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REVIEW

Genotoxicity biomarkers associated with exposure to traffic and near-road atmospheres: a review

David M. DeMarini*

Integrated Systems Toxicology Division, US Environmental Protection Agency, B105-03, Research Triangle Park, NC 27711, USA

*To whom correspondence should be addressed. Tel: (919) 541 1510; Fax: (919) 541 0694; Email: demarini.david@epa.gov

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Diesel and gasoline emissions, which are the primary components of traffic exhaust, are known or possible human carcinogens, respectively, and working or living near high-traffic roads is associated with various health effects, including cancer. To help understand the mechanistic basis for this observation, the present article reviews 63 studies on genotoxicity biomarkers in traffic-exposed subjects, with office workers being the typical control subjects. The six primary biomarkers used in these studies were the traditional cytogenetic end points, chromosome aberrations (CAs), micronucleus (MN) and sister chromatid exchange, and the standard molecular end points for DNA damage, ^{32}P-postlabeling, the comet assay and urinary 8-hydroxydeoxyguanosine. These six assays accounted for 74 of the 87 biomarker assessments reported in the studies; all six effectively distinguished traffic-exposed from control populations, giving an average 89% positive results among exposed versus control subjects. In addition, three genomic biomarkers effectively distinguished between the exposed and control populations; these assays measured changes in gene expression, leukocyte telomere length and DNA methylation. Nearly half of all of the studies included exposure assessments involving blood (primarily protein adducts), urine (primarily 1-hydroxypyrene) or air (primarily polycyclic aromatic hydrocarbons); these assays distinguished the exposed from the control subjects for the vast majority of the studies. All but three of the 63 reports were environmental studies that investigated 18 general exposure categories, such as traffic police and automobile/bus mechanics. The studies were performed in 20 countries; however, nearly all of the environmental studies were performed in Europe and Asia, with only one each from Africa, North America and South America. Given that several of the biomarkers are associated with increased cancer risk, including CAs, MNs and altered telomere length, the data reviewed here provide strong mechanistic support for the ability of chronic exposure to traffic exhaust to increase cancer risk.

Don't play in the traffic!

Everybody's mother

Introduction

Along with the obvious and immediate hazard of working in or near traffic, as indicated by the admonition above, there are also long-term health effects that may accrue from being exposed chronically to traffic exhaust. Urban air pollution is a complex mixture of particles and gases derived from a variety of sources that is altered by the sun and temperature to produce a range of atmospheric transformation products. Traffic is an important source of this air pollution, contributing carbon dioxide, carbon monoxide, various hydrocarbons, nitrogen dioxide, particulate matter (PM), volatile organics (e.g. benzene, acetaldehyde, 1,3-butadiene and formaldehyde), heavy metals and secondary reaction products such as ozone, nitrates and organic acids (1).

The exhaust from gasoline and diesel vehicles is frequently a major source of the PM in urban air (2), especially $PM_{2.5}$ (fine particulates with a median aerodynamic diameter of 2.5 μm), which enters the respiratory tract and potentially the circulatory system more easily; it is generally more genotoxic than larger particles (3). Not surprisingly, the concentrations of $PM_{2.5}$, as well as other constituents of air pollution, are generally highest near roads with high levels of vehicular traffic (4).

As a consequence, working or living near such roads is associated with numerous health effects, including asthma, decreased lung function, respiratory and increased cardiovascular disease, allergy, adverse birth outcomes and cancer (1). The particulate fraction alone has been estimated to cause 3.1 million excess deaths annually worldwide as a result of cardiopulmonary disease and lung cancer in adults and acute respiratory infections in children (5).

Biomarkers of exposure and effect, especially of genotoxicity, inflammation, lung function, asthma and oxidative stress, have been examined in people working near roads with high levels of vehicular traffic and compared with subjects working farther from such roads or, most typically, nearby indoor office workers. The present review summarises the results for genotoxicity biomarkers such as DNA damage and chromosomal mutation that have been evaluated in traffic-exposed subjects relative to control subjects. If reported, exposure assessments and the influence of genotype/phenotype are also reviewed.

A search was done in PubMed and Scopus using the following words or phrases in combination with the word 'traffic' or 'near road': human, biomarker, automobile/diesel exhaust, genotoxicity, DNA adducts, DNA damage, cytogenetic, chromosome aberrations, micronucleus, sister chromatid exchange (SCE), oxidative damage and mutation. This resulted in 63 articles that are reviewed here in terms of the (i) nature of the control and exposed populations, (ii) quantitative results of various biomarkers and exposure assessments and (iii) association between exposure assessment and biomarker. These studies involve 12 different biomarkers and ~20 different occupations studied in subjects from 20 countries. The results illustrate consistent increases in genotoxic effects incurred by people living or working in or near heavy traffic for much of their workday.

DNA damage

The literature describes three primary assays used to evaluate DNA damage in traffic-exposed subjects: ^{32}P-postlabeling for DNA adducts, the comet assay for DNA damage and

concentration of urinary 8-hydroxydeoxyguanosine (8OHdG). Although SCEs can also be considered a measure of DNA damage, the literature for that end point is classified under the section on cytogenetic effects.

DNA adducts detected by ^{32}P-postlabeling

DNA adduct analysis by ^{32}P-postlabeling has been the most sensitive method for measuring largely aromatic or bulky DNA adducts due to exposure to complex mixtures, especially combustion emissions (6,7). Although it is an expensive and technically difficult assay to perform, it has been used more than any other method as a detector of a genotoxicity biomarker in traffic-exposed people. The advantages and disadvantages of ^{32}P-postlabeling, its various modifications and the types of adducts that it detects have been reviewed (6,7). This assay is especially useful for quantifying adducts due to exposure to complex mixtures, such as ambient air. The applicability of the ^{32}P-postlabeling assay to traffic-exposed populations is clearly illustrated by the studies reviewed below, which demonstrate the general ability of this method to distinguish between traffic-exposed and control populations.

Table I shows the various categories of exposure and controls where ^{32}P-postlabeling was used to determine the presence of stable DNA adducts associated with exposure to traffic; all studies evaluated DNA adducts in peripheral blood lymphocytes. As noted in Table I, all but one study used the nuclease P1 digestion procedure, which involves post-incubation of DNA with *Penicillium citrinum* nuclease P1 before ^{32}P-labeling. This can enhance the sensitivity to one adduct in 10^{10} nucleotides. Nuclease P1 cleaves deoxyribonucleoside 3′-monophosphates of normal nucleotides to deoxyribonucleosides, which do not serve as substrates for polynucleotide kinase, whereas most adducted nucleotides are totally or partially resistant to the 3′-dephosphorylating action of nuclease P1. One study used the butanol extraction procedure for enrichment of adducted nucleotides, and one used both P1 and butanol. The ^{32}P-postlabeling method has been used to evaluate 11 different categories of traffic-exposed subjects, which are more than have been evaluated by any other biomarker used to assess genotoxic effects associated with exposure to traffic.

Among the 21 reported assessments of DNA adducts by ^{32}P-postlabeling (Table I), all but four showed increased levels of DNA adducts in the exposed versus control populations. Among the four negative studies, two (8,9) found significant differences in exposure between their respective control and exposed groups but did not find any significant difference in DNA adduct levels among their control and exposed groups (Table II). The other two negative studies (10,11) did not perform an exposure assessment. Among the 21 assessments by ^{32}P-postlabeling, 16 included exposure assessments (Table II), and among these, all 16 found significant differences in exposure between the control and exposed subjects. Among the 16 studies that incorporated an exposure assessment, 7 found an association between the levels of DNA adducts and exposure; the other 9 either did not find such an association or did not perform a statistical analysis to determine such an association.

Traffic police. Two studies in Genoa, Italy, found significantly higher levels of DNA adducts in traffic police or police working outdoors compared with those of office/indoor workers in the same city (12,13). Although both studies found significant differences in exposure among their respective control and exposed populations based on the concentration of benzo[*a*]

Table I. Traffic-associated DNA damage determined by ^{32}P-postlabeling of DNA adducts

Country	Control		Exposed		*P* value	Call	References
	Description (*n*)	Result[a]	Description (*n*)	Result[a]			
Italy	Indoor workers (52)	1.01 ± 0.63	Traffic police (94)	1.48 ± 1.35	0.007	+	(12)
Italy	Office workers (36)	0.94	Outdoor police (34)	1.3	<0.05	+	(13)
Denmark	Rural residents (60)	0.074	Bus drivers (90)	0.636	<0.001	+	(15)
Sweden	Mechanical shop workers (22)	1 ± 0.32	Suburban bus drivers (23)	1.4 ± 0.48	<0.01	+	(10)
			Urban bus drivers (26)	0.9 ± 0.35	>0.05	–	
			Taxi drivers (21)	1.6 ± 0.91	<0.01	+	
	Mechanical shop workers (22)	2.08 ± 0.73	Bus maintenance (44)	3.10 ± 1.11	0.025	+	(17)
			Truck terminal (24)	2.7 ± 10.07	0.044	+	
	Mechanical shop and lab workers (27)	2.3	Bus maintenance (47)	3.2	<0.001	+	(18)
Denmark	Office workers (12)	0.26 butanol 0.08 P1	Bus garage (10)	0.84 butanol 0.65 P1	0.012 0.0004	+ +	(19)
Hungary	Unexposed (55)	3.1 ± 2.4 butanol	Garage mechanics (47)	2.7 ± 1.5 butanol	>0.05	–	(8)
Italy	Never-smoker residents of Florence (38)	0.87 ± 0.11	Never-smoker traffic-exposed workers (29)	1.49 ± 0.23	0.03	+	(23)
	Light-smoker residents of Florence (7)	0.47 ± 0.16	Light-smoker traffic-exposed workers (6)	2.52 ± 0.29	0.003	+	(23)
	Resident of Florence (152)	1.04 ± 1.16	Traffic-exposed workers (62)	1.52 ± 1.49	0.02	+	(24)
	Suburban newspaper vendors (22)	2.2	Urban newspaper vendors (31)	2.2	>0.05	–	(11)
Thailand	Rural school children (69)	0.09 ± 0.00	Urban school children (107)	0.45 ± 0.03	<0.001	+	(21,22)
Estonia	Surface diesel drivers (10)	3.5	Miner diesel drivers (10)	4.6	0.18	–	(9)
Denmark	Low traffic (23)	2.91 ± 1.78	High traffic (23)	4.29 ± 2.06	<0.01	+	(25)
Bénin	Rural/suburban residents (37)	2.6 ± 0.7	Taxi–bike drivers (13)	24.6 ± 6.2	<0.001	+	(16)
			Roadside residents (11)	23.78 ± 6.9	<0.001	+	
			Street vendors (16)	34.7 ± 9.8	<0.001	+	
			Gas station attendants (17)	37.2 ± 8.1	<0.001	+	

[a]Results are from DNA from white blood cells and are expressed as relative adduct levels/10^8 nucleotides except for those from (15,19,20), which are expressed as fmol/μg of DNA; all results are with nuclease P1 digestion (as explained in the text) except for references (8,19), which used butanol to enrich for adducted nucleotides.

