Biomarkers for Exposure to Ambient Air Pollution—Comparison of Carcinogen–DNA Adduct Levels with Other Exposure Markers and Markers for Oxidative Stress

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Human exposure to genotoxic compounds present in ambient air has been studied using selected biomarkers in nonsmoking Danish bus drivers and postal workers. A large interindividual variation in biomarker levels was observed. Significantly higher levels of bulky carcinogen–DNA adducts (75.42 adducts/10^8 nucleotides) and of 2-amino-apidic semialdehyde (AAS) in plasma proteins (56.7 pmol/mg protein) were observed in bus drivers working in the central part of Copenhagen, Denmark. In contrast, significantly higher levels of AAS in hemoglobin (55.8 pmol/mg protein), malondialdehyde in plasma (0.96 nmol/ml plasma), and polycyclic aromatic hydrocarbon (PAH)–albumin adduct (3.38 fmol/μg albumin) were observed in the suburban group. The biomarker levels in postal workers were similar to the levels in suburban bus drivers. In the combined group of bus drivers and postal workers, negative correlations were observed between bulky carcinogen–DNA adduct and PAH–albumin levels (p = 0.005), and between DNA adduct and γ-glutamyl semialdehyde (GGS) in hemoglobin (p = 0.11). Highly significant correlations were found between PAH–albumin adducts and AAS in plasma (p = 0.001) and GGS in hemoglobin (p = 0.001). Significant correlations were also observed between urinary 8-oxo-7,8-dihydro-2′-deoxyguanosine and AAS in plasma (p = 0.001) and PAH–albumin adducts (p = 0.002). The influence of the glutatione *S*-transferase (GST) M1 deletion on the correlation between the biomarkers was studied in the combined group. A significant negative correlation was only observed between bulky carcinogen–DNA adducts and PAH–albumin adducts (p = 0.02) and between DNA adduct and urinary mutagenic activity (p = 0.02) in the GSTM1 null group, but not in the workers who were homozygotes or heterozygotes for GSTM1. Our results indicate that some of the selected biomarkers can be used to distinguish between high and low exposure to environmental genotoxins. *Key words*: adducts, air pollution, biomarkers, genotoxic exposure, human, oxidative stress. *Environ Health Perspect* 107:233–238 (1999). [Online 9 February 1999]
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A number of epidemiological studies have indicated that people living in urban areas have an increased risk of lung cancer compared to people living outside metropolitan areas, but the extent to which urban air pollution contributes to the excess risk remains unknown (*1*). In addition, a synergistic effect of smoking and ambient air pollution has been observed in people living in urban areas. An increased risk of cancer has also been reported in occupations with heavy exposure to traffic-generated air pollution (*2*). A recent Danish cohort study suggested that smoking habits explained about 60% of the excess lung cancer risk for men in the Copenhagen area (*3*).

Ambient air contains a complex mixture of chemical compounds either in the gaseous form or bound to particulate matters. Some of these compounds are carcinogenic in experimental animals. Ambient air pollution in urban areas originates mostly from automobile exhaust, but there is also some contribution from residential heating and industrial emissions. Incomplete combustion of fossil fuels is a major problem in terms of air pollution. Heavy diesel engines, buses, and trucks contribute approximately 50–60% of the traffic emission in urban areas, and exhaust from diesel-powered engines has been classified as a Group 2A carcinogen by the International Agency for Research on Cancer (IARC), whereas gasoline-engine exhaust has been classified as a Group 2B carcinogen (*4*). A source apportionment study suggested that compounds present in automotive exhaust account for 55% of the estimated cancer cases due to ambient air pollution, and that most of the carcinogenic material was bound to particulate matters (*5*). The level and type of carcinogenic compounds depends on the engine, fuel type, and driving conditions (*6*).

