Chromosomal Aberrations in Humans Induced by Urban Air Pollution: Influence of DNA Repair and Polymorphisms of Glutathione *S*-Transferase M1 and *N*-Acetyltransferase 2[1]

Lisbeth E. Knudsen,[2] Hannu Norppa, Michael O. Gamborg, Per S. Nielsen,[3] Henrik Okkels,[4] Helle Soll-Johanning, Edith Raffn, Hilkka Järventaus, and Herman Autrup

National Institute of Occupational Health, DK 2100 Copenhagen Ø, Denmark [L. E. K., M. O. G., H. S-J.]; Finnish Institute of Occupational Health, FIN-00250 Helsinki, Finland [H. N., H. J.]; Institute of Environmental Medicine, DK 8000 Århus, Denmark [P. S. N., H. O., H. A.]; and Clinic of Occupational Medicine, Hillerød Hospital, DK 3400 Denmark [E. R.]

Abstract

We have studied the influence of individual susceptibility factors on the genotoxic effects of urban air pollution in 106 nonsmoking bus drivers and 101 postal workers in the Copenhagen metropolitan area. We used the frequency of chromosomal aberrations in peripheral blood lymphocytes as a biomarker of genotoxic damage and dimethylsulfate-induced unscheduled DNA synthesis in mononuclear WBCs, the glutathione *S*-transferase M1 (*GSTM1*) genotype, and the *N*-acetyltransferase 2 (*NAT2*) genotype as biomarkers of susceptibility. The bus drivers, who had previously been observed to have elevated levels of aromatic DNA adducts in their peripheral mononuclear cells, showed a significantly higher frequency of cells with chromosomal aberrations as compared with the postal workers. In the bus drivers, unscheduled DNA synthesis correlated negatively with the number of cells with gaps, indicating a protective effect of DNA repair toward chromosome damage. Bus drivers with the *GSTM1* null and slow acetylator *NAT2* genotype had an increased frequency of cells with chromosomal aberrations. *NAT2* slow acetylators also showed elevated chromosomal aberration counts among the postal workers. Our results suggest that long-term exposure to urban air pollution (with traffic as the main contributor) induces chromosome damage in human somatic cells. Low DNA repair capacity and *GSTM1* and *NAT2* variants associated with reduced detoxification ability increase susceptibility to such damage. The effect of the *GSTM1* genotype, which was observed only in the bus drivers, appears to be associated with air pollution, whereas the *NAT2* genotype effect, which affected all subjects, may influence the individual response to some other common exposure or the baseline level of chromosomal aberrations.

Introduction

Urban air pollution consists of a complex mixture of organic and inorganic compounds, many of which are genotoxic and potentially carcinogenic. An association has been suggested between high levels of urban air pollution and increased risk of lung cancer; the yearly excess of lung cancer due to air pollution in Western industrialized countries has been estimated to range between 30 and 150 cases per million people (1).

Some occupational groups, such as professional drivers working in urban areas, are exposed to a high level of ambient air pollution (2). An increased risk of several types of cancer, including that of the lungs and bladder, was observed among men working in urban, suburban, and interurban passenger transport and in trucking freight (3–10). In addition, truck drivers and railroad workers showed increased mortality due to cerebrovascular diseases and arteriosclerosis, and it was suggested that exposure to diesel exhaust played a contributing role (11).

In epidemiological studies, detailed information on exposure is usually scarce, and because several confounding factors exist, the actual contribution of ambient air pollution as a risk factor of cancer is difficult to assess. Biomarkers such as adducts in DNA and proteins and cytogenetic alterations may help in identifying the exposure and revealing its early effects (12–14). In this context, structural chromosomal aberrations are of special interest, because high chromosomal aberration levels in peripheral lymphocytes have been associated with an increased risk of cancer (15, 16). Because exhaust from diesel engines is a major contributor to the mutagenicity of urban air (17) and induces cytogenetic damage in both mammalian cells *in vitro* and rodents *in vivo* (18), exposure to urban air polluted with diesel exhaust may also increase the level of chromosome damage in humans. Egyptian traffic policemen showed an increased frequency of chromosomal aberrations and SCEs[5] in their peripheral lymphocytes (19), and an increase in SCEs was also seen in a similar Indian study (20). Policemen from the city of Genoa demonstrated exposure to benzo(*a*)pyrene, but no effect on SCEs or micronuclei in peripheral lymphocytes could

Received 10/6/98; revised 2/11/99; accepted 2/17/99.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] Supported in part by a grant from The Danish Strategic Environmental Research Program.
[2] To whom requests for reprints should be addressed, at Danish National Institute of Occupational Health, Lersø Parkalle 105, DK 2100 Copenhagen Ø, Denmark. Phone: 45-39165200; Fax: 45-39165201.
[3] Present address: Department of Occupational Health, Århus Kommune Hospital, DK 8000 Århus, Denmark.
[4] Present address: Department of Clinical Chemistry, Ålborg Hospital North, DK 9000 Ålborg, Denmark.

[5] The abbreviations used are: SCE, sister chromatid exchange; GSTM1, glutathione *S*-transferase M1; NAT2, *N*-acetyltransferase 2; BMI, body mass index; UDS, unscheduled DNA synthesis; DMS, dimethylsulfate.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024

be observed (21, 22). No association between occupational exposure to diesel exhaust and chromosome aberration frequencies could be seen in two Swedish studies (23, 24) or in a Welsh study (25).

Several studies have found associations between increased cancer risk and impaired DNA repair (26, 27). Therefore, individual differences in DNA repair capacity may generally influence the rate of genotoxic damage and cancer susceptibility from exposure to urban air pollution. Another potential source of individual susceptibility is the polymorphism of xenobiotic-metabolizing enzymes. A large number of chemical carcinogens present in ambient air are metabolized to the ultimate carcinogenic form or detoxified to less toxic or nontoxic metabolites that are excreted in the urine. These reactions are primarily mediated by members of the cytochrome P-450 family of enzymes and by conjugating enzymes such as glutathione *S*-transferases and *N*-acetyltransferases. High rates of metabolic activation and defective detoxification have been associated with an increased risk for certain types of cancer, with one example being lung cancer in individuals who lack GSTM1 activity due to a homozygous allelic loss of the *GSTM1* gene (28).