Table II. Exposure assessment and association with traffic-associated biomarkers

Exposure	Exposure assessment (control value/exposed value)			Significance of exposure		Association of exposure with biomarker			References
	Blood	Urine	Air	*P* value	Call	Biomarker	*P* value	Call	
Bus garage workers	22.1/33.3 pmol/g Hb			0.0065	+	^{32}P	>0.05	–	(19)
		0.05/0.11 µmol OHPy/mol creatinine		0.0038	+	^{32}P	>0.05	–	
Traffic police			0.03/3.3 ng B[*a*]P/m^3	<0.001	+	^{32}P	0.65	–	(13)
			0.15 ± 0.30/4.55 ± 3.44 ng PAH/m^3			^{32}P	0.94	–	(12)
						MN	0.14	–	
Urban bus drivers	119/232 fmol B[*a*]P-eq/mg albumin			>0.05	–	^{32}P			(10)
		13.0/11.5 nM OHPy		>0.05	–	^{32}P			
Suburban bus drivers	119/120 fmol B[*a*]P-eq/mg albumin			>0.05	–				
		13.0/12.0 nM OHPy		>0.05	–	^{32}P			
Taxi drivers	119/318 fmol B[*a*]P-eq/mg albumin			<0.001	+				
		13.0/10.2 nM OHPy		>0.05	–				
Traffic workers			PM_{10}		+	^{32}P	0.02	+	(24)
Garage mechanics		0.17 ± 0.18/0.30 ± 0.26 18 µmol OHPy/mol creatinine		0.001	+	^{32}P	>0.05	–	(8)
Traffic police			0.62–0.85/51–184 ng B[*a*]P/m^3	<0.05	+	^{32}P		–	
			74.94 ± 40.09/115.40 ± 46.17 µg $PM_{2.5}$/m^3	<0.01	+	Comet	<0.01	+	(31)
Diesel drivers in mine			85/637 pg of particle-associated 1NP in breathing zone/m^3		+	^{32}P, comet		–	(9)
Gate guards			4.86/18.27 µg metals/m^3		+	8OHdG	<0.05	+	(40)
			0.31/0.76 µg PAH/m^3		+				
Airport workers			9494/27,703 µg PAH/m^3		+	SCE, CA, comet, MN			(28)
		0.34 ± 0.26/0.32 ± 0.19 µg OHPy/g creatinine		0.978	–	SCE, CA, comet, MN		–	
Taxi–bike drivers, roadside residents, street vendors and gas station attendants		3.9 ± 2.3/254.3 ± 27.6 µg OHPy/g creatinine		<0.001	+	^{32}P		+	(16)
		11.3 ± 1.5/155.5 ± 15.6 mg phenol (benzene)/g creatinine		<0.001	+	^{32}P		+	
Taxi drivers		0.2 ± 0.14/0.33 ± 0.2 µg OHPy/g creatinine		<0.001	+	8OHdG	0.002	+	(39)
	40.0 ± 7.3/45.6 ± 8.7 µM NO_x			<0.001	+	8OHdG	0.280	–	
Bicyclists			More cycling increases exposure to 10–100 nm air particles	0.01	+	Fpg comet	0.0003	+	(34)
						Comet	>0.05	–	
Traffic conductors			70.82/82.87 µg $PM_{2.5}$/m^3	0.054	–	8OHdG	>0.05	–	(30)
						Comet	>0.05	–	
			8.24/13.07 ng PAH/m^3	<0.001	+	8OHdG	>0.05	–	
						Comet	>0.05	–	
		0.18/0.33 µg OHPy/g creatinine		<0.001	+	8OHdG	0.023	+	
						Comet	>0.05	–	

Table II. *Continued*

Exposure	Exposure assessment (control value/exposed value)			Significance of exposure		Association of exposure with biomarker			References
	Blood	Urine	Air	*P* value	Call	Biomarker	*P* value	Call	
Near-road school children		0.09 ± 0.01/0.020 ± 0.02 µmol OHPy/mol creatinine		<0.001	+	^{32}P, comet, challenge		+	(21,22)
			1.18 ± 0.09/4.13 ± 0.21 ng PAH/m^3	<0.001	+	^{32}P, comet, challenge		+	
	46.23 ± 4.32/77.97 ± 11.67 ppt benzene			<0.01	+	^{32}P, comet, challenge		+	
			8.10 ± 0.73/17.55 ± 1.29 µg benzene/m^3	<0.01	+	^{32}P, comet, challenge		+	
		0.06 ± 0.01/0.17 ± 0.03 mg t,t-muconic acid/g creatinine		<0.01	+	^{32}P, comet, challenge		+	
Mail carriers		0.57/0.75 µmol OHPy/mol creatinine		<0.001	+	Urine mut	>0.05	–	(67)
Bus drivers		1.14/1.60 µmol OHPy/mol creatinine for slow versus fast NAT2		<0.001	+	Urine mut	>0.05	–	
Diesel emission inspectors			PAHs in $DEP_{2.5}$ (ng/m^3) modeled			8OHdG	0.026	+	(41)
Traffic police		0.48 ± 0.27/0.98 ± 0.35 µmol OHPy/mol creatinine		<0.05	+	CA	>0.05	–	(44)
Taxi drivers		0.48 ± 0.27/0.20 ± 0.11 µmol OHPy/mol creatinine		<0.05	+	CA	>0.05	–	(44)
Traffic police			0.14/10.95 ng B[*a*]P/m^3	0.000	+	SCE	0.07	–	(64)
			0.14/10.95 ng B[*a*]P/m^3	0.000	+	MN	>0.05	–	(56)
			0.094 ± 0.033/0.260 ± 0.068 mg inhaled particles/m^3	<0.05	+	MN, SCE			(57)
			0.30 ± 0.09/0.75 ± 0.23 mg NO_x/m^3	<0.05	+	MN, SCE			
			1.55 ± 0.31/4.43 ± 2.11 mg CO/m^3	<0.05	+	MN, SCE			
			0.110 ± 0.037/0.192 ± 0.038 mg hydrocarbons/m^3	<0.05	+	MN, SCE			
			0.055 ± 0.020/0.095 ± 0.033 µg Pb/m^3	<0.05	+	MN, SCE			
Low versus high traffic			121.4 ± 1105.3/3960.7 ± 4140.3 vehicle km/24 h	<0.01	+	^{32}P	<0.01	+	(25)
						MN	0.02	+	
Residents of Massachusetts, USA			Modeled exposure to black carbon			Difference in telomere length for interquartile range (0.25 µg/m^3) increase in black carbon concentration	0.008	+	(74)

Table II. *Continued*

Exposure	Exposure assessment (control value/exposed value)			Significance of exposure		Association of exposure with biomarker			References
	Blood	Urine	Air	*P* value	Call	Biomarker	*P* value	Call	
Truck drivers			183.4/213.9 µg personal $PM_{2.5}/m^3$ (90th percentile; 8-h average on day of exam)	<0.001	+	↑Telomere length	0.007	+	(75)
			18.4/26.2 µg personal elemental carbon/m^3 (90th percentile; 8-h average on day of exam)	<0.001	+	↑Telomere length	0.01	+	
			181.0/188.0 µg ambient PM_{10} on day of exam (90th percentile)	0.29	–	↑Telomere length	<0.001	+	
			141.6/142.4 µg ambient PM_{10}/m^3 average over 14 days prior to exam (90th percentile)	0.45	–	↓Telomere length	0.02	+	
Traffic officers			13.0/31.8 µg benzene/m^3			↓Telomere length with ↑ benzene	0.004	+	(76)
			43.4/128.7 µg toluene/m^3			↓Telomere length with ↑ toluene	0.008	+	
Residents			Modeled for exposure to black carbon and $PM_{2.5}$			↓LINE-1 methylation after recent exposure to BC	0.002	+	(72)
						↓LINE-1 methylation after recent exposure to BC	<0.001	+	
Emission inspectors		0.061 ± 0.094/0.298 ± 0.212 µmol OHPy/mol creatinine		<0.001	+	Comet		+	(33)
		1.924±3.441/5.894±468.3 µmol 2-Naphthol/mol creatinine		<0.001	+	Comet		+	

^{32}P, ^{32}P-postlabeling; OHPy, hydroxypyrene; urine mut, urine mutagenicity; challenge, challenge assay, which measures DNA damage/repair.

pyrene (B[*a*]P) or polycyclic aromatic hydrocarbons (PAHs) in the air, neither found an association between exposure level and level of DNA adducts (Table II). One study (13) found that the levels of DNA adducts were higher in the summer, suggesting a possible role for atmospheric transformation products in the induction of DNA adducts. A study of traffic police in Prague, Czech Republic, found that the levels of B[*a*]P-like DNA adducts correlated with the levels of carcinogenic PAHs in the air determined by personal samples; however, no control population was used in this study (14).

Bus drivers. The levels of DNA adducts in bus drivers in Copenhagen, Denmark, were higher than in the controls, who were rural residents of Denmark (Table I); no exposure assessment was performed on these subjects (15). An investigation of bus drivers in the Stockholm region of Sweden (10) found that suburban drivers had significantly higher levels of DNA adducts, but urban bus drivers did not, compared with mechanical shop workers (Table I). Exposure assessments found no differences between the controls and the drivers for either urinary 1-hydroxypyrene (OHPy) or PAH-plasma protein adducts (Table II).