Different biomarkers have been used to assess exposure to genotoxic compounds in the general and occupational environment, ranging from the presence of mutagenic activity in urine to induction of chromosomal damage in peripheral lymphocytes. Polycyclic aromatic hydrocarbons (PAHs) are a major group of carcinogenic compounds in ambient urban air, and most recent biomarker studies have focused on assessing PAH exposure. Urinary PAH metabolites, particularly 1-hydroxypyrene (1-HOP), have been detected in a high percentage of human urine samples, especially from smokers and people occupationally exposed to PAHs (*7*). Carcinogenic PAHs will, following metabolic activation, form adducts with cellular macromolecules, and these reaction products have been used extensively to assess exposure. PAH exposure in urban and rural environments has been determined by PAH–albumin adducts using a competitive immunoassay (*8,9*). Surprisingly, the highest adduct level was observed in people living in rural areas, and it was postulated that combustion of cereal straws and wood for energy production was the major PAH source. The ^{32}P-postlabeling method has been used extensively to detect bulky carcinogen–DNA adducts in peripheral lymphocytes from occupationally exposed people and people living in areas with different levels of ambient air pollution (*9–11*). An increased level of adducts has been observed in nonsmoking bus drivers working in the center of Copenhagen (*12*). A result of primary damage to DNA is gene mutation or chromosomal mutations, e.g., aberrations. An increased level of chromosomal aberration has been reported in Danish bus drivers compared with postal workers (*13*).

Urban air pollution contains large amounts of oxidants, including NO_x and compounds that induce the generation of reactive oxygen species (ROS). During the metabolism of oxygen, e.g., P450-mediated and intracellular processes, several ROS are

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formed, e.g., free radicals. Excessive generation of ROS within tissues can damage macromolecules such as DNA, lipid, and protein. The oxidation of membrane lipids, one of the primary events in the oxidative cellular damage, can be assessed by measurement of plasma malondialdehyde (MDA), a breakdown product of lipid peroxides. Increased levels of MDA have been observed in smokers (*14*) and in workers exposed to heavy metals, e.g., lead (*15*). ROS produces irreversible modifications in DNA, e.g., 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), a promutagenic lesion (*16,17*). An increased level of 8-oxodG has been detected in urine from smokers (*18*), and administration of diesel exhaust to mice resulted in an increased level of 8-hydroxydeoxyguanosine in lung DNA (*19*). ROS damage to proteins is often complex and irreversible, leading to amino acid oxidation, decarboxylation, and deamination (*20*), but none of these lesions have been linked causally to human disease. Chemically induced stress in experimental animals resulted in an increased level of 2-amino-adipic semialdehyde (AAS) and γ-glutamyl semialdehyde (GGS), which are oxidation products of lysine and argine/proline, respectively (*21*).

The objective of this study was to evaluate the burden with environmental toxicants present in ambient air using various biomarkers and to compare the predictive values of these biomarkers. The selected population consisted of nonsmoking bus drivers and mail carriers—people assumed to be exposed extensively to ambient air pollution, mostly generated by incomplete combustion of fossil fuels.

Materials and Methods

Study subjects. The study group consisted of nonsmoking bus drivers employed by the Copenhagen bus company that operates in the metropolitan area, and nonsmoking mail carriers employed in the metropolitan area. The study participants volunteered to participate in the study after written invitation. The group of bus drivers included a total of 107 healthy Caucasians (81 men and 26 women), with a mean age of 45 years (range 27–60). The drivers were classified into two groups, i.e., city center (high) and suburban and semirural areas (low), by two independent investigators according to estimated exposure based upon traffic densities and street configurations. The group of mail carriers included a total of 102 persons (70 men and 32 women), with a mean age of 38 years (range 20–60). The mail carriers covered both city center and suburban areas and walked or bicycled during duty hours. As the mail carriers were spending less time outdoors than the bus drivers, their exposure could be classified as medium to low. Based on operational street pollution models, it was estimated that the pollution in the city center was significantly higher than in the suburban/rural areas.

All participants filled in a questionnaire concerning potential confounders that might influence biomarker levels, e.g., exposure to tobacco smoke, eating habits, residence, and activities involving exposure to combustion products. The local ethics committee, in accordance with the Helsinki declaration, approved the study and the participants signed an informed consent form.