In a Danish national research program to evaluate genetic biomarkers to assess exposure to the genotoxic compounds present in urban air, bus drivers and postal workers in the Copenhagen were selected as the study population. To exclude contribution by active smoking, only nonsmokers were chosen. City bus drivers, in comparison with rural and suburban bus drivers, were observed to have elevated levels of bulky DNA adducts in their mononuclear WBCs (14). An analysis of air pollution from personal air samplers demonstrated that bus drivers were exposed up to three times higher levels of naphthalene than mail carriers (29). The present study reports results from the genetic monitoring of chromosomal aberrations as a measure of genotoxic damage and UDS as a measure of DNA repair. The findings are correlated with the genetic polymorphisms of two important xenobiotic-metabolizing enzymes, GSTM1 and NAT2.

Materials and Methods

Subjects. Nonsmoking bus drivers and postal workers from Copenhagen and its suburbs were recruited to the study with the help of the employers and unions. The participation of each subject was voluntary and could be canceled by the subject at any time during the study (according to the Helsinki II declaration). All participants signed an informed consent form, and the study protocol was approved by the the local ethical committee.

The study groups consisted of 81 male and 25 female bus drivers and 70 male and 31 female postal workers (Table 1). On the basis of bus routes driven during the past month, two researchers categorized the exposure of 100 bus drivers as high (city of Copenhagen), medium (suburbs), and low (countryside).

Half of the postal workers were city mail carriers with some exposure to urban air pollution, although at a lower level than the bus drivers in the city. The other half of the postal worker group worked in offices and was therefore not considerably exposed to air pollution.

In connection with a health examination, heparinized blood samples were collected (on Tuesdays) from the bus drivers in August to November 1994 and from the postal workers in August to November 1995, with 6–12 persons being sampled per week. A self-administrated questionnaire concerning present and previous occupations, environmental urban air exposure, symptoms and diseases, familiar disposition, smoking, drinking and eating habits, physical activity at work and leisure, and contact with the health care system was filled in by all selected subjects and was completed during the health examination.

The nonsmoking status of the subjects was confirmed by serum cotinine analysis using a kit (STC Diagnostics Care, Bethlehem, PA). Sixty-one bus drivers had never smoked, whereas 45 bus drivers were ex-smokers with an average of 16 smoking years and an average time since quitting of 14 years [range, 0.5–34 year(s)]. Thirty-nine of the ex-smokers stated that they had smoked, on average, 20 cigarettes/day. Seventy-four postal workers had never smoked, and 27 were ex-smokers. The ex-smokers had smoked, on average, 15 cigarettes/day, with an average of 14 smoking years and an average time since quitting of 11 years (range, 1.5–27 years).

In comparison with the postal workers (Table 1), the bus drivers were, on average, older (mean, 45 years *versus* 38 years) and showed a higher mean BMI [weight (in kilograms) divided by the square of height (in meters)]; 47% of bus drivers were classified as overweight ($25 < \text{BMI} < 30$), and 31% were classified as heavily overweight ($\text{BMI} \geq 30$), whereas the respective percentages for postal workers were 34% and 16%.

UDS in Peripheral Mononuclear WBCs. Peripheral mononuclear WBCs were isolated from 60 ml of whole blood (collected in sodium heparin) within 4 h after sampling by gradient centrifugation in Lymphoprep (Nycomed Pharma, Oslo, Norway), as described previously (30). The isolated cells were washed twice in RPMI 1640 (Grand Island Biological Co., Paisley, United Kingdom) and once in PBS [137 mM NaCl, 3 mM KCl, 8 mM Na_2HPO_4, and 2 mM KH_2PO_4 (pH 7.4)] with centrifugation at $1200 \times g$ for 10 min.

The ability of the cells to repair induced DNA damage was determined as UDS by applying the method described by Pero *et al.* (31) and modified by Knudsen (30). Two million cells were resuspended in 1 ml of RPMI 1640 containing 1% (v/v) L-glutamine (200 mM; Merck 100289; Darmstadt, Germany) and 1% penicillin-streptomycin (10,000 IU/ml penicillin and 10,000 μg/ml streptomycin; Grand Island Biological Co.) and treated at 37°C with 100 μM DMS (99% purity; Merck) for 30 min. The cells were then transferred to microtiter plates (200,000 cells/well) and incubated for 18 h at 37°C with 0.4 μM [^{3}H]thymidine (925 GBq/mmol; Amersham Life Science, Buckinghamshire, United Kingdom) in RPMI 1640 supplemented as described above with the addition of 20% autologous plasma. To minimize replicative DNA synthesis, the medium also contained 10 mM hydroxyurea (Sigma Chemical Co., St. Louis, MO). UDS was determined as the cpm [^{3}H]thymidine incorporated into 200,000 cells by eight duplicate measurements in a 1205 Betaplate liquid scintillation counter (Wallac, Turku, Finland), using glass fiber filters to which the cells had been transferred using a Mach II cell harvester 96 (Wallac). Background UDS, which was determined in parallel cultures exposed to DMSO (99.8% purity; Merck), was subtracted from the results.

To control interindividual differences due to the variation of T- and B-lymphocyte content in the mononuclear cell fraction used for the UDS assay, the proportion of T- and B-lymphocytes and monocytes was studied by immunofluorescence. Specific monoclonal antibodies (Simultest CD3/CD19; Becton Dickinson, San Jose, CA) toward B-lymphocytes (anti-Leu-12, CD19; fluorescein conjugated) and T-lymphocytes (anti-Leu-4, CD3; phycoerythrin conjugated) were simulta-

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024

Table 1 Characteristics of the bus drivers and postal workers studied and DMS-induced UDS in peripheral mononuclear WBCs

Group	No. of subjects	No. of men/ women	Mean age ± SD (yr)	Mean BMI ± SD[a]	Mean anciennity ± SD[b]	Mean DMS-induced UDS ± SD[c]
Bus drivers[d]						
High exposure	55	41/14	44 ± 8	27 ± 5	11 ± 6	147 ± 117
Low + medium exposure	45	35/10	46 ± 8	29 ± 4	12 ± 8	201 ± 157
Total	106	81/25	45 ± 8	28 ± 4	12 ± 7	171 ± 138
Postal workers						
Mail carriers	60	50/10	38 ± 11	26 ± 4	13 ± 10	172 ± 106
Office workers	41	20/21	38 ± 10	26 ± 5	9 ± 9	153 ± 136
Total	101	70/31	38 ± 11	26 ± 4	11 ± 10	164 ± 119

[a] BMI, weight (in kilograms) divided by the square of height (in meters).
[b] Years employed as a bus driver or postal worker.
[c] UDS after a 30-min treatment with 100 μM DMS expressed as cpm of [^{3}H]thymidine incorporated into 200,000 cells incubated with 10 mM hydroxyurea to suppress replication.
[d] Exposure classified according to bus driving experience during the 3 weeks preceding the sampling: low, in the countryside; medium, in suburbs; high, in the center of Copenhagen. Six people had not driven a bus during the preceding 3 weeks.

neously added on microscopic specimens of mononuclear cells. A separate cell sample was reacted with antibodies toward monocytes (anti-Leu-M3, CD14; phycoerythrin conjugated; Becton Dickinson). The percentage of B cells (orange-red) and T cells (green) and, separately, the percentage of monocytes (orange-red) were counted from a minimum of 200 cells in a fluorescence microscope.