Taxi drivers. Taxi drivers in the Stockholm region of Sweden had higher levels of DNA adducts compared with mechanical shop workers (10) (Table I). Exposure assessment by PAH-plasma protein level showed significantly higher exposure among the taxi drivers relative to the control population (Table II); however, urinary OHPy levels were not significantly different between the two groups (Table II). Taxi–bike drivers in Contonou, Bénin, had higher levels of DNA adducts compared with rural/suburban residents of Bénin (16) (Table I). The taxi–bike drivers had higher levels of exposure compared with the controls based on concentrations of urinary OHPy and urinary phenol (Table II).

Garage mechanics. Two studies have evaluated the induction of stable DNA adducts by ^{32}P-postlabeling in bus maintenance workers, both in Stockholm, Sweden (Table I). Both found elevated levels of DNA adducts in these workers relative to hospital mechanics (17) or a combination of hospital mechanics and laboratory workers as control populations (18). Neither study included an exposure assessment.

DNA adducts were increased among a group of bus garage workers in Copenhagen, Denmark, compared with a group of office workers (19) (Table I). Adduct analysis was performed by both the P1 and butanol extraction methods, and although higher adduct levels were obtained by the butanol method, the difference in adduct levels between the exposed and control populations was more significant by the P1 method (Table I). No exposure assessment was performed on these populations.

Using only the butanol extraction method, no difference was found between the levels of DNA adducts of a group of garage mechanics compared with a group of unexposed subjects (occupations not stated) in Hungary (8) (Table I). Although this was a negative study relative to the biomarker, two types of exposure assessments, urinary OHPy and B[*a*]P concentration in the air, found higher exposure in the mechanics versus the controls (Table II).

Truck terminal workers. A study of truck terminal workers in Stockholm, Sweden, found elevated levels of DNA adducts among this population compared with a group of hospital mechanics (Table I) (17). No exposure assessment was performed in this study.

Street vendors. One study found no difference in DNA adduct levels between newspaper vendors in Milan, Italy, compared with those in a suburban area of Milan (11) (Table I); no exposure assessment was performed in this study. However, another study of newspaper vendors in Milan, which did not measure a genotoxicity biomarker, did assess exposure by measuring B[*a*]P-diol epoxide adducts of haemoglobin (Hb) based on the concentration of B[*a*]P tetrahydrotetrols released from Hb after acid hydrolysis and quantified by GC/MS after immunoaffinity chromatography (20). The authors found higher levels of B[*a*]P-Hb adducts among non-smoker vendors who had high exposure to traffic (>1300 vehicles/h) compared with non-smoker vendors who had low exposure (<1300 vehicles/h) (P = 0.02) (20). A third study of street vendors found higher levels of DNA adducts in vendors in Contonou, Bénin, compared with rural/suburban residents of Bénin (16) (Table I). Exposure assessments showed differences between the two groups for levels of urinary OHPy and urinary phenol (as a measure of benzene exposure) (Table II). Although no statistical analysis was performed to show an association between exposure and DNA adducts, the >65-fold difference in exposure levels suggests that such an association is likely.

Gas station attendants. One study examined DNA adducts among gas station attendants in Contonou, Bénin, and found higher levels among these workers compared with rural/suburban residents of Bénin (16) (Table I). Exposure assessment also found higher levels of urinary OHPy and urinary phenol among the gas station attendants relative to the controls (Table II).

School children. Higher levels of DNA adducts were found among primary school children whose schools were located within 500 m of main roads in Bangkok, Thailand, compared with children whose schools were located in Banghra or Vornapa districts in rural Chonburi province, which is ~110 km from Bangkok (21,22) (Table I). Extensive exposure assessments found that the urban children had higher levels of urinary OHPy and benzene as well as higher levels of PAHs in the ambient air than did the rural children (21,22) (Table II).

Traffic workers and roadside residents. Several studies evaluated DNA-adduct levels in so-called traffic workers or people living near high-traffic areas versus various controls; the occupations of the traffic workers were not specified. Two studies found elevated levels of DNA adducts among traffic workers in Florence, Italy, compared with general residents of Florence (23,24) (Table I). One of these studies included exposure assessment and found that the traffic workers were exposed to higher concentrations of PM with a diameter of 10 μm (PM_{10}) in the air than were the control subjects (24). Roadside residents in Contonou, Bénin, had higher levels of DNA adducts compared with rural/suburban residents of Bénin (16) (Table I); exposure assessments showed that the roadside residents had high levels of urinary OHPy and urinary phenol compared with the controls (Table II).

Traffic-exposed mothers/umbilical cord blood. One study found higher levels of DNA adducts in mothers and umbilical cord blood from those mothers who lived in high- versus

low-traffic areas of Denmark (25) (Table I). The correlation between maternal and cord blood DNA adducts was $P < 0.01$. The exposure was higher among the high-traffic areas relative to the low-traffic areas based on the vehicle km/24 h (Table II).

Diesel drivers in a mine. An evaluation of miners exposed to diesel exhaust found no increase in adducts compared with the levels in diesel drivers on the surface (9) (Table I). Nonetheless, exposure assessment found a higher concentration of particle-associated 1-nitropyrene (1NP) in the breathing zone of the miners compared with the surface diesel drivers (Table II).

Comet assay

The comet assay detects a wide range of DNA damage, including single-strand DNA (ssDNA) breaks and double-strand DNA breaks, apurinic or apyrimidinic sites, oxidised and fragmented bases and specific lesions such 8OHdG (26,27). It has been used for more than two decades as a means of measuring DNA damage in subjects exposed to a wide variety of agents, including traffic. As reviewed below, the comet assay has largely shown an ability to distinguish traffic-exposed subjects from controls using several different tissues/cell types from subjects in varied occupations.

The comet assay has been used to assess traffic-associated DNA damage among seven exposure groups (Table III). Of the 10 sets of study groups reviewed here (Table III), 9 involved evaluation of comets in blood cells, one of comets in buccal cells (28), and one of comets in nasal epithelia of children (29). Nine of the 10 studies found higher levels of DNA damage in the exposed versus control populations. Six of the nine studies included exposure assessments, and all found higher levels of exposure among the exposed versus control populations. Of the six studies incorporating an exposure assessment, all but one (9) found an association between exposure and DNA damage.

Traffic police. A study in Taipei City, Taiwan, found higher levels of DNA damage in traffic conductors compared with office workers (30) (Table III); no exposure assessment was conducted in this study. A study in Shanghai, China, found higher frequencies of DNA damage in traffic police compared with residents of the city (31) (Table III). An exposure assessment showed that the traffic police had higher exposure to $PM_{2.5}$ in the air compared with that of the control population, and the level of exposure was associated with the level of DNA damage (Table II). Higher levels of DNA damage were found among traffic police from eight districts in Guangzhou compared with police who work in offices (32); no exposure assessment was done in this study.

Children. Greater amounts of ssDNA damage were found in nasal epithelia of children in Mexico City, Mexico, compared with that from children from a relatively unpolluted coastal area of Mexico (29) (Table III). Higher levels of DNA damage were found in children who attended schools within 500 m of a high-traffic road in Bangkok, Thailand, compared with children who went to a rural school (21,22) (Table III). Extensive exposure assessments found that, relative to the rural school children, the near-road children had higher levels of urinary OHPy and t,t-muconic acid as well as of benzene in their blood; the air breathed by these children also had high concentrations of benzene and PAHs (Table II).

Emission inspectors. Higher levels of DNA damage were found in both mononuclear and polynuclear blood cells among auto emission inspectors in Korea compared with volunteer controls (33) (Table III). Exposure assessments found that the

Table III. Traffic-associated DNA damage determined by the comet assay

Country	Control		Exposed		*P* value	Call	References
	Description (*n*)	Result[b]	Description (*n*)	Result[a]			
Korea	Healthy volunteers (84)	1.34 ± 0.16 OTM (mononuclear)	Emission inspectors (44) OTM	1.71 ± 0.23 OTM (mononuclear)	<0.001	+	(33)
		2.72 ± 0.59 OTM (polynuclear)	(mononuclear)	2.85 ± 0.49 OTM (polynuclear)	<0.001	+	
Mexico	Children near coast (12)	19 ± 9 ss-breaks (μm tail)	Children in Mexico City (87)	56.4 ± 14 ss-breaks (μm tail)	<0.05	+	(29)
Taiwan	Office workers (82)	9.46% T	Traffic conductors (47)	15.37% T	<0.001	+	(30)
China	Residents (101)	10.05 ± 3.45% T	Traffic police (175)	15.2 ± 3.46% T	<0.01	+	(31)
		0.86 ± 0.22 OM		1.25 ± 0.29 OM	<0.05	+	
	Office workers (130)	3.23 μm	Traffic police (682)	4.20 μm	<0.001	+	(32)
Estonia	Surface diesel drivers (30)	60 (out of score of 400)	Miner diesel drivers (41)	145 (out of score of 400)	<0.001	+	(9)
Denmark	Indoor cyclists (15) 14 days	0.02 inferred 8OHdG/106 bp	Outdoor cyclists (15) 74 days	0.08 inferred 8OHdG/10^6 bp	0.0003	+	(34)
Thailand	Rural school children (69)	0.16 ± 0.01 OTM	Urban school children (115)	0.23 ± 0.01 OTM	<0.001	+	(21,22)
Italy	Indoor airport workers (31)	Buccal: 68.2 TM – Fpg; 78.32 TM + Fpg	Outdoor airport workers (41)	Buccal: 118.87 TM – Fpg; 146.11 + Fpg	0.001	+	(28)
						+	
		Blood: 55.86 TM – Fpg; 43.98 TM + Fpg		Blood: 43.01 TM – Fpg; 55.86 TM + Fpg	0.136	–	
					0.003	+	
	Non-exposed (34)	2.85 ± 0.18% tail DNA	Tunnel workers (39)	3.08 ± 0.29% tail DNA	>0.05	–	(35)
		1.16 ± 0.33 + Fpg		1 ± 0.38 + Fpg	>0.05	–	
		0.82 ± 0.24 EndoIII		0.41 ± 0.29 EndoIII	>0.05	–	

OTM, olive tail moment; OM, olive moment; TM, tail moment; T%, % of DNA in the tail; EndoIII, endonuclease III; bp, base pair.
[a]Results are from DNA from white blood cells except for those from (35), which are from nasal epithelium, and from (28), which are from buccal and blood. All studies scored 100 cells or nuclei/sample, except for studies (28,29,30,31), which scored 50 cells or nuclei/sample.

emission inspectors had higher levels of urinary OHPy and 2-naphthol than did the control subjects, and there was an association between the levels of DNA damage and exposure (Table II).