Samples from both groups were collected in the period August–November. Samples from bus drivers were collected in 1994 and from mail carriers in 1995. Blood samples (total 90 ml) were collected in nine tubes and processed into plasma, serum, erythrocytes, and lymphocytes. Heparinized blood (60 ml) was used to isolate peripheral mononuclear white blood cells (PMBC) by the standard Lymphoprep (Nycomed-DAK, Copenhagen, Denmark) procedure. All samples were processed within 4 hr of collection and distributed to the participating laboratories for analyses. Twenty-four-hour urine samples were collected. The processed blood samples were kept at -70°C and urine samples at -20°C until analysis. All samples were analyzed blinded.

Quantification of B and T lymphocytes. Specific monoclonal antibodies toward B lymphocytes (anti-Leu-12, CD19) and toward T lymphocytes (anti-Leu-4, CD3) were added to a cell sample of the PMBC (Simultest CD3/CD19, Becton-Dickinson, San Jose, CA). The monoclonal antibodies are conjugated to different fluorochromes. When the reacted cells are excited by a single light source from a fluorescence microscope, T cells display green fluorescence and B cells display orange-red fluorescence. A minimum of 200 cells was counted, and the subpopulations were given as percentage of the number of cells counted in the sample.

^{32}P-Postlabeling of DNA adducts. DNA was extracted from PMBC using a DNA extraction kit (Nuclitips, Amersham, Little Chalfont, Buck, UK). The ^{32}P-postlabeling procedure was performed as previously described (*12*) using the butanol enrichment procedure. A benzo[*a*]pyrene-diol-epoxide (BPDE)–DNA standard was included in the analysis to correct for assay variability. The reported bulky carcinogen adduct level is the average of at least two completely independent assays (less than twofold variation was observed in 85% of the analyzed samples). The results are given as adducts/10^8 nucleotides.

PAH–albumin adduct. The level of benzo[*a*]pyrene (BaP) bound to serum albumin was determined by a competitive ELISA as previously described (*8*) using an antibody (8E11) recognizing BaP tetrols. The level of adducts was estimated from the standard inhibition curve and expressed as femtomoles BaP equivalents per microgram albumin. The same control serum sample was included on each plate in order to adjust for interplate and day-to-day variation.

Oxidized proteins. AAS and GGS levels in hemoglobin (Hb) and plasma were analyzed as described by Daneshvar et al. (*21*). The protein carbonyl group was derivatized with 6-aminofluorescein and hydrolyzed by HCl (6 M, 110°C for 24 hr). The hydrolysate was separated by HPLC, and the AAS and GGS products were quantitated by a UV detector. The results are expressed as picomoles per milligram hemoglobin and milligrams plasma protein, respectively.

Determination of MDA. Malondialdehyde was determined in plasma by HPLC as described by Nielsen et al. (*14*). The method is based on the principle of the thiobarbiturate assay. The results are expressed as nanomoles per milliliter plasma.

Determination of 8-oxodG. The urinary level of 8-oxodG was determined by HPLC, using electrochemical detection as previously described (*18*). The results are expressed as nanomoles per millimole creatinine.

Determination of 1-hydroxypyrene. Reverse-phase HPLC was used for the quantitative analysis of 1-HOP in urine using a previously published method (*22*). The results are expressed as micromoles per millimole creatinine.

Mutagenic activity. The urine samples were analyzed principally according to the procedure of Yamasaki and Ames (*23*), using the YG1021 *Salmonella typhimurium* strain (*24*). The mutagenicity test used preincubation with S9 (from Arachlor 1254-induced rats) as described by Kado et al. (*25*). Each urine sample was tested on 2 separate days. On each of these days, duplicates of four concentrations of urine corresponding to 0.30, 0.62, 1.25, and 2.5 ml urine were tested.

The mutagenic activity was calculated by a regression model as

$$\text{Number of Colonies} = K_3 + K_1 \times \text{Volume Urine}/(1+K_2 \times \text{Volume Urine}),$$

where Number of Colonies is the number of colonies at a given concentration urine, K_1 is the calculated initial slope of the curve, K_2 is a calculated factor describing the saturation effect of the curve, and K_3 describes the calculated intercept of the

curve. It was found that pooling the raw data gave the least variation. The mutation rate is estimated by the slope of the regression curves at 1 ml urine and is expressed as revertants per micromole creatinine

Determination of GSTM1 genotype. DNA was isolated from whole blood and the genotype was determined as described by Okkels et al. (*26*) using glutathione *S*-transferase (GST) M4 as an internal positive control.