Chromosomal Aberration Analysis. From each donor, a 5-ml sample of heparinized blood was sent by air to Finland for the analysis of chromosomal aberrations. The majority of the shipments arrived at the Finnish Institute of Occupational Health approximately 24 h after sampling. During the transfer, the samples were refrigerated and were not X-rayed. Two phytohemagglutinin-stimulated whole-blood lymphocyte cultures/sample were established in cell culture tubes (Falcon 3033; Becton Dickinson Labware, Lincoln Park, NJ) and cultured and harvested as described previously (32). For the analysis of chromosomal aberrations, 100 metaphases were scored from microscopic preparations using Cytoscan DF (Applied Imaging, Sunderland, United Kingdom) for metaphase finding. Chromatid gaps were defined to be wider than a chromatid but smaller than the width of two chromatids. All analyses were performed on coded slides by one observer.

Genotype Determination. DNA was isolated from whole blood as described by Spurr *et al.* (33). The genotyping of *NAT2* and *GSTM1* was performed in duplicate, using techniques based on PCR, as described by Okkels *et al.* (34, 35). The *NAT2* genotypes were classified as slow acetylators (any combination of the variant alleles *M1*, *M2*, and *M3*) or rapid acetylators (heterozygous wild-type/variant and the rare homozygous wild-type), and the *GSTM1* genotypes were classified as *GSTM1* null (both alleles deleted) or *GSTM1* positive (at least one undeleted allele).

Statistical Analysis. The data were processed in a Clarion database (Top Speed Corp., Pompano Beach, FL) and transferred into the SAS/STAT software (Version 6.12, Fourth Edition, Vol. 2; SAS Institute, Inc., Cary, NC). The nonparametric Wilcoxon rank-sum test was performed to compare two samples. Because the discrete frequencies of chromosomal aberrations did not significantly deviate from the Poisson distribution, the data were further analyzed with a multiple log-linear Poisson regression model estimating the relative risks and their 95% confidence intervals (36). The level of significance was $P < 0.05$.

Results

The genotype analysis showed roughly expected proportions of both *NAT2* slow and *GSTM1* null individuals among the bus drivers (54% and 57%, respectively) and the postal workers (67% and 53%). The genotype distribution did not differ markedly between never smokers and ex-smokers. In the group of bus drivers, *NAT2* slow and *GSTM1* null proportions were 53% and 62% in ex-smokers and 54% and 52% in never smokers. The respective proportions in the group of postal workers were 56% and 59% in ex-smokers and 72% and 52% in never smokers.

The proportion of B-lymphocytes, T-lymphocytes, and monocytes in the mononuclear cell fractions of all subjects ranged from 2–28%, 30–77%, and 2–51%, respectively.

The DMS-induced UDS (after subtraction of the DMSO background) ranged from −92 to 563 cpm/200,000 cells. In the bus drivers, the DMS-induced UDS activity correlated positively with the age of the subjects ($r = 0.19$; $P = 0.05$). No such correlation was observed in the analysis of the postal workers. In the bus drivers and postal workers, a significantly lower UDS value was observed in women (117 ± 123 and 129 ± 146, respectively) in comparison with men (189 ± 140 and 180 ± 102, respectively). No effect of the exposure category of the bus drivers on UDS was observed, and the bus drivers did not differ significantly from the postal workers (Table 1). Previous smoking had no influence on UDS.

The results of the chromosomal aberration analysis are shown in Tables 2 and 3. In the bus drivers, log-linear Poisson regression analysis showed a significant negative dependence between DMS-induced UDS and the number of cells with gaps ($P < 0.0005$; Table 4).

Tables 5 and 6 show the relative risk point estimates for different classes of chromosomal aberrations by Poisson regression models including metabolic genotypes, DNA repair, age, and sex as possible confounders. This multivariate analysis indicated a statistically significant difference between the postal workers and bus drivers in the total number of aberrant cells, gaps included (Table 5). Besides gaps, chromosome-type aberrations also contributed to the observed difference (Tables 2 and 3). No significant effects on chromosomal aberrations were seen for sex, age (categories, ≤45 and >45 years), or previous smoking (comparison of ex-smokers and never smokers; data not shown).

In the bus drivers, a significant effect of both the *GSTM1*

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024

Table 2 Mean frequencies (%; ±SD) of cells with structural chromosomal aberrations in peripheral lymphocytes[a] of bus drivers

Group	No. of subjects	Chromatid-type					Chromosome-type					Total	
		Gaps	Breaks	Exchanges	Total − gaps	Total + gaps	Gaps	Breaks	Exchanges	Total − gaps	Total + gaps	−Gaps	+Gaps
Nonsmokers	61	0.79 ± 0.82	0.95 ± 0.96	0.05 ± 0.28	1.00 ± 1.02	1.79 ± 1.52	0.05 ± 0.22	0.21 ± 0.49	0.49 ± 0.77	0.70 ± 1.01	0.75 ± 1.07	1.69 ± 1.41	2.51 ± 1.92
Ex-smokers	45	0.87 ± 0.76	0.93 ± 1.07	0.11 ± 0.32	1.04 ± 1.02	1.91 ± 1.29	0.02 ± 0.15	0.27 ± 0.50	0.58 ± 0.72	0.84 ± 0.88	0.87 ± 0.87	1.87 ± 1.31	2.73 ± 1.48
Exposure[b]													
High	55	0.96 ± 0.84	1.15 ± 0.97	0.07 ± 0.33	1.22 ± 1.01	2.18 ± 1.48	0.04 ± 0.19	0.25 ± 0.48	0.42 ± 0.66	0.67 ± 0.86	0.71 ± 0.96	1.85 ± 1.41	2.84 ± 1.87
Medium	26	0.73 ± 0.72	0.42 ± 0.76	0.08 ± 0.27	0.50 ± 0.76	1.23 ± 1.07	0.04 ± 0.20	0.12 ± 0.33	0.65 ± 0.80	0.77 ± 0.95	0.81 ± 0.94	1.27 ± 1.04	2.04 ± 1.34
Low	19	0.63 ± 0.68	1.15 ± 1.21	0.05 ± 0.23	1.21 ± 1.18	1.84 ± 1.54	0.05 ± 0.23	0.32 ± 0.67	0.37 ± 0.60	0.68 ± 0.89	0.74 ± 0.87	1.89 ± 1.45	2.53 ± 1.84
Low + medium	45	0.69 ± 0.70	0.73 ± 1.03	0.07 ± 0.25	0.80 ± 1.01	1.49 ± 1.31	0.04 ± 0.21	0.20 ± 0.50	0.53 ± 0.73	0.73 ± 0.91	0.78 ± 0.90	1.53 ± 1.25	2.24 ± 1.57
Total	106	0.82 ± 0.79	0.94 ± 1.00	0.08 ± 0.30	1.02 ± 1.01	1.84 ± 1.42	0.04 ± 0.19	0.24 ± 0.49	0.53 ± 0.75	0.76 ± 0.95	0.80 ± 0.99	1.77 ± 1.35	2.60 ± 1.74