Diesel drivers in a mine. Increased levels of DNA damage were found among diesel drivers in a mine in Estonia compared with surface workers (9) (Table III). Exposure assessment showed that the miners had elevated levels of particle-associated 1NP in their breathing zone compared with that of surface workers (Table II).

Bicyclists. An assessment of DNA damage in bicyclists in Denmark found that cyclists outdoors in Copenhagen had higher levels of DNA damage than indoor cyclists when formamido pyrimidine glycosylase (Fpg) was used to infer the production of 8OHdG (indicative of oxidative damage) (34) (Table III). Exposure assessment found increased levels of urinary NO_x associated with this type of DNA damage, and more cycling outdoors increased exposure to 10–100 nm air particles (Table II). No association was found between exposure and DNA damage minus Fpg, which detects primarily ssDNA breaks. Thus, oxidative damage seemed to be the primary class of DNA damage induced by cycling in the traffic.

Airport workers. Elevated levels of DNA damage were found in buccal cells with and without Fpg among outdoor versus indoor workers at the international airport in Rome, Italy; however, increased amounts of DNA damage in blood were found only with Fpg among the outdoor versus indoor workers, indicating that the outdoor atmosphere (which contained uniquely jet-fuel emissions) caused oxidative damage to DNA (28) (Table III). Exposure assessments showed that the outdoor workers had higher levels of PAHs in the air compared with indoor workers; however, the concentrations of urinary OHPy were not different between indoor and outdoor workers (Table II).

Tunnel workers. No induction of DNA damage was found with the comet assay with or without Fpg or endonuclease III, which identifies damaged pyrimidines, among road tunnel construction workers in the Umbrian Apennine Mountains in Italy, relative to non-exposed controls (35) (Table III). No exposure assessment was performed on these subjects.

8OHdG analyses

Reactive oxygen species generate many oxidative modifications in DNA bases, and 8OHdG is one that has been measured most often as a biomarker of oxidative damage (36). 8OHdG is a potentially pre-mutagenic lesion that is typically measured in urine; however, as reviewed below, it can be detected by staining nasal epithelia. Although this lesion is produced as a consequence of normal metabolic processes, its frequency can be increased by a wide variety of genotoxic exposures, including traffic exhaust.

Analysis of 8OHdG has been used to investigate the induction of oxidative DNA damage due to exposure to traffic among six exposure groups (Table IV). Among the seven studies reviewed here, six determined the concentration of urinary 8OHdG, and one study determined 8OHdG by staining of nasal epithelia (29). All seven studies reported increased levels of 8OHdG among the exposed groups compared with the control subjects, suggesting that 8OHdG might be an especially sensitive biomarker of traffic exposure. Four of the studies included exposure assessments, and all found an increased level of 8OHdG among the exposed subjects compared with the controls. Among these four studies, three showed an association between exposure and induction of 8OHdG.

Traffic police. The levels of urinary 8OHdG were higher in traffic police in Hyderabad, India, compared with that in office workers (37) (Table IV). No exposure assessment was done on this population. Another study also found increased concentrations of urinary 8OHdG among traffic conductors in Taipei City, Taiwan, compared with office workers (30) (Table IV). Extensive exposure assessments found that the air in the areas in which the traffic conductors worked had higher concentrations of PAHs, although not of $PM_{2.5}$, than did that of the control subjects; the traffic conductors had higher concentrations of urinary OHPy than did the controls (Table II). The concentration of urinary OHPy was associated with the concentration of urinary 8OHdG.

Urban bus drivers. The concentration of urinary 8OHdG was higher among urban bus drivers in Copenhagen, Denmark, compared with that of rural/suburban bus drivers (38) (Table IV). No exposure assessment was performed on these subjects.

Table IV. Traffic-associated DNA damage determined by concentration of 8OHdG

Country	Control		Exposed		*P* value	Call	References
	Description (*n*)	Result[a]	Description (*n*)	Result[a]			
Taiwan	Office workers (38)	1.73	Diesel emission inspectors (28)	2.49	0.033	+	(41)
Mexico	Children near coast (12)	210 ± 122	Children in Mexico City (87)	602 ± 195	<0.05	+	(29)
China	Pre-shift gate guards (2), 29 samples	1.83 ± 0.52	Post-shift gate guards (2), 29 samples	6.92 ± 3.67	<0.001	+	(40)
Denmark	Rural/suburban bus drivers (20)	146 ± 89	Urban bus drivers (29)	190 ± 108	<0.05	+	(38)
Taiwan	Office workers (46)	0.91	Traffic conductors (83)	1.37	<0.001	+	(30)
India	Office workers (135)	3.57 ± 0.63	Traffic police (148)	9.23 ± 2.24	<0.05	+	(37)
Korea	Non-urban residents (75)	4.57	Taxi drivers (95)	5.32	0.002	+	(39)

[a]With the exception of references (29) and (38), results are concentrations of urinary 8OHdG expressed as μmol/mol creatinine. The results for (38) are expressed as the concentration of urinary 8OHdG in terms of pmol/kg 24 h. The results from (29) are based on the staining of nasal epithelium and are the average intensity of nuclear staining expressed as the average absorbance × 10^3. The data from references (30,39,41) were published as micrograms of 8OHdG/g creatinine and was converted to μmol 8OHdG/mol creatinine by multiplying the reported value by 0.0035 and then dividing by 0.0088.

Taxi drivers. The levels of urinary 8OHdG were higher among taxi drivers in Seoul, Korea, compared with those in non-urban residents (39) (Table IV). Exposure assessments demonstrated higher concentrations of urinary OHPy and plasma NO_x among the taxi drivers relative to the controls (Table II). The concentrations of urinary 8OHdG were associated with the concentrations of urinary OHPy but not with the concentrations of plasma NO_x (Table II).

Gate guards. A study of gate guards at the west gate entrance to Peking University, China, found that the concentrations of urinary 8OHdG were higher post-shift than pre-shift (40) (Table IV). Exposure assessment demonstrated that the concentrations of various metals and PAHs in the air were higher at the gate entrance than on the roof of a six-storey building in the middle of the campus (Table II). There was an association between the urinary concentration of 8OHdG and the air concentrations of metals and PAHs.

Diesel emission inspectors. The concentrations of urinary 8OHdG were higher among diesel emission inspectors in Taiwan compared with those from office workers (41) (Table IV). Exposure assessment modeled the concentration of PAHs in the diesel exhaust particles $(DEP)_{2.5}$ and found that this was associated with the urinary concentrations of 8OHdG (Table II).

Children. The amount of 8OHdG based on staining of nasal epithelia was higher in children in Mexico City, Mexico, compared with that from children from a relatively unpolluted coastal area of Mexico (29) (Table IV). No exposure assessment was performed on these populations.

Cytogenetic effects

Chromosome aberrations

The frequency of chromosome aberrations (CAs) in peripheral blood lymphocytes has been shown to be predictive of the risk of cancer (42). It is the oldest genotoxicity biomarker, developed in the 1960s, and it has been used relatively frequently to assess genotoxicity in traffic-exposed subjects. All of the studies reviewed here have used standard staining and scoring methods, and two thirds were published prior to the year 2000. This is because the CA assay has been eclipsed in the past decade by the micronucleus (MN) assay. However, as described below, CAs reliably distinguished traffic-exposed from non-traffic-exposed subjects and is the biomarker most predictive of cancer risk (42).

Table V summarises the studies in which CAs were evaluated as a biomarker of traffic exposure. The eight studies reviewed here cover seven categories of traffic-exposed workers evaluated for this biomarker, and all showed an association between CAs and traffic exposure although this was the case for two exposure groups only when the data were stratified by genotype/phenotype (43). Two of the eight studies included exposure assessments, covering three exposure categories, and both studies found higher levels of exposure among the traffic-exposed subjects than among the controls (28,44) (Table II). However, neither study showed an association between levels of exposure and frequencies of CAs.

Among the studies noted in Table V, three (28,43,45) cultured the lymphocytes for 48 h; however, two (44,46) cultured the cells for 72 h and one for 69 h (60). Culturing cells for more than 48 h can result in increased frequencies of CAs formed during the extended period of growth in culture. However, only one of these studies with long culturing times (46) appears to have an elevated frequency of CAs among the controls. Most notable, however, is the high frequency of CAs among the controls in this study (48). Nonetheless, all of the studies in Table V reported significantly higher frequencies of CAs among the exposed relative to control populations.

Traffic police. Four studies have found increased frequencies of CAs among traffic police compared with their respective control populations (Table V). Thus, traffic police in Cairo, Egypt, had higher frequencies of CAs than police trainers (45); as did those in Ankara, Turkey, compared with office workers (44); as did traffic police in Hyderabad, India, compared with subjects who did not work in traffic (46); as did traffic police in Heibei City, Henan, China compared with police who worked in offices (47). The study from Turkey included an exposure assessment, which found higher concentrations of urinary OHPy among the traffic police relative to the control population (44) (Table II). However, no association was found between the levels of exposure and levels of the biomarker (Table II).