Statistical methods. The Wilcoxon Rank Sum test was used to compare the biomarker levels in the different groups of exposed individuals. Pearson correlation analysis was used to study the association between the biomarker levels.

Results

A panel of biomarkers for oxidative stress and genotoxic action was used to assess the exposure in two occupational groups assumed to be exposed to high levels of ambient air pollution. The exposure was assessed using markers for the internal dose: 1-HOP and mutagenicity in urine; biologically effective dose; protein and DNA adducts; early biological effects; chromosomal aberrations; and different markers of oxidative stress. The levels of different biomarkers in bus drivers working in the center of Copenhagen and in suburban and semirural areas are shown in Table 1. A large interindividual variation in the biomarker levels could be seen. A significant higher level of bulky carcinogen–DNA adducts (*12*) was observed in the group classified as the high exposure group, whereas the levels of PAH–albumin adduct and markers of oxidative stress, MDA and AAS, in hemoglobin were significantly higher in the suburban/semirural group. The level of the biomarkers in the postal worker group was similar to the levels in the suburban/semirural bus driver group (Table 1). Another marker for oxidative stress, MDA, was significantly higher in the bus driver group combined than in the postal workers. A statistically significant correlation was observed between the content of total monocytes and the bulky carcinogen–DNA adduct level (r = 0.1564; p = 0.040; 192 cases), whereas no significant correlation was observed between adduct level and the content of B lymphocytes (r = -0.097394; p = 0.20; 192 cases) or between T lymphocytes and the DNA adduct levels (r = 0.0268; p = 0.71; 192 cases).

Correlation between the various biomarkers for genotoxic and oxidative damages was analyzed by Pearson correlation analysis in the combined group of nonsmoking bus drivers and postal workers (Table 2) and in subgroups of bus drivers divided according to exposure (Table 3). The effect of GSTM1 genotypes on the correlation between selected biomarkers was analyzed in the combined group of bus drivers and postal workers (Table 4). A negative correlation between PAH–albumin and bulky carcinogen–DNA adduct levels was observed in both the high exposure group and in the combined group of bus drivers. After subdividing the combined group according to the GSTM1 genotype, the negative association between the two biomarkers was only significant in the GSTM1 null group. No other significant correlation between DNA adduct levels and markers for genotoxic exposure, i.e., mutagenic activity in urine or 1-HOP, was observed either in the combined group or in the subgroups. However, in the postal worker group, a borderline significant correlation between DNA adduct levels and 1-HOP was observed (r = 0.17; p = 0.11; n = 86). A highly significant correlation existed between the two markers for oxidation of proteins GGS-Hb and AAS-Hb (r = 0.95; p = 0.0001). No other association between biomarker levels in postal workers was observed.

A negative association was observed between carcinogen–DNA adduct level and the level of AAS in Hb in the bus driver groups, especially in the low exposure group. In the combined bus driver group, the correlation was statistically significant in the GSTM1 null group. The bulky carcinogen–DNA adduct level did not correlate with the other markers of oxidative stress.

8-OxodG in urine is considered a marker for genotoxic exposure, but no correlation between the level of 8-oxodG and

Table 1. Biomarker levels in bus drivers and postal workers

Biomarker	Bus drivers				
	Rural/suburban	City center	p-Value[a]	Postal workers	p-Value[b]
DNA adducts (12)	25.99 ± 40.57 (47)	75.42 ± 112.04 (49)	0.012	30.88 ± 51.84 (97)	0.0002
PAH–albumin	3.38 ± 1.35 (44)	2.81 ± 1.41 (56)	0.016	4.01 ± 1.38 (101)	0.0001
MDA	0.96 ± 0.25 (45)	0.87 ± 0.19 (55)	0.093[c]	0.71 ± 0.16 (101)	0.0001
AAS-Hb	59.77 ± 8.99 (45)	52.81 ± 12.65 (56)	0.0009	56.59 ± 14.61 (101)	0.59
AAS-plasma	42.44 ± 13.86 (44)	55.80 ± 24.11 (56)	0.0016	30.99 ± 5.29 (101)	0.0001
GGS-Hb	36.50 ± 5.54 (27)	35.70 ± 11.54 (60)	0.23	49.24 ± 17.39 (90)	0.0001
8-oxodG	1.54 ± 0.96 (20)	1.74 ± 0.87 (29)	0.41	2.25 ± 1.13 (82)	0.86
1-HOP	0.25 ± 0.30 (27)	0.24 ± 0.16 (30)	0.36	0.15 ± 0.13 (91)	0.87

