Urine (nmol l^{-1})	9.3 ± 4.3 (71)	12.1 ± 5.3 (40)	+ 30	0.003	Tagesson, *et al.* (1996)
Glass work, male [b]	Urine (nmol l^{-1})	12.6 ± 5.7 (142)	14.1 ± 6.6 (75)	+ 12	0.078	
Asbestos [c]	Leucocytes (8OHdG /10^5 dG)	—	—	+ 0.22 [g]	NS	Takahashi, *et al.* (1997)
Benzene	Urine (μmol mol^{-1} creatinine)	1.32 ± 0.50 (22)	1.41 ± 0.46 (33)	+ 7	0.74	Lagorio, *et al.* (1994)

[a] Retired miners with a history of coal dust exposure.
[b] Includes both the control group and glass workers.
[c] Working and retired asbestos-factory workers with asbestosis.
[d] 8OHdG was determined by HPLC-EC in all studies.
[e] Values are mean± SD or SE as presented by authors except as noted.
[f] Percentage difference in 8OHdG (smokers–non-smokers / non-smokers ×100).
[g] Spearman correlation coefficient, smoking status vs 8OHdG.
[h] *P* value not reported, NS = No significant difference between smokers and non-smokers, NT = not tested for statistical significance.

habits vary greatly. For example Gao *et al.* (1994) noted that the smokers in their study were not heavy smokers and smoked between 10 and 20 cigarettes per day; Nilsson *et al.* (1996) noted that some smokers in their study only smoked two cigarettes per shift. Furthermore, Shen *et al.* (1997) reported that 8OHdG levels correlated positively with cotinine (biomarker for nicotine) levels, supporting the conclusion that dose is important. Therefore, lumping cigarette smokers into a single group and comparing them with non-smokers may be inadequate for determining the role of tobacco smoke as confounder of occupational exposures affecting oxidative DNA damage.

Asami *et al.* (1996) also found that in addition to increased oxidative DNA damage, tobacco smoking increased DNA repair capacity, suggesting activation of a compensatory mechanism. Increased damage and repair should be reflected as an increase in byproducts of repair in studies that analyse urine for 8OHdG. The present assessment of studies of smokers, however, does not support this conclusion. This is due to the fact that urinary analysis for 8OHdG was no more effective in demonstrating that smoking increased oxidative DNA damage than analysis of peripheral lymphocytes. An additional factor that needs to be considered is that the repair of oxidative DNA damage is rapid with the potential for significant repair occurring within 1 h of damage (Collins *et al.* 1997a). It may be that investigation of occupational hazards, with the focus on the workplace exposure, do not play close enough attention to how factors such as smoking can impact results.

Nonetheless, data from smokers have revealed several important elements that have to be included in any experimental design using oxidative DNA damage as a biomarker of biologically effective dose. These are that oxidative DNA damage can occur very rapidly following exposure. Once the damage occurs it can be repaired very rapidly. Exposed individuals may have induction of repair capacity so that net damage may not be increased, and lastly inter-individual variability is quite high and this includes the individual's response to exposure.

Gender (table 3)

Few studies have considered sex in the assessment of chemically induced oxidative DNA damage. Tagesson *et al.* (1993) reported that urinary excretion of 8OHdG was comparable in control men and women, and asbestos exposure was associated with significantly increased urinary 8OHdG excretion in women, but not with men. In contrast to the sex discrepancy among men and women exposed to asbestos, the same study reported that 8OHdG was significantly increased to a comparable degree in both men and women working in the vulcanized rubber industry. In a study of benzene-exposed workers, there was no difference between the levels of lymphocyte 8OHdG in male and female controls (Liu *et al.* 1996). However, in the benzene-exposed groups, 8OHdG in women was significantly higher than in men at the same level of exposure. Although statistical analysis was not conducted, the response in women with exposure to glass work was 5–10-fold greater than the response in comparably exposed men. However, this effect was seen only in women who smoked. Non-smoking women were less affected by glasswork than non-smoking men. In the small data set shown in table 3, 8OHdG was higher in women than in men in five out of eight comparisons. In terms of response to exposure (net change) 8OHdG was increased to a greater extent in

Table 3. Comparison of 8OHdG between men and women with comparable workplace exposures.