Table V. Traffic-associated chromosome aberrations

Country	Control		Exposed		*P* value	Call	References
	Description (*n*)	Result[a]	Description (*n*)	Result[a]			
Egypt	Police trainers (15)	0±0	Traffic police (28)	0.4±0.7	<0.05	+	(45)
Turkey	Office workers (23)	0.26±0.73	Traffic police (15)	1.29±1.59	<0.05	+	(44)
			Taxi drivers (17)	1.81±1.79	<0.01	+	
India	Non-traffic workers (115)	3.35	Traffic police (136)	6.48	<0.05	+	(46)
China	Office police (30)	0.4%	Traffic police (45)	0.98%	<0.01	+	(47)
Brazil	Non-traffic workers (24)	16.7	Gas station attendants (49)	46.9	<0.05	+	(48)
India	Non-traffic workers (40)	0.47±0.12	Car repair workers (40)	1.15±0.22	<0.01	+	(60)
Italy	Indoor airport workers (31)	0	Outdoor airport workers (41)	0.37	0.005	+	(28)
Denmark	Low-exposed bus drivers (19)	Stratified by genotype	High-exposed bus drivers (55)	Stratified by genotype		+	(43)
	Office workers (41)		Postal workers (60)			+	

[a]With the exception of reference (48), results are expressed as the % cells with aberrations; all studies examined 100 metaphases/subject except for references (46,48), which studied 150 metaphases/subject. The data for reference (48) are reported as the percentage of subjects with chromosome aberrations (gaps not included) and not as % cells with aberrations.

Taxi drivers. The frequencies of CAs were higher in taxi drivers in Ankara, Turkey, compared with office workers (44) (Table V), and the taxi drivers had higher concentrations of urinary OHPy than did the control subjects (Table II). No association was found between the levels of exposure and the levels of CAs (Table II).

Gas station attendants. A study of gas station attendants in Sao Paulo and Rio de Janeiro, Brazil, found higher frequencies of CAs in station attendants compared with subjects not exposed to traffic (48) (Table V). No exposure assessment was performed on these populations.

Car mechanics. Higher frequencies of CAs were found among car mechanics in Chandigarh, India, compared with subjects not exposed to traffic (49) (Table V). No exposure assessment was performed in this study.

Airport workers. An investigation of outdoor airport workers at the international airport in Rome, Italy, found higher frequencies of CAs in this population compared with the frequencies found among airport office workers (28) (Table V). Exposure assessments found higher concentrations of PAHs in the air outdoors compared with the air in the offices, but there was no difference in the concentrations of urinary OHPy among the exposed and control groups (Table II).

Bus drivers. A study of bus drivers categorised the exposure groups as high for drivers within the city of Copenhagen, Denmark, and medium or low for drivers in the suburbs or countryside, respectively (43) (Table V). When stratified by genotype/phenotype, those bus drivers who were *GSTM1-null* and had the slow-acetylator *NAT2* genotype exhibited an exposure-related increase in CAs (43). (The role of genotype/phenotype is discussed later in this review.)

Postal workers. Comparison of mail carriers in Copenhagen, Denmark, versus office workers found that the mail carriers who were *NAT2* slow acetylators had higher frequencies of CAs than did those who were fast acetylators (43) (Table V). This result suggested that the *NAT2* genotype may influence responses to other common exposures or may influence the baseline frequencies of CAs (43). No exposure assessment was performed on these subjects.

Micronucleus

An increased frequency of MN in peripheral blood lymphocytes has been found to have some evidence of being predictive of risk for cancer; however, it is not as predictive as CAs (50). Nonetheless, it is much simpler to perform than CA analysis, and it is the second most frequent biomarker (after ^{32}P-postlabeling) used to assess genotoxicity in traffic-exposed people. The MN assay can identify two classes of chromosomal damage: chromosomal breakage and aneuploidy. Because of the ease of performance and scoring, the MN assay has largely replaced CA assay for genotoxicity biomonitoring, as indicated by the fact that 6 of the 12 papers in Table VI were published after the year 2000.

Twelve studies have used the MN assay to assess genotoxicity in traffic-exposed populations covering nine categories of exposure (Table VI). Of these 12 studies, six included exposure assessments, and all of these found higher levels of exposure among the exposed group compared with the controls (Table II). However, only one study found an association between

Table VI. Traffic-associated micronuclei

Country	No. of cells[a]	Control		Exposed		*P* value	Call	References
		Description (*n*)	Result[b]	Description (*n*)	Result[b]			
Turkey	1000	Workers (28)	0.05 ± 0.04	Engine repairers (34)	0.07 ± 0.05	>0.05	–	(51)
		Workers (20)	0.03 ± 0.03	Taxi drivers (17)	0.12 ± 0.05	<0.0001	+	
				Traffic police (15)	0.10 ± 0.0	<0.05	+	
Philippines	2000	City residents (18)	6.5	Gas station attendants (18)	18.9	<0.05	+	(52)
				Traffic police (18)	17.07	<0.05	+	
China	N/R	Office police (49)	1.97 ± 0.21	Traffic police (65)	4.27 ± 0.68	<0.05	+	(57)
India	3000	City residents (100)	2.94	Gas station attendants (110)	12.76	<0.001	+	(53)
China	1000	Police office workers (34)	3.22 ± 1.31	Traffic police (67)	5.72 ± 2.57	<0.05	+	(55)
	2000	Unexposed (38)	0.084	Diesel train attendants (51)	0.166	<0.01	+	(54)
				Wheel–axel workers (30)	0.356	<0.01	+	(54)
Hungary	N/R	Unexposed (60)	19.9 ± 6.5	Garage mechanics (48)	23.5 ± 5.7	0.02	+	(8)
	1000	Outdoor workers away from traffic (34)	4.71 ± 0.28	Tunnel workers (39)	6.31 ± 0.61	<0.05	+	(35)
Italy	2000	Indoor workers (54)	4.49 ± 2.0	Traffic police (94)	3.75 ± 1.65	0.02	+	(12)
	2000	Office workers (31)	Buccal: 0.064 ± 0.054	Outdoor airport workers (41)	Buccal: 0.064 ± 0.098	0.251	–	(28)
	1000		Blood: 0.710 ± 0.421		Blood: 0.815 ± 0.37	0.129	–	
	1000	Lab workers (34)	4.3	Traffic police (82)	3.73	>0.05	–	(56)
Denmark	2000	Low traffic (23)	2.91 ± 1.78	High traffic (23)	4.29 ± 2.06	<0.01	+	(25)

[a]Number of cells analysed per sample; N/R, not reported.
[b]Results are expressed as % cells with micronuclei. Data are from buccal cells for (51–53) and where noted for (28). Data are from peripheral blood for (8,12,35,55,56) and where noted for (28). Data from (25) are from cord blood.

exposure and MN frequency (25). Three studies evaluated MN in buccal cells (51–53), seven studies evaluated MN in lymphocytes (8,12,35,54–57), one evaluated MN in both cell types (28) and one evaluated MN in maternal lymphocytes and cord blood (25). Of the total of 17 measurements of MN reported in the articles reviewed here, 13 found increased frequencies of MN in the exposed versus control populations. All but one (55) of the studies in lymphocytes used the cytokinesis-blocked version of the MN assay; those studies that used buccal cells did not (28,51–53).

Traffic police. Six studies have investigated the induction of MN in traffic police relative to controls not exposed chronically to traffic (Table VI). Traffic police in Ankara, Turkey, had higher frequencies of buccal-cell MN than did the controls (details of controls not specified) (51) (Table VI). No exposure assessment was performed on these populations. Likewise, higher frequencies of buccal-cell MN were found in traffic police in Manila, Philippines, compared with residents of Manila (52) (Table VI). No exposure assessment was done in this study. Increased frequencies of MN were found in buccal cells of traffic police in Lanzhou, China, compared with the frequencies found in police who work in offices (55) (Table VI). No exposure assessment accompanied this study.

A study of MN in lymphocytes in traffic police in Genoa, Italy, found increased frequencies in this group relative to a group of indoor workers (12) (Table VI). Exposure assessment found a 30-fold higher concentration of PAHs in the air outdoors compared with that in the office space; however, no statistical analysis was reported (Table II). Another study in lymphocytes of traffic police in Genoa found no increase in MN frequencies in traffic police compared with a group of laboratory workers (56) (Table VI). Nonetheless, exposure assessment showed higher levels of B[*a*]P in the air outdoors compared with that in the laboratories (Table II). However, there was no association between the levels of exposure and the frequencies of MN. Higher frequencies of MN were found among traffic police in Heibei, China, compared with police who worked in offices (57) (Table VI), and exposure assessments found increased concentrations of inhaled particles in the air breathed by the exposed compared with the control subjects (Table II). Increased concentrations of nitrogen oxides (NO_x), carbon monoxide (CO), hydrocarbons and lead were also found among the exposed versus control populations (Table II).

Garage mechanics. The frequencies of buccal-cell MN were not different in engine-repair workers in Ankara, Turkey, compared with controls (details of occupation of controls were not specified) (51) (Table VI). No exposure assessment was performed in this study. In contrast, increased frequencies of MN were found in lymphocytes of garage mechanics in Budapest, Hungary, relative to those of unexposed controls (8) (Table VI). Exposure assessment found higher concentrations of urinary OHPy among the garage mechanics, but the level of exposure was not associated with the frequency of MN (Table II).

Gas station attendants. Gas station attendants in Manila, Philippines, had higher frequencies of buccal-cell MN compared with residents of Manila (52) (Table VI); no exposure assessment was performed in this study. The frequencies of buccal-cell MN were higher in gas station attendants in Coimbatore City, India, compared with residents of Coimbatore City (53) (Table VI). No exposure assessment accompanied this study.

Taxi drivers. The frequencies of buccal-cell MN were higher in taxi drivers in Ankara, Turkey, compared with control subjects (51) (Table VI); no exposure assessment was reported.

Airport workers. No increase in either buccal or lymphocyte MN frequencies were found among outdoor airport workers at the international airport in Rome, Italy, compared with airport office workers (28) (Table VI). Exposure assessments found that the concentration of PAHs was higher in the outdoors than in the offices, but concentrations of urinary OHPy were not different between the two groups of workers (Table II).

Tunnel workers. There were higher frequencies of lymphocyte MN in tunnel workers in the Umbrian Apennine Mountains, Italy, compared with outdoor workers away from traffic (35) (Table VI). No exposure assessment was performed in this study.

Traffic-exposed mothers/umbilical cord blood. One study found higher frequencies of MN in lymphocytes of mothers and umbilical cord blood from those mothers who lived in high- versus low-traffic areas of Denmark (25) (Table VI). The exposure was higher among the high-traffic areas relative to the low-traffic areas based on the vehicle km/24 h (Table II).