Abbreviations: PAH, polycyclic aromatic hydrocarbon; MDA, malondialdehyde; AAS, 2-amino-apidic semialdehyde; Hb, hemoglobin; GGS, γ-glutamyl semialdehyde; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 1-HOP, 1-hydroxypyrene. Values shown are mean ± standard deviation (number of cases).
[a]Wilcoxon 2-sample test.
[b]Postal workers vs. combined bus drivers; Wilcoxon 2-sample test.
[c]Two-tailed.

Table 2. Correlation between biomarker levels in the combined bus driver and postal worker group

Biomarker	DNA adduct	PAH–albumin	AAS-Hb	AAS-plasma	GGS-Hb	MDA
PAH–albumin	-0.1998[a] p = 0.005 n = 192					
AAS-Hb	-0.1341 p = 0.06 n = 193	0.0351 p = 0.61 n = 207				
AAS-plasma	0.1290 p = 0.07 n = 192	-0.2179 p = 0.001 n = 206	-0.1117 p = 0.10 n = 207			
GGS-Hb	-0.1350 p = 0.11 n = 134	0.2547 p = 0.001 n = 147	0.8427 p = 0.0001 n = 148	-0.2168 p = 0.008 n = 147		
MDA	-0.0470 p = 0.51 n = 192	0.0270 p = 0.69 n =206	-0.008 p = 0.90 n = 207	-0.1252 p = 0.07 n = 206	-0.1946 p = 0.01 n = 148	
8-oxodG	-0.09751 p = 0.32 n = 102	0.2833 p = 0.002 n = 114	0.0556 p = 0.55 n = 115	-0.2942 p = 0.001 n = 114	0.1063 p = 0.19 n = 151	-0.0855 p = 0.36 n = 115
1-HOP	0.0883 p = 0.31 n = 134	-0.0582 p = 0.48 n = 147	-0.0338 p = 0.68 n = 148	0.1282 p = 0.12 n = 147	-0.1586 p = 0.03 n = 186	0.00211 p = 0.97 n = 148
Urine mutagen	-0.1124 p = 0.12 n = 185	0.04493 p = 0.52 n = 199	-0.1105 p = 0.11 n = 200	0.1560 p = 0.03 n = 189	-0.1015 p = 0.17 n = 183	0.07084 p = 0.39 n = 146

Abbreviations: PAH, polycyclic aromatic hydrocarbon; AAS, 2-amino-apidic semialdehyde; Hb, hemoglobin; GGS, γ-glutamyl semialdehyde; MDA, malondialdehyde; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; 1-HOP, 1-hydroxypyrene.
[a]Pearson correlation coefficient.

other markers for genotoxic damage could be observed.

The activities of antioxidative enzymes (glutathione peroxidase, glutathione reductase, and superoxide dismutase) were analyzed as part of the study, but the activity of these enzymes did not influence the level of biomarkers for genotoxic exposure (data not shown).

Discussion

Several different biomarkers have been used to assess human exposure to genotoxic compounds present in ambient air. In this study, selected biomarkers were applied to assess exposure in a country with supposedly low levels of ambient air pollution in order to evaluate the validity of these markers as predictors of exposure.

The level of ambient air pollutants is routinely measured in Denmark, and a recent study showed that the level of BaP in a busy street of Copenhagen was 3.9 ng/m^3 compared with 0.08–0.5 ng/m^3 in rural areas (*27*). The level in Copenhagen is considerably lower than in some eastern European countries, where levels of BaP were more than 10-fold higher (*11*). In the latter countries, stationary sources are the major contributors, whereas vehicle exhaust is the predominant source of genotoxic air pollutants in Denmark.