[a] Whole-blood lymphocyte cultures were cultured for 48 h, and 100 metaphases were scored per person.
[b] Exposure was classified according to bus driving experience during the 3 weeks preceding the sampling: low, in the countryside; medium, in suburbs; high, in the center of Copenhagen. Six people had not driven a bus during the preceding 3 weeks.

Table 3 Mean frequencies (%; ±SD) of cells with structural chromosomal aberrations in peripheral lymphocytes[a] of postal workers

Study group	No. of subjects	Chromatid-type					Chromosome-type					Total	
		Gaps	Breaks	Exchanges	Total − gaps	Total + gaps	Gaps	Breaks	Exchanges	Total − gaps	Total + gaps	−Gaps	+Gaps
Nonsmokers	74	0.64 ± 0.84	1.21 ± 1.11	0.11 ± 0.31	1.32 ± 1.14	1.96 ± 1.48	0	0.14 ± 0.34	0.24 ± 0.46	0.38 ± 0.59	0.38 ± 0.59	1.68 ± 1.28	2.31 ± 1.56
Ex-smokers	26	0.81 ± 0.96	0.89 ± 1.09	0	0.89 ± 1.09	1.70 ± 1.61	0	0.19 ± 0.40	0.30 ± 0.54	0.48 ± 0.70	0.48 ± 0.70	1.33 ± 1.41	2.11 ± 1.89
Mail carriers[b]	60	0.65 ± 0.73	1.05 ± 1.00	0.07 ± 0.25	1.12 ± 1.03	1.77 ± 1.29	0	0.18 ± 0.39	0.22 ± 0.42	0.40 ± 0.62	0.40 ± 0.62	1.50 ± 1.16	2.12 ± 1.38
Office workers[c]	41	0.73 ± 1.05	1.24 ± 1.26	0.10 ± 0.30	1.34 ± 1.28	2.07 ± 1.78	0	0.10 ± 0.30	0.32 ± 0.57	0.41 ± 0.63	0.41 ± 0.63	1.73 ± 1.53	2.46 ± 1.98
Total	101	0.68 ± 0.87	1.13 ± 1.11	0.08 ± 0.27	1.21 ± 1.13	1.89 ± 1.51	0	0.15 ± 0.36	0.26 ± 0.48	0.41 ± 0.62	0.41 ± 0.62	1.59 ± 1.32	2.26 ± 1.65

[a] Whole-blood lymphocyte cultures were cultured for 48 h, and 100 metaphases were scored per person.
[b] Half of the working day was spent indoors, and the other half was spent outdoors.
[c] Subjects worked indoors at the post office.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024

Table 4 DMS-induced UDS in mononucleate leukocytes of bus drivers with different numbers of cultured lymphocytes with gaps[a]

No. of gaps[b]	No. of subjects	UDS[c]
0	40	229 ± 155
1	42	149 ± 121
2+	23	113 ± 103

[a] Log-linear Poisson regression analysis showed significant negative dependence between DMS-induced UDS and the number of gaps ($P < 0.0005$). One person with a missing UDS value was not included.
[b] The number of cells with chromatid and chromosome gaps in 100 metaphases.
[c] Mean (±SD) cpm of [^{3}H]thymidine incorporated into 200,000 cells incubated with 10 mM hydroxyurea to suppress replication.

Table 5 Effect of urban air exposure on the frequency of cells with chromosomal aberrations in peripheral lymphocytes of bus drivers in comparison with postal workers, according to Poisson regression models including metabolic genotypes

Group, type of aberration	No. of subjects	Risk ratio	95% Confidence interval
Postal workers, total + gaps	101	1.00	
Bus drivers, total + gaps	106	1.21	1.01–1.45

null genotype and *NAT2* slow genotype was seen in total aberrant cells with and without gaps (Table 6). The relative risks were especially increased for these aberration categories in the bus drivers with high exposure. In the postal workers, no clear effects of the *GSTM1* genotype could be observed, but the relative risk for aberrant cells (with or without gaps) was significantly increased by the *NAT2* slow genotype (Table 6). This effect was particularly clear for cells with gaps.

To investigate the effect of the combinations of the metabolic genotypes, the bus drivers were divided into four groups according to the available *GSTM1* (null and positive) and *NAT2* (slow and rapid) genotypes (Table 7). A statistically significantly higher frequency was found for cells with chromatid-type aberrations and for the total number of aberrant cells (with or without gaps) in the *GSTM1* null, *NAT2* slow genotype group in comparison with the *GSTM1* positive, *NAT2* rapid genotype group. The *GSTM1* null, *NAT2* rapid and *GSTM1* positive, *NAT2* slow groups showed intermediate frequencies.

Discussion

A number of different biomarkers of genotoxicity have been applied to assess human exposure to the genotoxic compounds present in ambient air. Increased levels of chromosomal aberrations and SCEs have been demonstrated in studies of populations exposed to heavy air pollution, *e.g.,* in Poland and in the Czech Republic (37, 38).

In the present study, chromosomal aberrations were used to assess genotoxic effects in bus drivers exposed to a high level of urban air pollution. Besides bus drivers, we also studied postal workers who are expected to be exposed to lower air pollution levels than bus drivers. All subjects were nonsmokers to exclude the effects of this common confounder that is known to affect chromosome aberration rates. Some of the individuals studied were ex-smokers, but no significant effects of previous smoking could be observed in the present study, although some of our previous data have suggested that the chromosome-damaging effect of smoking can persist for several years (32).