Exposure	Source (units)	Men 8OHdG (N)[a, b]	Woman 8OHdG (N)[a,b]	%Difference W vs M[c]	$P <$	Reference
Control	Urine (μmol mol^{-1} creatinine)	1.04 ± 0.41 (21)	1.10 ± 0.50 (20)	+ 6	N T[d]	Tagesson, *et al.* (1993)
Asbestos	Urine (μmol mol^{-1} creatinine)	1.22 ± 0.40 (18)	1.93 ± 0.78 (9)	+ 58	N T	
Net change 8OHdG		*+0.18*	*+ 0.83*	+ 361	N T	
Control	Lymphocytes (8OHdG/10^{5}dG)	3.22 + 1.49 (17)	4.19 + 1.69 (13)	+ 30	N S[d]	Liu, *et al.* (1996)
Benzene	Lymphocytes (8OHdG/10^{5}dG)	8.96 + 1.95 (13)	33.99 + 2.48 (11)	+ 279	0.05	
Net change 8OHdG		*+ 5.74*	*+ 29.8*	+ 419	N T	
Control (non-smoker)	Urine (nmol l^{-1})	11.1 + 5.6 (37)	9.56 + 4.4 (63)	–14	N T	Tagesson, *et al.* (1996)
Glass Work (nonsmoker)	Urine (nmol l^{-1})	13.1 + 5.7 (105)	7.29 + 3.8 (8)	–44	N T	
Net Change 8OHdG		+ 2.0	–2.27	–214	N T	
Control (smoker)	Urine (nmol l^{-1})	13.7 + 6.1 (12)	11.0 + 4.9 (31)	–20	N T	Tagesson, *et al.* (1996)
Glass Work (smoker)	Urine (nmol l^{-1})	14.2 + 7.0 (63)	15–9 + 4.8 (9)	+ 12	N T	
Net Change 8OHdG		*+ 0.5*	*+ 4.9*	+ 880	N T	

[a] 8OHdG was determined by HPLC-EC in all studies
[b] Values are mean ± SD or SE as presented by authors
[c] Percentage difference in 8OHdG (Women–Men / Men ×100)
[d] P value not reported, NS = No significant difference between women and men, NT = Not tested for statistical significance

women than in men in three out of four comparisons. It may well be that women experience more oxidative DNA damage from a specific exposure than do men, but additional data will be needed to substantiate this proposal.

Age

Fraga *et al.* (1990) were the first to examine the relationship between oxidative DNA damage and aging. They found an age-dependent increase in 8OHdG in liver, kidney and intestine of rats. The increase in organ 8OHdG was accompanied by a decrease in urinary excretion of 8OHdG. The authors (Fraga *et al.* 1990) attributed these results to a reduction in DNA repair, but could not rule out an increase in the rate of oxidative DNA damage. Although the role of decreased repair activity is controversial, there is considerable evidence that elevated DNA damage occurs in senescence (Mullaart *et al.* 1990, Shaddock *et al* . 1993, Barnett and King 1995, Bohr and Anson 1995). Hertog *et al.* (1997) reported decreased excretion in 8OHdG in humans with increasing age, which is consistent with a decreased repair capacity. However, Nakajima *et al.* (1996) found no association between age and steady state 8OHdG in human leucocytes; suggesting repair capacity is not hindered. One possible explanation for the disparity among studies is the non-linear relationship that may occur between aging and steady state levels of 8OHdG (Kaneko *et al.* 1996). Hayakawa *et al.* (1993), who also found a non-linear relationship between aging and 8OHdG, proposed that the sharp increase in 8OHdG that occurs in senescence is correlated with a change in mitochondrial electron transport chain function. Regardless of the mechanism, it is evident that some consideration must be give to age as a potential confounder in occupational studies using 8OHdG as a biomarker. Only three studies reviewed here considered age as a possible confounder. Lagorio *et al.* (1994) found a significantly lower concentration of 8OHdG excretion in urine among workers aged 40–45 in comparison with younger and older workers. However, regression analysis of age and 8OHdG did not result in a significant correlation. Interestingly, in a study of industrial art glass workers, individuals aged between 36 and 49 also had lower urinary 8OHdG levels than younger and older workers, but the differences were very small and non-significant. This contrasted with an earlier study (Tagesson *et al.* 1993) which found a significant positive increase in urinary 8OHdG excretion with age in the control population–a finding consistent with animal studies (Fraga *et al.* 1990). However, the effect was not evident in controls who also smoked cigarettes. Furthermore, the trend with aging in non-smoking workers exposed to asbestos, rubber compounds or azo-dyes varied depending on the exposure. Therefore, it is evident that additional human studies are necessary to clarify the issue of age and its contribution to occupational studies.