Conventionally, exposure assessment has been based on environmental monitoring of standard air pollutants. The objective of this study has been to obtain a better estimate of human exposure and to identify groups with aberrant exposure, using biomarkers. As representatives for people spending a large proportion of their day in the city center, nonsmoking bus drivers and postal workers were selected. Bus drivers in Copenhagen have an increased risk of developing cancer in lung, bladder, and liver (*28*), and it is assumed that exposure to genotoxic compounds present in ambient air plays a role in the pathogenesis. Transportation workers have an increased exposure to carcinogenic PAHs, e.g., truck drivers (*29*). Increased exposure to genotoxic compounds have been reported in bus drivers in Copenhagen using the ^{32}P-postlabeling method to measure bulky carcinogen–DNA adducts (*12*). Drivers who spent most of their working hours in central Copenhagen had a higher adduct level than suburban and semirural drivers. In contrast, no difference in DNA adduct level could be observed in street vendors in Milan, Italy, working in areas of high and low traffic density (*30*). However, the Italian study included both smokers and nonsmokers, and smoking is known to increase the adduct level (*31*). In the present study an interesting effect of the PMBC composition was observed, as individuals with a high content of B lymphocytes had a lower DNA adduct level. This could potentially be a result of different DNA repair capacity of the two different subtypes of lymphocytes, as the B lymphocytes have a higher rate of DNA repair (*32*). The adduct level in long-lived T lymphocytes correlated better with exposure data based on environmental monitoring than did short-lived granulocytes (*33*). As the B/T-lymphocyte ratio may be influenced by confounding factors and environmental exposure, this could contribute to the large interindividual variation in DNA adduct levels reported in different studies. The level of bulky carcinogen–DNA adducts in lymphocytes is only a biomarker for exposure, not for risk, as no association between the adduct level in these surrogate cells and the target cells for the carcinogenic action has been demonstrated.

The biologically active form of PAH, including BaP, will react with other cellular macromolecules. An increased level of PAH albumin has previously been observed in workers occupationally exposed to PAHs, e.g., foundry workers (*34*), using a competitive ELISA to detect BaP tetrols released by acid hydrolysis. Bus drivers in the center of Copenhagen had a significantly lower PAH–albumin level than bus drivers in the suburban/rural area. This observation is similar to previous studies in which nonsmoking young males living in rural areas had a higher adduct level than young males living in the city center, and young women living in the country had a higher adduct level than women living in suburban areas or the city center (*8,9*). Because these samples were collected during the winter period, it was assumed that the higher PAH–albumin level was due to the emission from local heating of the houses using cereal straw or wood. There is no obvious reason why the PAH–albumin adduct levels should be higher in suburban/semirural groups in the present study. Most of the carcinogenic PAHs associated with particulate matter are produced by diesel engines. The composition and the amount of different PAHs bound to the particulate depends on driving conditions, fuel type, and engine type (*6*). Benzo[*a*]pyrene, detectable as BaP tetrols by the ELISA assay, is not a major PAH in diesel exhaust, and other PAHs not detectable by the ELISA assay may be responsible for the bulky carcinogen–DNA adducts. In an Italian study, BPDE–Hb adducts were used to monitor newspaper vendors exposure to traffic exhaust. A nonsignificantly higher adduct level was observed in street vendors working in areas with high traffic density (*35*). In this study, a significant negative correlation was observed between the levels of DNA adduct and BaP–albumin in both the combined group and the city center bus driver group. This observation is similar to an observation in young males living in urban and rural areas (*9*). In experimental systems the covalent binding to serum proteins is, in most cases, related to binding to DNA. However, the relationship between protein and DNA binding is usually different for each particular compound. The negative correlation between the two parameters could be an indication that the ratio of BaP to other DNA adduct-forming PAHs will depend on the source. An alternative explanation is that highly modified albumin from highly exposed bus drivers could easily be recognized by proteases and degraded. In animal

Table 3. Correlation of carcinogen–DNA adducts and other biomarkers in the bus driver group