Wheel–axle workers. Higher frequencies of MN were found among wheel–axle workers in Heibei, China, compared with unexposed controls (54); no exposure assessment was performed on these subjects.

Diesel train attendants. Elevated frequencies of MN were measured among diesel train attendants in Heibei, China, compared with unexposed controls; no exposure assessment accompanied this study (54).

Sister chromatid exchanges

SCEs have been used extensively as a biomarker of genotoxicity (58); however, unlike CAs and MNs, SCEs have not turned out to be predictive of cancer (59). Nonetheless, it is a sensitive indicator of exposure to a variety of genotoxic agents; and as reviewed below, this includes exposure to traffic exhaust.

There were 10 studies in which SCEs in lymphocytes were investigated as a biomarker associated with exposure to traffic exhaust (Table VII). Of these 10 reports, all but 2 found increased frequencies of SCEs in the exposed population compared with the control subjects. Among the 10 reports, 3 exposure groups were studied; 2 of the 10 studies included exposure assessments, both of which found difference between the exposure levels of the exposed and control populations. All of the studies cultured the lymphocytes for 72 h, except for reference (46), which cultured cells for 70 h, and reference (28), which cultured for 48 h.

Traffic police. There were nine studies of SCEs in traffic police, all but one of which found higher frequencies of SCEs in the traffic police relative to the control populations (Table VII); the one negative study included an exposure assessment (Table II). Traffic police in Cairo, Egypt, had higher frequencies of SCEs than did police trainers (45); the same was true for traffic police

Table VII. Traffic-associated SCEs

Country	No. of metaphases[a]	Control		Exposed		*P* value	Call	References
		Description (*n*)	Result[b]	Description (*n*)	Result[b]			
Egypt	40	Police trainers (10)	4.8	Traffic police (21)	7.5	<0.10	+	(45)
India	25	Not exposed (23)	5.67 ± 0.37	Traffic police (23)	12.78 ± 0.68	<0.001	+	(60)
Italy	50	Lab workers (35)	7.36 ± 1.35	Traffic police (54)	7.47 ± 1.28	>0.05	–	(64)
China	100	Office working police (34)	3.73 ± 1.51	Traffic police (67)	8.81 ± 1.83	<0.05	+	(55)
Italy	50	Indoor airport workers (31)	3.84 ± 0.58	Outdoor airport workers (41)	4.61 ± 0.80	<0.001	+	(28)
India	50	Office workers (60)	4.18 ± 1.85	Traffic police (85)	9.31 ± 5.29	<0.05	+	(46)
Thailand	N/R	University students (20)	0.24 ± 0.12	Traffic police (30)	4.40 ± 0.93	<0.05	+	(63)
China	N/R	Office police (49)	2.69 ± 0.35	Traffic police (65)	4.32 ± 0.58	<0.05	+	(57)
Italy	30	Outdoor workers away from traffic (34)	4.88 ± 0.08	Tunnel workers (39)	5.07 ± 0.11	>0.05	–	(35)
India	25	Office workers (25)	6.49 ± 0.31	Traffic police (56)	10.62 ± 0.57	<0.001	+	(62)

[a]Number of metaphases analysed per sample; N/R, not reported.
[b]Results are from blood cells and are expressed as SCEs/cell.

in Madras, India, compared with subjects not working in traffic (60). There were higher frequencies of SCEs in traffic police in Lanzhou, China, compared with police who worked in offices (55); the same was true for traffic police in Hyderabad, India (61), or Chennai city, India (62), compared with office workers. Traffic police in Bangkok, Thailand, had higher frequencies of SCEs than university students who were used as the control population (63). Although traffic police in Genoa, Italy, did not have elevated frequencies of SCEs compared with laboratory workers as controls (64), an exposure assessment found that the concentration of B[*a*]P in the outdoor air was higher than in the laboratory spaces (Table II). Traffic police in Heibei, China, had higher frequencies of SCEs compared with police who worked in offices (57) (Table VII), and exposure assessments showed that the exposed populations had higher concentrations in their air compared with the controls for a variety of compounds: inhaled particles, NO_x, CO, hydrocarbons and lead (Table II).

Airport workers. Outdoor workers at the international airport in Rome, Italy, had higher frequencies of SCEs than did indoor workers at the airport (28) (Table VII), and exposure assessments found that the outdoor air had higher concentrations of PAHs than did the indoor air (Table II). Nonetheless, there was no difference in the urinary concentration of OHPy between the outdoor and indoor workers (Table II).

Tunnel workers. The frequencies of SCEs were not higher among tunnel workers in the Umbrian Apennine Mountains, Italy, compared with outdoor workers away from traffic (35) (Table VII). No exposure assessment accompanied this study.

Miscellaneous biomarkers

This section reviews 14 articles covering a variety of biomarkers, such as *HPRT* mutation, urinary mutagenicity, gene expression, telomere length and DNA methylation and repair. Seven of these studies included exposure assessment, and all seven found higher levels of exposure in the exposed versus control populations. The exposure groups are many of the same ones used in the studies reviewed above, such as bus maintenance workers, car mechanics, mail carriers, truck drivers, traffic officers and children in schools located near high-traffic roads. However, unique to the articles reviewed in this section are three reports that did not involve occupational exposures but, instead, involved exposure of subjects in chambers to known amounts of diesel exhaust or ultrafine carbon particles; all of these were performed in the USA.

Mutation and DNA repair

There were no differences in the mutant frequencies at the *HPRT* locus in lymphocytes from bus maintenance workers versus hospital mechanics in the Stockholm area of Sweden (18) (Table VIII). Sequencing of the mutations did not reveal any obvious differences in mutation spectra except that the frequencies of splicing errors were 24% among 29 bus maintenance workers compared with 9.5% among 14 hospital mechanics; no statistical analysis of these results was reported (65).

Three studies investigated the influence of traffic on urinary mutagenicity, which represents a measure of systemic exposure to mutagens. A study of railroad workers in the Northeast of the USA found no difference in the levels of urinary mutagenicity among these workers who had a moderate exposure to diesel exhaust compared with people with no or low exposure to diesel exhaust (66) (Table VIII). Likewise, no differences in urinary mutagenicity were found among mail carriers in Denmark compared with indoor mail workers (67) (Table VIII). However, exposure analysis found higher concentrations of urinary OHPy in the mail carriers compared with the indoor workers (Table II). No differences were found in urinary mutagenicity among car mechanics compared with office workers in the Netherlands (68) (Table VIII).

The challenge assay measures global DNA repair by 'challenging' cells *in vitro* to a genotoxic agent, where the cells are from an exposed versus control population, and quantifying the differences in the induction of a response to the genotoxin, such as chromosomal aberrations. Application of this assay to lymphocytes of children from urban versus rural schools in Thailand found enhanced frequencies of deletions per metaphase among the urban children compared with the rural children (21,22). This indicated that the DNA repair capacity of the urban children was not as high as that from rural children, presumably because the DNA repair systems of the urban children were already induced as far as possible and were not able to perform adequate DNA repair after the lymphocytes were exposed *in vitro* to a genotoxin (in this case, after exposure to 100 cGy using a ^{137}Cs source at a dose rate of 5 Gy/min). Extensive exposure analyses

Table VIII. Miscellaneous biomarkers and their association with exposure to traffic

Endpoint	Country	Control		Exposed		*P* value	Call	References
		Description (*n*)	Result	Description (*n*)	Result			
HPRT	Sweden	Hospital mechanics (22)	Mutant Frequency = 8.4/106 survivors	Bus maintenance workers (47)	Mutant Frequency = 8.6/10^6 survivors	>0.05	–	(18)
Urine mutagenicity	USA	No or low diesel exposure non-smoker RR workers (66)	0.43 rev/μmol/creatinine	Moderate diesel exposure non-smoker RR workers (105)	0.28 rev/μmol creatinine	>0.05	–	(66)
Urine and faecal mutagenicity	Netherlands	Office workers (9)	No average value reported	Car mechanics (8)	No average value reported	Not reported	–	(68)
Urine mutagenicity	Denmark	Indoor mail workers (37)	Stratified for genotype	Outdoor mail carriers (56)	Stratified for genotype	>0.05	–	(67)
Expression of oxidative stress genes	USA	Exposure to filtered air (14)		Exposure to diesel exhaust 300 μg/m^3 for 1- and 2-h exposures (14)	Oxidative stress response pathway	<0.05	+	(69)
		Exposure to filtered air (3)		Exposure to ultrafines at 50 μg/m^3 2 h (3)	Oxidative stress response and inflammation pathways	<0.05	+	(70)
		Exposure to filtered air (5)		Exposure to diesel exhaust 200 μg/m^3 $PM_{2.5}$ 2 h (5)	Oxidative stress response and inflammation pathways	<0.05	+	(71)
Telomere length	China	Office workers (9120)	0.79 T/S ratio[a]	Truck drivers (120)	0.87 T/S ratio[a]	0.002	+	(75)
	USA	165 subjects modeled for exposure to black carbon		165 subjects modeled for exposure to black carbon	↓Telomere length with ↑black carbon	0.003	+	(74)
	Italy	Office workers (57)	1.27 T/S ratio[a]	Traffic officers (77)	1.10 T/S ratio[a]	<0.001	+	(76)
DNA methylation	USA	718 residents (1097 blood samples) modeled exposure		718 residents (1097 blood samples) modeled exposure	↓LINE-1 methylation with ↑ black carbon	0.002	+	(72)
					↓LINE-1 methylation with ↑ $PM_{2.5}$	<0.001	+	(72)
Challenge assay[b]	Thailand	Rural school children (55)	0.26 ± 0.001 deletions/metaphase	Urban school children (91)	0.45 ± 0.01 deletions/metaphase	<0.001	+	(21,22)
ELISA PAH-DNA adducts	Bangladesh	Non-rickshaw drivers (11)	2.03 adducts/108 nt	Rickshaw drivers (19)	8.56 adducts/10^8 nt	0.04	+	(77)

[a]T/S is the ratio of T, which is the telomere repeat copy number, and S, which is the single-copy gene, which in these studies was the human beta-globin gene.
[b]The challenge assay measures DNA damage/repair as described in references (21,22).

found that the urban children had higher levels of urinary OHPy and urinary t,t-muconic acid, as well as higher concentrations of plasma benzene; in addition, their ambient air had higher concentrations of PAHs and benzene (Table II).