Dietary supplements

Only one study (Howard *et al.* 1998) reviewed here considered the impact of diet or dietary supplements on 8OHdG levels. They recruited only individuals that had not used vitamin supplements for at least 6 weeks prior to the study. Furthermore, they measured serum vitamin C, α-tocopherol, and β-carotene in the study volunteers. There was a non-significant increase in β-carotene and a significant increase in α-tocopherol in the participants exposed to ETS. The

authors suspected that these antioxidants would be decreased in the ETS group. They speculated that the significant increase in α-tocopherol could be the result of a biological over-compensation to the oxidative stress of ETS. The unexpected results in this study illustrate the complexity of the issue and the need to understand all the factors that must be considered when assessing an individual's antioxidant status.

Despite the conflicting information on the role of antioxidants in preventing cancer (ATBC 1994), antioxidant status must be considered as a possible confounder in molecular epidemiological studies concerned with oxidative DNA damage. This is because *in vitro* studies have consistently demonstrated that antioxidants such as vitamin E, vitamin C or carotenoids can prevent the formation of oxidative DNA damage either in isolated DNA or cultured cells (Duthie *et al.* 1996, Cozzi *et al.* 1997, Sweetman *et al.* 1997). Furthermore, studies with experimental animals administered dietary supplements have demonstrated protective effects of antioxidants against increases in 8OHdG (Cho *et al.* 1995, Hasegawa *et al.* 1995). These studies have been supported by some human studies that also show that diets rich in antioxidants can impact oxidative DNA damage (Pool-Zobel *et al.* 1997, Verhagen *et al.* 1997). However, other studies have failed to detect an effect of dietary antioxidants on 8OHdG levels (Nakajima *et al.* 1996, Hertog *et al.* 1997); attempts to define which specific constituent of the diet such as vitamin E, vitamin C or β-carotene is responsible for the protective effect results have been inconsistent and even contrary to the notion that antioxidant supplements can protect against elevated oxidative DNA damage (Umegaki *et al.* 1993, Prieme *et al.* 1997). These results led Prieme *et al.* (1997) to conclude that the cancer-protective effect of fruits and vegetables seems to rely not on the action of a single antioxidant but rather on other anticarcinogenic compounds or on a combination of several micronutrients present in foods. This latter conclusion is consistent with the findings of Chen (1989) that vitamin C could act as an prooxidant or antioxidant and that the antioxidant capacity of vitamin C was dependent upon vitamin E status. An additional factor that must be considered is the individuals whose diets include a high percentage of fats. Shuker and Benford (1997) note in their review that women who consumed at least 30 % of their total caloric intake as fat had significantly elevated oxidative DNA damage. These studies make it evident that the antioxidant status of an individual appears to be a critical factor affecting the outcome of exposure to oxidants and/or chemicals producing reactive intermediates that can result in oxidative damage.