The group of bus drivers studied here has previously been examined for aromatic DNA adducts in peripheral mononuclear cells; the results showed a clear exposure, with a 15-fold increase in the level of DNA adducts in comparison with a rural control group (14). The present findings also suggest that the bus drivers have experienced genotoxic exposure. Compared with the postal workers in a multivariate Poisson regression model adjusting for the modifying effect of age, sex, and DNA repair, a significantly increased risk of chromosome aberrations was seen.

During the last 20 years, several nonpositive and positive studies on the association between exposure to diesel exhaust and cytogenetic damage have been published (19–25), all of which included both smokers and nonsmokers and relatively small numbers of subjects. The present results showed that an effect of traffic-generated urban air exposure on chromosomal aberrations can be demonstrated in a carefully conducted study. The sensitivity of our analysis was increased by the relatively high number of study subjects and the exclusion of smokers, enabling us to show an effect at exposure levels that are presumably lower than those in the studies cited above.

An important factor that has not been taken into account in previous studies of urban air pollution and chromosome damage is the polymorphism of enzymes involved in the metabolism of air-borne carcinogens. The *GSTM1* null genotype, resulting in total lack of GSTM1 enzyme activity, was associated with an increased level of chromosome damage in the bus drivers (especially those driving in the center of the city), but not in the postal workers, indicating an association with exposure. This pattern resembles our recent findings in a group of pesticide-exposed greenhouse workers and control persons in which the effect of smoking, another complex exposure, on lymphocyte chromosomal aberrations was seen only in *GSTM1* null subjects (39, 40). In epidemiological studies, the *GSTM1* null genotype has been associated with an increased risk of developing lung cancer by smoking (28). Our previous study on the bus drivers showed no statistically significant effects of *GSTM1* or *NAT2* genotypes, alone or in combination, on the level of DNA adducts in mononuclear leukocytes, although the *GSTM1* null genotype tended to be associated with higher adduct levels in all groups studied, including rural controls (14).

Our present results on the bus drivers showed significantly higher levels of chromosomal aberrations in subjects with *NAT2* slow, *GSTM1* negative genotypes compared with those with *NAT2* rapid and *GSTM1* positive genotypes, the two other combinations of these genotypes giving intermediate results. The *GSTM1* null, *NAT2* slow combination has previously been described to enhance the level of thioguanine-resistant mutant T-lymphocytes and aromatic DNA adducts in nonsmoking male bus maintenance workers and controls (41, 42), urine mutagenicity in coke oven workers (43), and the risk of malignant mesothelioma (44). Interestingly, in our results, only the *NAT2* slow (but not the *GSTM1*) genotype was a significant risk factor in the group of postal workers. This indicates that the *NAT2* genotype effect is not due to exposure to a high level of urban air pollution but to something else common to all subjects. Alternatively, the *NAT2* genotype might influence the baseline level of chromosome damage. Acetylation occurs in folate catabolism (45) and in various cellular processes such as the function of chromosomal proteins (46) and the interconversion of polyamines (44, 47), although the possible involvement of NAT2 in this connection is unknown. The *NAT2* slow genotype has been reported to increase the risk of bladder cancer in exposure to aromatic amines (48).

Another interesting result in the present study was that DNA repair, measured as DMS-induced UDS, was found to influence the frequency of chromosomal aberrations. There was

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024

Table 6 Effect of metabolic genotypes on the frequency of cells with chromosomal aberrations (CAs) in peripheral lymphocytes of bus drivers and postal workers, according to Poisson regression models including significant confounders

Group Variable	*NAT2* rapid[a]	*NAT2* slow			*GSTM1*[+c]	*GSTM1*^{−}		
	No. of subjects	No. of subjects	Risk ratio[a]	95% CI[b]	No. of subjects	No. of subjects	Risk ratio	95% CI
Bus drivers								
CA + gaps	49	56	1.39	1.09–1.78	45	60	1.29	1.00–1.66
CA − gaps	49	57	1.48	1.09–2.00	46	60	1.42	1.05–1.93
Bus drivers, city								
CA + gaps	23	31	1.43	1.01–2.03	23	31	1.41	1.00–1.99
CA − gaps	23	32	1.32	0.87–2.01	24	31	1.74	1.13–2.68
Postal workers								
CA + gaps	33	68	1.68	1.23–2.31	47	54	1.08	0.83–1.41
CA − gaps	33	68	1.52	1.05–2.19	47	54	1.05	0.77–1.45
Gaps	33	68	2.31	1.22–4.35	47	54	1.14	0.70–1.86

[a] Reference groups (risk ratio, 1.00) were *NAT2* rapid for *NAT2* slow and *GSTM1*$^+$ for *GSTM1*$^-$.
[b] The 95% confidence interval for risk ratio.
[c] *GSTM1*$^+$, positive (at least one undeleted allele); *GSTM1*$^-$, GSTM1 null (both alleles deleted).

Table 7 Influence of *GSTM1* and *NAT2* genotypes on the frequency of cells with chromosomal aberrations in peripheral lymphocytes of bus drivers

Genotype[a]	No. of subjects	Mean (±SD) frequency of cells with chromosomal aberrations[b]		
		Gaps	Total, gaps excluded	Total, gaps included
GSTM1$^-$, *NAT2* slow	30	1.00 ± 1.05	2.27 ± 1.53[c]	3.20 ± 2.06[c]
GSTM1$^-$, *NAT2* rapid	30	0.90 ± 0.76	1.73 ± 1.05	2.63 ± 1.40
GSTM1$^+$, *NAT2* slow	27	0.85 ± 0.72	1.81 ± 1.55	2.67 ± 1.82
GSTM1$^+$, *NAT2* rapid	19	0.58 ± 0.69	0.95 ± 0.85	1.53 ± 1.07

[a] *GSTM1*$^-$, null genotype (both alleles deleted); *GSTM1*$^+$, positive genotype (at least one undeleted allele); *NAT2* slow, variant genotype associated with slow acetylation; *NAT2* rapid, heterozygous wild-type/variant or homozygous wild-type, associated with rapid acetylation.
[b] Whole-blood lymphocyte cultures were cultured for 48 h, and 100 metaphases were scored per person.
[c] $P < 0.0005$ in Poisson test compared with the *GSTM1*$^+$ and *NAT2* rapid genotype.

a statistically significant negative association between the DMS-induced UDS in mononuclear cells and the number of (mostly chromatid) gaps in cultured peripheral lymphocytes. This indicates a protective role of high UDS activity toward chromosome damage. Comparable results were previously obtained in patients with psoriasis and skin cancer who had lower UDS activity and higher DNA damage than healthy subjects, in agreement with the idea that a low rate of DNA repair increases DNA damage (49). Our findings suggest that (chromatid) gaps are the result of unrepaired lesions in DNA. Gaps, as we defined them (50), have both an upper size limit (the width of two chromatids) to arbitrarily distinguish them from larger breaks and a lower size limit (the width of a chromatid) to avoid recording any small unstained areas in chromosomes as gaps. This probably means that many of our gaps are true chromatid breaks.