	Exposure	
Biomarkers	Rural/suburban	City center
DNA adducts vs. PAH–albumin	-0.1968[a] p = 0.22 n = 40	-0.3531 p = 0.01 n = 49
DNA adducts vs. MDA	-0.1201 p = 0.45 n = 41	-0.2638 p = 0.07 n = 48
DNA adducts vs. AAS-Hb	-0.3121 p = 0.04 n = 41	-0.1676 p = 0.24 n = 49
DNA adducts vs. 8-oxodG	-0.1690 p = 0.51 n = 17	0.04658 p = 0.83 n = 23
DNA adducts vs. urine mutagen	-0.0720 p = 0.74 n = 22	-0.2015 p = 0.34 n = 24

Abbreviations: PAH, polycyclic aromatic hydrocarbon; MDA, malondialdehyde; AAS, 2-amino-apidic semialdehyde; Hb, hemoglobin; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine.
[a]Pearson's correlation.

Table 4. Comparison of biomarkers in the combined group: effect of the GSTM1 genotype

	Genotype	
Biomarkers	GSTM1 –/–	GSTM1 +/+ and+/–
DNA adducts vs. PAH–albumin	-0.2144[a] p = 0.02 n = 104	-0.1920 p = 0.07 n = 88
DNA adducts vs. MDA	-0.0041 p = 0.96 n = 104	-0.09188 p = 0.39 n = 88
DNA adducts vs. AAS-Hb	-0.1138 p = 0.24 n = 105	-0.1793 p = 0.09 n = 88
DNA adducts vs. urine mutagen	-0.2289 p = 0.02 n = 99	-0.0008 p = 0.99 n = 86
8-oxodG vs. AAS-Hb	0.1790 p = 0.09 n = 90	0.1578 p = 0.16 n = 78

Abbreviations: PAH, polycyclic aromatic hydrocarbon; MDA, malondialdehyde; AAS, 2-amino-apidic semialdehyde; Hb, hemoglobin; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine.
[a]Pearson's correlation.

experiments, the half-life of a serum protein depended on the level of modification.

The active metabolite of BaP that reacts with DNA and proteins to form covalently bound adducts is detoxified by, for example, GSTM1. Bus drivers with a deficiency in GSTM1 activity due to a homozygous deletion did not have a significantly higher DNA adduct level (*12*). In this study, the negative correlation between albumin and bulky carcinogen–DNA adduct level seen in the combined group was only statistically significant in the GSTM1 null group. This suggests that the metabolism of compounds binding to DNA and not detectable by the immunoassay are influenced by the GSTM1 phenotype.

1-Hydroxypyrene has frequently been used to assess occupational exposure for PAHs. In the combined group, no association between the level of carcinogen–DNA adduct and urinary 1-HOP could be established. This lack of association may in part be explained by the different kinetics of the two parameters. Urinary 1-HOP represents the exposure within the last 24 hr, whereas DNA adducts represent the accumulated exposure over a longer time period. In people eating charbroiled beef, the urinary 1-HOP glucuronide concentration during and immediately after the feeding period was significantly correlated with the mean PAH–DNA adduct level during the same period (*36*). In smokers, a statistically nonsignificant association between 1-HOP glucuronide and a specific adduct in urothelial cells was observed (*37*). The source of the pyrene may be an important factor. We have previously reported a lack of association between DNA adducts and urinary 1-HOP in garage workers exposed to automobile exhaust (*38*), but the adduct level correlated with urinary 1-HOP in foundry workers (*31*). Similarly, as in our observation for DNA adducts, we did not observe any correlation between urinary 1-HOP and the PAH–albumin adduct level.

No significant correlation between the level of carcinogen–DNA adducts and urinary mutagenicity could be detected in the present study. Urinary mutagenicity has frequently been used to evaluate lifestyle and occupational exposure to mutagens and potential carcinogens. Urinary mutagenicity is nonspecific but has the advantage of detecting the mixture of mutagenic compounds present in urban air, similar to the ^{32}P-postlabeling method to detect DNA adducts. The lack of association could be a result of the kinetics of the two markers, as discussed for 1-HOP. However, a positive correlation between urinary mutagenicity and a specific adduct in urothelial cells has been reported in workers exposed to benzidine (*39*).