Occupational health and biomonitoring of workers for oxidative DNA damage

A critical element of occupational safety and health is early detection of health effects arising from hazardous work-place conditions. Enhanced knowledge of molecular mechanisms of disease will make it increasingly possible to detect intermediate stages of the disease process well before the disease has manifested itself. In terms of chemical toxicity, biomarkers have become increasingly sensitive and specific (Shaham and Ribak 1996, Perera 1987). They provide a measure of the internal dose, the biologically effective dose, and early biological effects (Perera *et al.* 1991, Hattis and Silver 1993, Schulte 1993). To date, the aflatoxin B_1–DNA adduct (AFB_1–DNA) is the only biomarker of DNA damage that has been used in prospective epidemiological studies (reviewed by Shuker and Benford 1997). There is ample evidence that AFB_1 is a heptocarcinogen. AFB_1 binds to liver DNA in a

linear dose-dependent manner and good correlations occur between administered AFB_1 and the AFB_1–DNA adduct. There is also a good correlation between dietary AFB_1 in humans and urinary excretion of AFB_1–Gua. DNA adducts formed by AFB_1 are associated with high incidences of a specific mutation in the p53 tumour suppressor gene, and case-control studies show a highly significant association between urinary AFB_1–Gua and hepatocellular carcinoma. Therefore, urinary AFB_1–Gua can be used as a biomarker for estimating the risk of cancer from AFB_1 exposure. Interestingly, administration of AFB_1 to rats results in increased 8OHdG in hepatic DNA (Shen *et al.* 1995). The authors concluded that oxidative DNA damage could constitute a pathway to AFB_1 hepatocarcinogenesis. If true, it illustrates how an environmental agent like AFB_1 can have multiple pathways to cancer, which can provide alternatives for biomonitoring. Despite oxidative DNA damage being a possible mechanistic route for cancer from AFB_1, using 8OHdG as a biomonitor for AFB_1 exposure probably does not constitute a method of choice because of the availability of urinary AFB_1–Gua. This example illustrates both the advantage and disadvantage of a non-specific biomarker like 8OHdG which serves as an index of increased oxidative damage and provides only circumstantial evidence as to the source of the damage. However, the biomarker AFB_1–Gua represents the exception rather than the norm, as most biomarkers of chemical exposure have not been validated to the extent that AFB_1–DNA has. From a practical perspective, a specific biomarker such as AFB_1–Gua, while providing important information for a specific exposure, cannot serve as a versatile biomonitoring tool applicable to a variety of occupational exposures.

The present review demonstrates the broad applicability of the 8OHdG adduct. While several of these studies have severe shortcomings, the investigation of Liu *et al.* (1996) on 8OHdG in benzene-exposed workers represents the most effective application of 8OHdG in a molecular epidemiological study and should serve as a paradigm for future studies. The study included strong biological plausibility based on theoretical grounds backed by *in vitro* studies and studies with experimental animals that demonstrate benzene's capacity to induce oxidative DNA damage. In the application of 8OHdG to determine if workplace benzene exposure posed a biological hazard, Liu *et al.* (1996) not only assessed environmental levels of benzene, but included a biomarker of internal dose (TTMA). The results of the study were convincing because the authors found a strong dose–response relationship between TTMA and 8OHdG.

The ultimate goal of occupational safety and health practice is prevention, and risk assessment is an important element of preventing adverse outcome of occupational chemical exposure. There are few applications of biomarker data to quantitative risk assessment of carcinogens (Calleman *et al.* 1978, Schulte 1991, La and Swenberg 1996). Despite limited characterization of the relationship between cancer and chemical–DNA adducts or other types of DNA damage the use of such surrogates in risk assessment is promising (Schulte and Mazzuckelli 1991, La and Swenberg 1996, Poirier and Weston 1996). This is especially important for cancer risk assessments which usually rely on animal bioassays. Dose–response data in humans obtained from biomarkers can provide a mechanistic link between animal studies and human exposures, which may reduce the assumptions and uncertainties that arise from high-dose to low-dose and interspecies extrapolations. They can also provide a means for gathering relevant epidemiological data that are more proximate in time to exposures and carry more statistically analysable information

for each observation. Again, the benzene study of Liu *et al.* (1996) is the only investigation reviewed here that provides a data base that meets the essential criteria (Perera and Santella 1993) for a cancer risk assessment, which includes adequate sample size, appropriate controls, low variation in assay results, consideration for confounding variables, and data on exposure.