In this study, no exposure-related effects on UDS were found, which suggests that the exposure of the subjects did not alter their capacity to perform the type of DNA repair measured in the assay. Similarly, the metabolic genotypes did not affect the individual UDS values. The observed positive correlation between UDS and the monocyte content of the mononuclear cell fraction indicates a higher DNA repair capacity in monocytes than in lymphocytes. Previously, a wide interindividual variation in UDS induced by chemicals or UV radiation has been observed (51–53). Besides interindividual variation in DNA repair capacity, the differential composition of peripheral mononuclear cells and differences among the leukocyte subsets in the ability to process DNA damage could account for this variation. Because induced UDS appears to be higher in monocytes and B-lymphocytes than in T-lymphocytes (54, 55), it is recommended that the samples be characterized with respect to the subsets of cell populations (30) or that UDS be recorded in one type of cells only.

The present study demonstrates a genotoxic effect of ambient air pollution, mostly attributable to traffic, in bus drivers driving in the center of a major city. An increased risk of cancer has been reported in Danish bus drivers (10), and our results further support the view that genotoxic exposure is partly responsible for this finding. Interindividual differences in susceptibility are becoming an increasingly important issue in environmental carcinogenesis (56–58). The present study shows that polymorphisms of major carcinogen-metabolizing enzymes affect the induced or spontaneous level of chromosome damage, suggesting that they should be taken into account when biomarkers of genotoxicity are determined. As in the present study, correlations between genotypes and effect biomarkers are usually observed at the group level, and such information can be used, *e.g.,* in assessing risks associated with exposure to urban air pollution. However, the existing knowledge is not enough to justify screening of risk genotypes at the individual level, because many scientific, social, and ethical questions are still unanswered (59). Studies like the present one enable prospective follow-up of the predictive value of biomarkers and metabolic genotypes/phenotypes. The predictivity may concern cancer risk, risk of cardiovascular diseases, or other diseases accessible in national registries. Such prospective follow-up studies have already been designed and performed by Nordic and Italian researchers (15, 16) and by the European Study Group of Cytogenetic Biomarkers and Health (60). An

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce049900303o.pdf by guest on 29 May 2024

association has been observed between high levels of chromosomal aberrations and increased risk of cancer.

Acknowledgments

We gratefully acknowledge Bo Christensen, Birgitte Korsholm, and Helle Binderup for efforts in collecting the samples and distributing information. Drs. Elsa Bach and Karsten Wassermann participated in the design and application of the National Strategic Environmental Program. The technical assistance of Lourdes M. Petersen, Lise Rud Nielsen, Birgitte Korsholm, Helle Binderup, and Bo Christensen is gratefully acknowledged. The recruitment of participating work places and persons was accomplished through active support from the labor organization and employers organizations.

References

1. Hemminki, K., and Pershagen, G. Cancer risk of air pollution: epidemiological evidence. Environ. Health Perspect., *102* (Suppl. 4)*:* 187–192, 1994.

2. Guillemin, M. P., Herrera, H., Huynh, C. K., Droz, P. O., and Vu Duc, T. Occupational exposure of truck drivers to dust and polynuclear aromatic hydrocarbons: a pilot study in Geneva, Switzerland. Int. Arch. Occup. Environ. Health, *63:* 439–447, 1992.

3. Silverman, D. T., Hoover, R. N., Mason, T. J., and Swanson, G. M. Motor exhaust-related occupations and bladder cancer. Cancer Res., *46:* 2113–2116, 1986.

4. Balarajan, R., and McDowall, M. E. Professional drivers in London: a mortality study. Br. J. Ind. Med., *45:* 483–486, 1988.

5. Hayes, R. B., Thomas, T., Silverman, D. T., Vineis, P., Blot, W. J., Mason, T. J., Pickle, L. W., Correa, P., Fontham, E. T., and Schoenberg, J. B. Lung cancer in motor exhaust-related occupations Am. J. Ind. Med., *16:* 685–695, 1989.

6. Guberan, E., Usel, M., Raymond, L., Bolay, J., Floretta, G., and Puissant, J. Increased risk for lung cancer and for cancer of the gastrointestinal tract among Geneva professional drivers. Br. J. Ind. Med., *49:* 337–344, 1992.

7. Steenland, K., Silverman, D., and Zaebst, D. Exposure to diesel exhaust in the trucking industry and possible relationships with lung cancer. Am. J. Ind. Med., *21:* 887–890, 1992.

8. Barbone, F., Bovenzi, M., Cavallieri, F., and Stanta, G. Air pollution and lung cancer in Trieste, Italy. Am. J. Ind. Med., *141:* 1161–1169, 1995.

9. Jakobsson, R., Gustavsson, P., and Lundberg, I. Increased risk of lung cancer among male professional drivers in urban but not rural areas of Sweden. Occup. Environ. Med., *54:* 189–193, 1997.

10. Soll-Johanning, H., Bach, E., Olsen, J. H., and Tüchsen, F. Cancer incidence in urban bus drivers and tramway employees: a retrospective cohort study. Occup. Environ. Med., *55:* 594–598, 1998.

11. Hansen, E. S. Shared risk factors for cancer and atherosclerosis: a review of the epidemiological evidence. Mutat. Res., *239:* 163–179, 1990.

12. Perera, F. P., Hemminki, K., Gryzbowska, E., Motykiewicz, G., Michalska, J., Santella, R. M., Young, T-L., Dickey, C., Brandt-Rauf, P., DeVivo, I., Blaner, W., Tasi, W-Y., and Chorazy, M. Molecular and genetic damage in humans from environmental pollution in Poland. Nature (Lond.), *360:* 256–258, 1992.

13. Autrup, H., Vestergaard, A. B., and Okkels, H. Transplacental transfer of environmental genotoxins: polycyclic aromatic hydrocarbon-albumin adducts in nonsmoking women, and the effect of maternal GSTM1 genotype. Carcinogenesis (Lond.), *16:* 1305–1309, 1995.

14. Nielsen, P. S., de Pater, N., Okkels, H., and Autrup, H. Environmental air pollution and DNA adducts in Copenhagen bus drivers: effect of GSTM1 and NAT2 genotype on adduct levels. Carcinogenesis (Lond.), *17:* 1021–1027, 1996.