Gene expression after exposure to diesel exhaust or ultrafine carbon particles in a chamber

Three studies from the USA have examined changes in gene expression in subjects before and after exposure to either diesel exhaust or ultrafine carbon particles in chambers (Table VIII) (69–71). Exposures to diesel exhaust caused changes in expression of genes in the following pathways: oxidative stress response (69,71), vascular homeostasis (69,71), inflammation and leukocyte activation (71) and proteosome pathway and mitochondrial dysfunction (69). Exposure to ultrafine carbon particles (70) altered expression of genes in the following pathways in addition to inflammation: tissue growth, insulin growth factor 1 signalling, insulin receptor signalling and the NF-E2-related factor 2-mediated oxidative stress response.

Methylation reduces gene expression, and a study of subjects from the Boston area of Massachusetts, USA, found that when exposure was modeled for levels of black carbon as an indicator of traffic-associated particles, then those subjects with recent exposure to high levels of black carbon and $PM_{2.5}$ had reduced methylation of repetitive element LINE-1 DNA (72). However, whether such modification mediates exposure-related health effects is unknown.

Leukocyte telomere length

Telomeres are regions of non-coding DNA at the end of chromosomes that protect against structural degradation, inappropriate recombination and end-to-end fusion of chromosomes (73). Telomeres shorten with each successive cell division and, thus, reflect biological aging. Shortened telomeres are associated with increased risk of chronic diseases (see reference 74). Three studies have evaluated leukocyte telomere length relative to exposure to traffic or related air pollution.

Truck drivers in Beijing, China, had longer telomeres than office workers (75) (Table VIII). For both the truck drivers and office workers together, an increase in telomere length was associated with personal $PM_{2.5}$ concentration, personal elemental carbon concentration and ambient PM_{10} concentration on the day that blood was sampled from the subjects (Table II). However, shorter telomere length was associated with the

ambient PM_{10} concentration averaged over the 2 weeks prior to the blood draw (Table II). These results indicate that longer telomere length is associated with short-term exposure to ambient PM, consistent with an effect of PM on telomeres during acute inflammatory responses. In contrast, long exposures to PM may shorten telomeres due to extended exposures to pro-oxidants.

A population living in Massachusetts, USA, was evaluated for telomere length that was compared with modeled exposure to carbon black as a marker for traffic-related particles (74). This study found that telomere length shortened as carbon black exposure increased (Table VIII). This result is consistent with the study in China, showing that prolonged exposure to ambient air particles is associated with shortened telomeres.

A third study of telomere length found shorter telomeres among traffic officers in Milan, Italy, compared with office workers (76) (Table VIII). Among the traffic officers, the adjusted mean telomere length was shorter in subjects working in high compared with low traffic intensity (Table II). An additional exposure assessment found that telomere length decreased with increasing concentrations of personal exposure to benzene and toluene. Collectively, these studies show that leukocyte telomere length is shortened in subjects exposed chronically to traffic pollution, which implies that such exposures result in early biological aging and an increased risk for disease.

PAH-DNA adducts determined by enzyme-linked immunosorbent assay

One study has been performed on rickshaw drivers in Dhaka City, Bangladesh, using an enzyme-linked immunosorbent assay (ELISA) that detects immunologically PAH-DNA adducts in white blood cells (77). Rickshaw drivers had higher levels of PAH-DNA adducts than did non-rickshaw drivers (Table VIII). No exposure assessment was done in this study.

Influence of genetic polymorphisms

Ten studies evaluated their subjects for some genotypic or phenotypic variations to assess the influence such variation might have on the measured biomarker and/or the exposure assessment. The results of these studies are summarised in Table IX, which shows that the data are quite limited and indicate no consistent evidence for genotypic or phenotypic modulation of the biomarkers or exposure assessments reviewed here relative to exposure to traffic exhaust.

A study of bus maintenance workers in Sweden found that among *NAT2* slow acetylators, *GSTM1-null* subjects had higher levels of DNA adducts determined by ^{32}P-postlabeling than did *GSTM1+* subjects (18). However, the authors found that *GSTM1* and *NAT2* genotype status had no influence on *HPRT* mutant frequency. The three major *NAT2* alleles were determined by restriction analysis.

The NAT2 slow acetylators among a group of bus drivers or mail carriers who worked outdoors in Copenhagen, Denmark, had higher concentrations of urinary OHPy than did slow acetylators; however, this phenotype did not influence the levels of urinary mutagenicity (67). The NAT phenotype was determined by analysis of urinary metabolites of caffeine.

Subjects in Florence, Italy, with occupational exposure to traffic exhaust who had at least one variant of the DNA nucleotide excision repair gene *XPD-Lys751/Gln* had increased levels of DNA adducts determined by ^{32}P-postlabeling (23).

GSTM1 and *NAT2* (*M1*, *M2* and *M3* alleles) had no effect on the levels of DNA adducts measured by ^{32}P-postlabeling in Copenhagen bus drivers (15). In another study, *GSTM1*, *GSTT1* and *GSTP1* had no effect on the levels of DNA adducts measured by ^{32}P-postlabeling or DNA damage measured by the comet assay in shale oil mine workers exposed to diesel exhaust (9). *CYP1A1* and *GSTM1* had no influence on the observed increase in MN in road-tunnel construction workers relative to office controls in Genoa, Italy (35).

For postal workers and bus drivers in Denmark, having the *GSTM1-null* and/or slow *NAT2* genotype increased the frequency of CAs (43). Another study of bus drivers in Denmark found that the phenotypic activity of CYP1A2 or NAT2 had no influence on the concentration of urinary 8OHdG; however, the level of CYP1A2 activity did influence this biomarker on the workday that it was measured (38).

The *CYP1A1*, *GSTT1* or *GSTM1* genotypes had no effect on the levels of DNA adducts determined by ^{32}P-postlabeling or on the levels of urinary OHPy among school children whose schools were located near a high-traffic road in Bangkok, Thailand, or in a rural area of Thailand (21,22). In contrast, *CYP1A1m* and *GSTM1-null* were found to influence the concentration of urinary 8OHdG among traffic police in India (37).

Table IX. Summary of studies on the influence of genotype or phenotype on traffic-associated biomarkers

Exposure group	Genotype or phenotype	Result	*P* value	References
Bus maintenance workers	*GSTM1-null* genotype	↑^{32}P DNA adducts	0.03	(18)
Bus drivers and mail carriers	NAT2 slow phenotype	No effect on *HPRT* but ↑ urinary OHPy	>0.05	(67)
Traffic-exposed subjects	*XP2-Lys751/Gln* genotypic variants	↑^{32}P DNA adducts	0.02	(23)
Bus drivers	*GSTM1*; *NAT2* genotypes	No effect on ^{32}P DNA adducts	>0.05	(15)
Diesel-driving miners	*GSTM1*; *GSTT1*; *GSTP1* genotypes	No effects on ^{32}P DNA adducts or comet results	>0.05	(9)
Tunnel workers	*CYP1A1*; *GSTM1* genotypes	No effect on MN	>0.05	(35)
Postal workers	*NAT2* slow genotype	↑CA	N/R[a]	(43)
Bus drivers	*GSTM1-null*; *NAT2* slow genotypes	↑CA	<0.0005	(43)
Bus drivers	CYP1A2 and NAT2 phenotypes	No effect on urinary 8OHdG except on the workday	>0.05	(38)
School children	*CYP1A1*; *GSTT1*; *GSTM1* genotypes	No effect on ^{32}P DNA adducts or urinary OHPy	>0.05	(21,22)
Traffic police	*CYP1A1m1*; *GSTM1-null* genotypes	↑Urinary 8OHdG	<0.01	(37)

[a]N/R, not reported.

Discussion

Qualitative summary

Table X summarises the main categories of exposure groups, the countries in which those groups were studied and the numbers of measurements of the main biomarkers reported in the studies reviewed here on traffic exposure biomarkers. Among the 63 articles, there were 18 general exposure categories in 20 countries that researchers have evaluated for 6 primary genotoxicity biomarkers of traffic exposure. A total of 87 assessments were reported, with ^{32}P-postlabeling for DNA adducts and the MN assay for chromosomal mutation being used most often. The six main biomarkers effectively detected genotoxic responses in subjects exposed to traffic exhaust. Considering all the assessments listed in Tables I and II–VII, the percentage of positive results was 90% (9/10) for the comet, 81% (13/16) for MN, 80% (8/10) for SCEs, 81% (17/21) for ^{32}P-postlabeling, 100% (7/7) for 8OHdG and 100% (10/10) for CAs, for an overall average of 89%. Urinary mutagenicity was the least sensitive biomarker and the only one not appearing to be useful to detect genotoxicity in traffic-exposed populations. Traffic police was the exposure group studied most frequently, with 26 assessments reported, followed by mechanics with 10 (Table X).

Among the six main biomarkers used in these studies, 36 assessments used cytogenetic (chromosomal) endpoints (MN, CA and SCE), and another 39 evaluated DNA damage *per se* (^{32}P-postlabeling, ELISA-based analysis for PAH-DNA adducts, comet and 8OHdG) (Table IX). All of the six main biomarkers used in these studies are well established and have been in routine use for 20–40 years. Three of these, CA (42), MN (59) and shortened telomere length (80), are associated with increased risk for cancer, and all have shown utility as biomarkers for a variety of other exposures. Among the cytogenetic assays, the MN assay has generally replaced the CA and SCE assays; among the molecular assays, the ^{32}P-postlabeling assay is used less frequently now than in the recent past.

Perhaps most interesting is the application of new genomic biomarkers, such as changes in gene expression, telomere length and DNA methylation (Table VIII). All three gene-expression studies were performed on subjects exposed to either diesel exhaust or ultrafine carbon particles under controlled conditions in a chamber; thus, they are the only studies reviewed here that were not done in people exposed to real-world traffic exhaust. Nonetheless, the results obtained, which included increases in expression of genes in oxidative stress and inflammation pathways, were consistent with other studies showing that ambient air pollution enhances oxidative stress and inflammation (79).