The studies presented here show promise for 8OHdG as a biomarker in investigations of the potential risk that may be associated with a chemical exposure. Before this promise can be fulfilled several questions need to be addressed. First and foremost the association between increased oxidative DNA damage and cancer or other disease needs to be more clearly defined. This will be accomplished only when case controls studies are completed. Adequate case control studies will be aided by a better understanding of the precise relationship between an occupational exposure and the formation of the 8OHdG adduct. Establishing this relationship will be promoted by establishing the normal or acceptable range of oxidative DNA damage and by knowing when elevated levels should be a concern of the individual. Having and using this knowledge will require standardization of methods for determining and recording oxidative DNA damage. In regard to this latter issue little attention has been focused on the relationship between urinary excretion of 8OHdG and the steady state levels in lymphocytes. All of the studies reviewed examined only one 8OHdG endpoint. Future studies should consider measuring both steady state oxidative DNA damage in blood or buccal cells and the byproducts of oxidative DNA damage repair in urine.

Future studies

Studies *in vitro* and with experimental animals have identified several types of exposures that may pose a risk for workers in terms of inducing elevated oxidative DNA damage. For example diesel exhaust particles produce oxygen radicals *in vitro* (Vogl and Elstner 1989) and can induce formation of the 8OHdG adduct in an aqueous DNA solution (Seto *et al.* 1994). Furthermore, a close correlation between lung 8OHdG and cancer was found in mice exposed to diesel exhaust particles (Ichinose *et al.* 1997). It is reasonable to speculate that diesel exhaust is responsible for the oxidative DNA damage occurring in volunteers exposed to auto emissions (Suzuki *et al* 1995). In occupations such as mining where diesel exhaust exposure is common, workers may be experiencing elevated oxidative DNA damage. A number of pesticides have been found to be rodent carcinogens at relatively high doses. These compounds have the potential to cause cancer in pesticide applicators. Although no human studies have assessed oxidative stress in pesticide applicators, a recent study has demonstrated that two of 15 pesticides encountered as residue in food increased 8OHdG levels in livers of exposed rats (Lodovici *et al.* 1997). In one study presented here, the effect of coal dust exposure was examined (Schins *et al.* 1995) and the authors noted that miners were also exposed to silica. Although this study was conducted long after exposures were terminated, a study using rats demonstrated that a single instillation of silica produced a statistically significant increase in 8OHdG in lung tissue but only a non-significant increase in 8OHdG in peripheral blood cells (Yamano *et al.* 1995). Therefore, 8OHdG has the potential to serve as biomarker of effect in silica-exposed workers, but an alternative to blood such as buccal or nasal swabs which contain cells directly exposed to silica will be required as a source of DNA.

Although studies investigating the effects of exercise on oxidative DNA damage have produced mixed results (Okamura *et al.* 1997, Sumida *et al.* 1997), they give credence to the idea that excessive physical activity at work could elevate an individual's oxidative DNA damage level. This is because during aerobic metabolism, oxygen is reduced to water and a small fraction of reactive oxygen species leak out during the process. Physical exercise increases oxygen uptake and metabolism thereby enhancing the leak of free radical species and subsequently increasing oxidative stress (Ji 1995). Consistent with this is the finding that oxidative DNA damage is associated with metabolic rate (Adelman *et al.* 1988). When this is considered in light of the recent observation that psychological stress elevates liver 8OHdG in rats (Adachi *et al.* 1993), it is apparent that using a non-specific biomarker of DNA damage such as 8OHdG will require close attention to experimental design and interpretation of results. Interpretation of results can be eased if investigators include in their experimental designs additional methods for assessing DNA damage such as the comet assay (Collins *et al.* 1993, 1996). Also appropriate are alternative biomarkers of oxidative stress such as the F_2 isoprostanes, which are stable peroxidation products of arachidonic acid that are excreted in the urine (Reilly *et al.* 1996). Use of specific biomarkers of exposure (best exemplified here by TTMA for benzene (Liu *et al.* 1996)) in conjunction with 8OHdG will enhance the understanding of the role 8OHdG plays in the exposure-disease continuum. Close attention should also be paid to the kinetics of oxidative DNA damage, as studies of smokers have demonstrated the rapid appearance of the 8OHdG adduct (Kiyosawa *et al.* 1990), which can be quickly excised (Yamaguchi *et al.* 1996).

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