15. Hagmar, L., Brøgger, A., Hansteen, I. L., Heim, S., Högstedt, B., Knudsen, L., Lambert, B., Linnainmaa, K., Mitelman, F., Nordenson, I., Reuterwall, C., Salomaa, S., Skerfving, S., and Sorsa, M. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res., *54:* 2919–2922, 1994.

16. Bonassi, S., Abbondandolo, A., Camurri, L., Dal Prá, L., De Ferrari, M., Degrassi, F., Forni, A., Lamberti, L., Lando, C., Padovani, P., Sbrana, I., Vecchio, D., and Puntoni, R. Are chromosome aberrations in circulating lymphocytes predictive of future cancer onset in humans? Cancer Genet. Cytogenet., *79:* 133–135, 1995.

17. Stevens, R. K., Lewis, C. W., Dzubau, T. G., Cupitt, L. T., and Lewtas, J. Sources of mutagenic activity in urban fine particles. Toxicol. Ind. Health, *6:* 81–94, 1990.

18. IARC. Diesel and Gasoline Engine Exhausts and Some Nitroarenes. Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 46*.* Lyon, France: IARC, 1989.

19. Anwar, W. A., and Kamal, A. A. M. Cytogenetic effects in a group of traffic policemen in Cairo. Mutat. Res., *208:* 225–231, 1988.

20. Chandrasekaran, R., Samy, P. L. P., and Murthy, P. B. K. Increased sister chromatid exchange (SCE) frequencies in lymphocytes from traffic policemen exposed to automobile exhaust pollution. Hum. Exp. Toxicol., *15:* 301–304, 1996.

21. Bolognesi, C., Gallerani, E., Bonatti, S., De Ferrari, M., Fontana, V., Valerio, F., Merlo, F., and Abbondandolo, A. Sister chromatid exchange induction in peripheral blood lymphocytes of traffic police workers. Mutat. Res., *394:* 37–44, 1997.

22. Bolognesi, C., Merlo, F., Rabboni, R., Valerio, F., and Abbondandolo, A. Cytogenetic biomonitoring in traffic police workers: micronucleus test in peripheral blood lymphocytes. Environ. Mol. Mutagen., *30:* 396–402, 1997.

23. Nordensson, I., Sweins, A., Dahlgren, E., and Beckman, L. A study of chromosomal aberrations in miners exposed to diesel exhaust. Scand. J. Work Environ. Health, *7:* 14–17, 1981.

24. Fredga, K., Dävring, L., Sunner, M., Bengtsson, B. O., Elinder, C-G., Sigtryggsson, P., and Berlin, M. Chromosome changes in workers (smokers and nonsmokers) exposed to automobile fuels and exhaust gases. Scand. J. Work. Environ. Health, *8:* 209–221, 1982.

25. Parry, E. M., Ballantine, J. A., Ellard, S., Evans, W. E., Jones, C., Kilic, N., and Lewis, R. I. Biomonitoring study of a group of workers potentially exposed to traffic fumes. Environ. Mol. Mutagen., *30:* 119–130, 1997.

26. Bohr, V. A., Evans, M. K., and Fornace, A. J. Biology of disease. DNA repair and its pathogenic implications. Lab. Investig., *61:* 143–161, 1989.

27. Pero, R. W., Miller, D. G., Lipkin, M., Markowitz, M., Gupta, S., Winawer, S. J., Enker, W., and Good, R. Reduced capacity for DNA repair synthesis in patients with or genetically predisposed to colorectal cancer. J. Natl. Cancer Inst., *70:* 867–875, 1983.

28. McWilliams, J. E., Sanderson, B. J. S., Harris, E. L., Richert-Boe, K. E., and Henner, W. D. Glutathione *S*-transferase M1 (GSTM1) deficiency and lung cancer risk. Cancer Epidemiol. Biomark. Prev., *4:* 589–594, 1995.

29. Hansen, A. M., Wallin, H., Wilhardt, P., and Knudsen, L. E. Monitoring of urban air exposure of bus drivers and mail carriers in Denmark. *In:* K. Chiyotani, Y. Hosoda, and Y. Aizawa (eds.), Advances in the Prevention of Occupational Respiratory Diseases, pp. 1005–1060. Tokyo, Japan: Elsevier, 1998.

30. Knudsen, L. E. Evaluation of unscheduled DNA synthesis (UDS) in human lymphocytes as an indicator of genotoxic exposure. Use and validation of a selected method for monitoring and screening for occupational genotoxic exposures (Thesis). Copenhagen, Denmark: National Institute of Occupational Health and the University of Copenhagen, 1993.

31. Pero, R. W., Seidegård, J., and Miller, D. G. Development of biochemical markers sensitive to ecogenetic variation and their use in assessing risk from genotoxic exposures. Genetic toxicology of environmental chemicals. B. Genetic effects and applied mutagenesis. Prog. Clin. Biol. Res., *209B:* 225–235, 1986.

32. Valjus, J., Norppa, H., Järventaus, H., Sorsa, M., Nykyri, E., Salomaa, S., Järvinen, P., and Kajander, J. Analysis of chromosomal aberrations, sister chromatid exchanges and micronuclei among power linesmen with long-term exposure to 50 Hz electromagnetic fields. Radiat. Environ. Biophys., *32:* 325–336, 1994.

33. Spurr, N. K., Gough, A. C., Smith, C. A. D., and Wolf, C. R. Genetic analysis of cytochrome P450 system. Methods Enzymol., *206:* 149–166, 1991.

34. Okkels, H., Sigsgaard, T., Wolf, H., and Autrup, H. Arylamine N-acetyltransferase 1 (NAT1), and 2 (NAT2) polymorphisms in susceptibility to bladder cancer: the influence of smoking. Cancer Epidemiol. Biomark. Prev., *6:* 225–231, 1997.

35. Okkels, H., Sigsgaard, T., Wolf, H., and Autrup, H. Glutathione *S*-transferase m as a risk factor of bladder tumours. Pharmacogenetics, *6:* 251–256, 1996.

36. Bonassi, S., Ceppi, M., Fontana, V., and Merlo, F. Multiple regression analysis of cytogenetic human data. Mutat. Res., *313:* 69–80, 1994.

37. Hemminki, K., Grzybowska, E., Chorazy, M., Twardowska-Saucha, K., Sroczynski, J. W., Putman, K. L., Randerath, K., Phillips, D. H., and Hewer, A. Aromatic DNA adducts in white blood cells of coke workers. Int. Arch. Occup. Environ. Health, *62:* 467–470, 1990.