Oxygen radicals are generated by environmental agents and by endogenous processes. These oxygen radicals cause extensive damage to DNA, including 8-oxodG in both leukocytes and potential target tissues such as lung (*40*). The damaged DNA is repaired, and the repair product can be detected in the urine. Smokers do have a higher level of 8-oxodG in urine than nonsmokers (*18*). Diesel exhaust particles, the main contributor of carcinogenic compounds in ambient air, do induce oxidative damage to DNA in experimental animals. The 8-oxodG level did not correlate with other markers for damage to DNA, e.g., chromosomal aberrations and bulky carcinogen–DNA adducts, although some of the compounds responsible for the latter DNA damage are expected to produce oxygen radicals during biotransformation processes. The nature of formation of 8-oxodG is more complex, involving a number of endogenous factors and modifying factors in the diet. Nevertheless, a number of occupational exposures, including benzene, reportedly increase 8-oxodG (*18,41*).

MDA, a bifunctional carbonyl compound, is a major product arising from the metal-catalyzed peroxidative degradation of the polyunsaturated fatty acid constituents of biological membranes. Lipid peroxidation can be induced by smoking (*14*) and occurs endogenously during normal metabolism. MDA reacts with macromolecules, including DNA, and it is assumed that this adduct is the major mutagenic and carcinogenic product of lipid peroxidation. In this study, MDA was measured in plasma: the level of MDA was slightly lower in the central Copenhagen bus driver group and in the postal worker group than in the suburban/semirural bus driver group. An inverse relationship between the DNA adduct levels and MDA was observed, suggesting that the compounds responsible for the DNA adducts are not the major factor in the induction of lipid peroxidation, although higher levels of MDA have been reported in smokers compared to nonsmokers (*14*).

Much attention has been focused on studying the introduction of carbonyl groups into amino acid residues of proteins for monitoring the oxidative modification by ROS (*42*). We have shown that among the oxidation products in both bovine serum albumin and bovine Hb, AAS was the most abundant naturally occurring semialdehyde in these proteins. Apart from *in vitro* oxidations, it has been reported that the oxidative stress induced in rats by chemicals, e.g., acrolein, resulted in the same product, confirming that *in vivo* exposure of plasma proteins to ROS could be quantified by measurement of oxidized lysine (*21*). In this study, the highest level of AAS in plasma proteins was observed in bus drivers working in the central part of Copenhagen. More over, a statistically significant difference in the level of Hb AAS levels was also observed between the two exposure groups, but with the highest levels in the rural and suburban areas.

Different biomarkers for genotoxic exposure and oxidative stress have been used in this study to evaluate the bus drivers' exposure to ambient air pollution. Only the carcinogen–DNA adducts and a potential biological consequence of adducts, chromosomal aberrations, showed a statistically significant difference between the two groups of drivers. This information shows that not all bus drivers are exposed to the same level of genotoxic compounds, and that information about working area, routes, and pollution are important when designing an epidemiological study to assess risk. Information about exposure cannot be obtained by environmental monitoring alone. Geographic information, traffic density, and other parameters should be used in model calculations, as in the Operational Street Pollution Model (*43*). It is important in future research to link this model with selected relevant biomarkers. Care has to be taken when selecting the markers, as some of the markers only represent exposure within a very short time frame, and repeated sampling would be required to obtain an estimate of an accumulated dose. The lack of correlation or, in some cases, negative correlation could indicate that additional biological effects not related to the compounds responsible for the formation of DNA adducts are caused by some compounds present in ambient air, e.g., indoor air pollutants. Moreover, the chosen biomarkers represent exposure to or effects of different components on target molecules in different organs and/or compartments, i.e., lymphocytes DNA or chromosomes, blood proteins, and lipids, as well as estimates of whole-body exposure to pyrene, mutagenic substances, and oxidative damage to DNA. Furthermore, the synergistic or agonistic effects of the complex mixture of toxic compounds present in ambient air on the relevant biomarkers should be investigated in model systems in order to support and plan complex human biomonitoring studies.

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