38. Sram, R. J., Benes, I., Binkova, B., Dejmek, J., Horstman, D., Kotesovec, F., Otto, D., Perreault, S. D., Rubes, J., Selevan, S. G., Skalik, I., Stevens, R. K., and Lewtas, J. Teplice program: the impact of air pollution on human health. Environ. Health Perspect., *104:* 699–714, 1996.

39. Scarpato, R., Migliore, L., Hirvonen, A., Falck, G., and Norppa, H. Cytogenetic monitoring of occupational exposure to pesticides: characterization of GSTM1, GSTT1 and *NAT2* genotypes. Environ. Mol. Mutagen., *27:* 263–269, 1996.

40. Scarpato, R., Hirvonen, A., Migliore, L., Falck, G., and Norppa, H. Influence of GSTM1 and GSTT1 polymorphisms on the frequency of chromosome aberrations in lymphocytes of smokers and pesticide-exposed greenhouse workers. Mutat. Res., *389:* 227–235, 1997.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024

41. Hou, S., Lambert, B., and Hemminki, K. Relationship between hprt mutant frequency, aromatic DNA adducts and genotypes for GSTM1 and NAT2 in bus maintenance workers. Carcinogenesis (Lond.), *16:* 1913–1917, 1995.

42. Lambert, B., Bastlova, T., Osterholm, A. M., and Hou, S. M. Analysis of mutation at the hprt locus in human T lymphocytes. Toxicol. Lett., *82/83:* 323–333, 1995.

43. Gabbani, G., Hou, S-M., Nardini, B., Marchioro, M., and Lambert, B. GSTM1 and NAT2 genotypes and urinary mutagens in coke oven workers. Carcinogenesis (Lond.), *17:* 1677–1681, 1996.

44. Hirvonen, A., Pelin, K., Tammilehto, L., Karjalainen, A., Mattson, K., and Linnainmaa, K. Inherited GSTM1 and NAT2 defects as concurrent risk modifiers in asbestos-related human malignant mesothelioma. Cancer Res., *55:* 2981–2983, 1995.

45. Minchin, R. F. Acetylation of p-aminobenzoylglutamate, a folic acid catabolite, by recombinant human arylamine *N*-acetyltransferase and U937 cells. Biochem. J., *307:* 1–3, 1995.

46. Grunstein, M. Histone acetylation in chromatin structure and transcription. Nature (Lond.), *389:* 349–352, 1997.

47. Seiler, N., Knodgen, B., and Bartholeyns, J. Polyamine metabolism and polyamine excretion in normal and tumour bearing rodents. Anticancer Res., *5:* 371–377, 1985.

48. Hein, D. W. Acetylator genotype and arylamine-induced carcinogenesis. Biochim. Biophys. Acta, *948:* 37–66, 1988.

49. Møller, P., Knudsen, L. E., Frenz, G., Dybdahl, M., Wallin, H., and Nexo, B. A. Seasonal variation of DNA damage and repair in patients with nonmelanoma skin cancer and referents with and without psoriasis. Mutat. Res., *407:* 25–34, 1998.

50. Brøgger, A., Norum, R., Hansteen, I-L., Clausen, K. O., Skårdal, K., Mitelman, F., Kolnig, A-M., Strömbeck, B., Nordenson, I., Andersson, G., Jakobsson, K., Mäki-Paakkanen, J., Norppa, H., Järventaus, H., and Sorsa, M. Comparison between five Nordic laboratories on scoring of human lymphocyte chromosome aberrations. Hereditas, *100:* 209–218, 1984.

51. Madden, J. J., Falek, A., Shafer, D. A., Donahoe, R. M., Eltzroth, D. C., Hollingsworth, F., and Bokos, P. J. Influence of demographic factors on unscheduled DNA synthesis as measured in human peripheral leukocytes and fibroblasts. Environ. Sci. Res., *30:* 359–371, 1986.

52. Pero, R. W., Bryngelsson, C., Bryngelsson, T., Lindholm, L., Schersten, B., Trell, E., Janzon, L., Franzen, J., and Johansson, B. W. The effects of smoking, hypertension and cardiovascular disease on the estimation of unscheduled DNA synthesis and covalent binding to DNA induced by N-acetoxy-2-acetylaminofluorene in resting human mononuclear leukocytes. Atherosclerosis, *48:* 119–129, 1983.

53. Knudsen, L. E., Boisen, T., Christensen, J. M., Jelnes, J. E., Jensen, G. E., Jensen, J. C., Lundgren, K., Lundsteen, C., Pedersen, B., Wassermann, K., Wilhardt, P., Wulf, H. C., and Zebitz, U. Biomonitoring of genotoxic exposure among stainless steel welders. Mutat. Res., *279:* 129–143, 1992.

54. Yew, F-H., and Johnson, R. T. Human B and T lymphocytes differ in UV-induced repair capacity. Exp. Cell Res., *113:* 227–231, 1978.

55. Knudsen, L. E., Ryder, L. P., and Wassermann, K. Induction of DNA repair synthesis in human monocytes/B-lymphocytes compared with T-lymphocytes after exposure to N-acetoxy-N-acetyl-aminofluorene and dimethylsulfate *in vitro.* Carcinogenesis (Lond.), *7:* 1285–1287, 1992.

56. Barrett, J. C., Vainio, H., Peakall, D., and Goldstein, B. D. 12th meeting of the Scientific Group on Methodologies for the Safety Evaluation of Chemicals: susceptibility to environmental hazards. Environ. Health Perspect., *105* (Suppl. 4)*:* 699–737, 1997.

57. Perera, F. P. Environment and cancer: who is susceptible. Science (Washington DC), *278:* 1068–1073, 1997.

58. Norppa, H. Cytogenetic markers of susceptibility: influence of polymorphic carcinogen-metabolizing enzymes. Environ. Health Perspect., *105* (Suppl. 4)*:* 829–835, 1997.

59. Van Damme, K., Casteleyn, L., Heseltine, E., Huici, A., Sorsa, M., van Larebeke, N., and Vineis, P. Individual susceptibility and prevention of occupational diseases: scientific and ethical issues. J. Occup. Environ. Med., *37:* 91–99, 1995.

60. Hagmar, L., Bonassi, S., Strömberg, U., Brøgger, A., Knudsen, L., Norppa, H., Reuterwall, C., Forni, A., Hansteen, I-L., Högstedt, B., Huici Montagud, A., Lambert, B., Mitelman, F., Nordenson, I., Salomaa, S., and Skerfving, S. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European Study Group on Cytogenetic Biomarkers and Health (ESCH). Cancer Res., *58:* 4117–4121, 1998.

Downloaded from http://aacrjournals.org/cebp/article-pdf/8/4/303/3255693/ce0499000303o.pdf by guest on 29 May 2024