Studies in China, the USA and Italy have examined telomere length in leukocytes relative to exposure to traffic exhaust and have found that chronic exposure to traffic exhaust shortened telomeres among residents of Massachusetts with increased exposure to black carbon (as a measure of exposure to traffic-related particles) (74), as well as among truck drivers in Beijing, China (75), and traffic officers in Milan, Italy (76), relative to controls. Moreover, these results are consistent with studies showing that oxidative stress and inflammation accelerate the shortening of telomeres (80), supporting the gene-expression studies described above of subjects exposed solely to diesel exhaust who had increased expression of genes in these pathways.

The one study of DNA methylation found that subjects with high recent exposures to black carbon and $PM_{2.5}$ had reduced methylation of repetitive element LINE-1 DNA (72), and a

Table X. Summary of exposure groups, countries and genotoxicity biomarkers studied relative to traffic exposure[a]

Exposure group	Country	Biomarker (number of measurements)							
		^{32}P	Comet	8OHdG	CA	MN	SCE	Other	Total
Traffic police	China, Egypt, India, Italy, Philippines, Taiwan, Thailand, Turkey and USA	2	3	2	4	6	8	1	26
Mechanics	Denmark, India, Sweden, Hungary and Netherlands	4	0	0	1	3	0	2	10
Residents/ traffic	Bénin, Denmark, Italy and USA	4	0	0	0	1	0	2	7
Children	Mexico and Thailand	1	2	1	0	0	0	1	5
Taxi drivers[b]	Bénin, Sweden, Turkey, Korea and Bangladesh	2	0	1	1	1	0	1	6
Bus drivers	Denmark and Sweden	3	0	1	1	0	0	0	5
Miners/tunnel	Italy and Estonia	1	2	0	0	1	1	0	5
Airport workers	Italy	0	1	0	1	1	1	0	4
Gas station	Bénin, Brazil and Philippines	1	0	0	1	2	0	0	4
Diesel exhaust	USA	0	0	0	0	0	0	3	3
Street vendors	Bénin and Italy	2	0	0	0	0	0	0	2
Postal workers	Denmark	0	0	0	1	0	0	1	2
RR workers	China and USA	0	0	0	0	1	0	1	2
Emission inspectors	Korea and Taiwan	0	1	1	0	0	0	0	2
Gate guard	China	0	0	1	0	0	0	0	1
Truck drivers	China	0	0	0	0	0	0	1	1
Bicyclists	Denmark	0	1	0	0	0	0	0	1
Truck terminal	Sweden	1	0	0	0	0	0	0	1
Total	19	21	10	7	10	16	10	12	87

[a]Number of measurements refers to the number of studies (not number of articles) of a particular exposure group; some articles contained studies of more than one exposure group.
[b]Includes taxi–bike and taxi–rickshaw drivers.

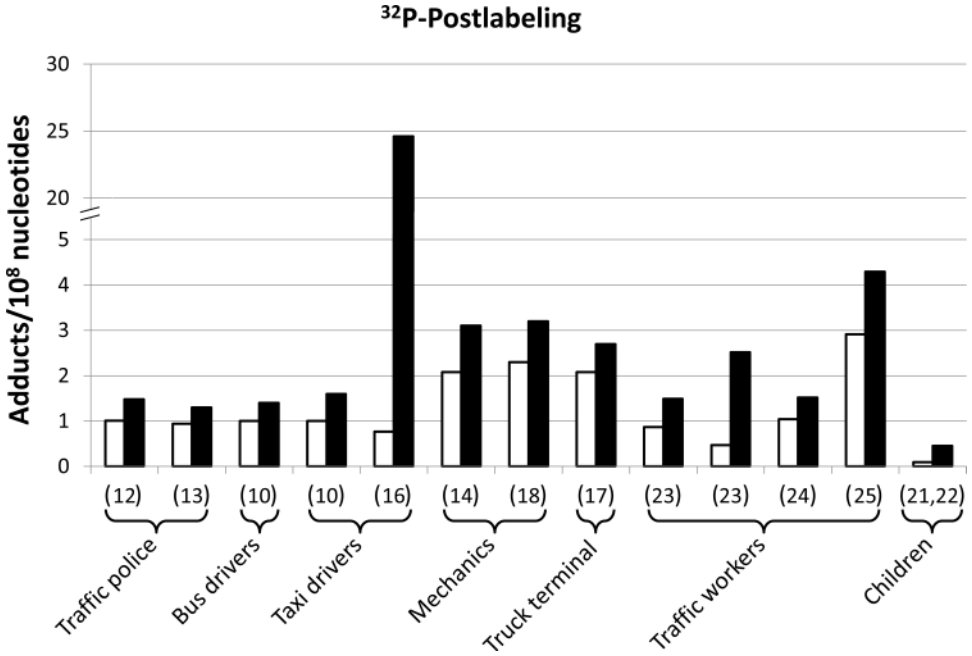

Fig. 1. Data sets from Table I of DNA adduct data determined by ^{32}P-postlabeling from control (open bars) and traffic-exposed (solid bars) populations; numbers below the bars are the references, and units are adducts/10^8 nucleotides.

recent study suggests that subjects with lower levels of such methylation are at increased risk of developing and dying from cancer (78). Thus, the limited data for this biomarker suggest that it also may be predictive of cancer risk along with the cytogenetic biomarkers CA and MN and might be useful in future studies of traffic-exposed populations.

Quantitative summary

The quantitative data summarised in Tables I and III–VII for the six main biomarkers reviewed here are shown graphically in Figures 1–6, which plot the control and exposed data for only positive studies and where common units of potency were reported to permit graphical comparison. For the 13 data sets for DNA adducts determined by ^{32}P-postlabeling data (Figure 1), only three (16,21–23) showed more than 2-fold differences between the control and exposed populations; however, all data plotted in Figure 1 from the exposed populations were significantly different from those of the control populations (Table I). The general category of traffic workers was the group studied most frequently for this biomarker. The highest level of DNA adducts was measured among taxi drivers in an urban area in Bénin, where the results among the taxi drivers were ~10 times greater than those among the control population of rural/suburban residents (16). Another group with relatively high levels of DNA adducts were mechanics (14,18).

The comet data in Table III were expressed in four different units; however, two data sets for traffic police expressed in terms of % of DNA in the tail are shown in Figure 2. Both found similar levels of DNA damage among traffic police in China and Taiwan. The lack of uniformity in expression of the results prevented further comparison of the comet data among the different exposure groups.

Figure 3 shows a plot of data of urinary 8OHdG; high levels of DNA damage were found among traffic police, gate guards and taxi drivers. Although all the differences in Figure 3 are

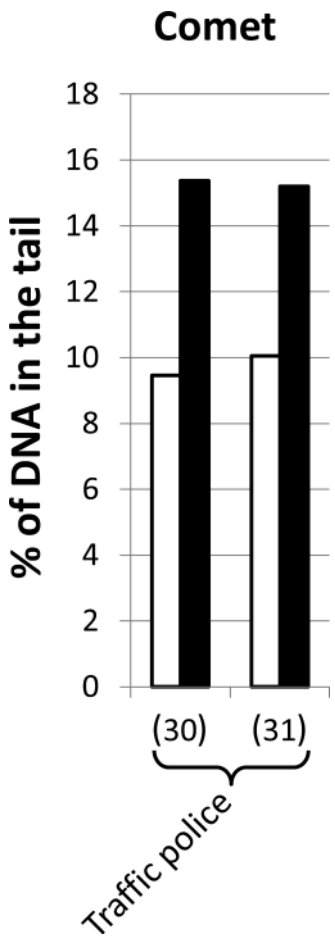

Fig. 2. Data sets from Table III of comet data from control (open bars) and traffic-exposed (solid bars) populations; numbers below the bars are the references; data are expressed as % DNA in tail.

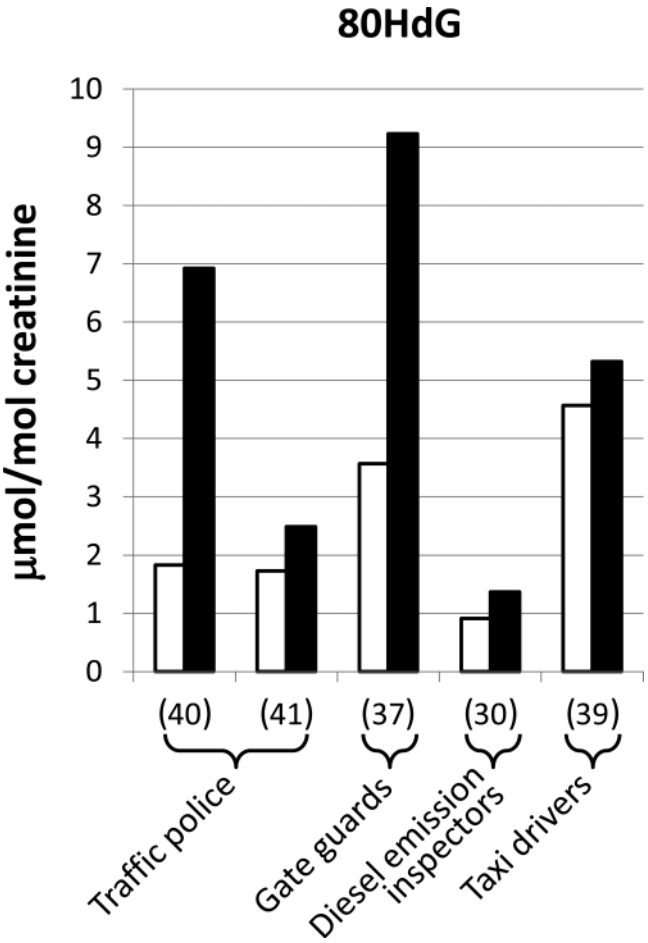

Fig. 3. Data sets from Table IV of 8OHdG data from control (open bars) and traffic-exposed (solid bars) populations; numbers below the bars are the references; data are expressed as µmol 8OHdG/mol of creatinine.

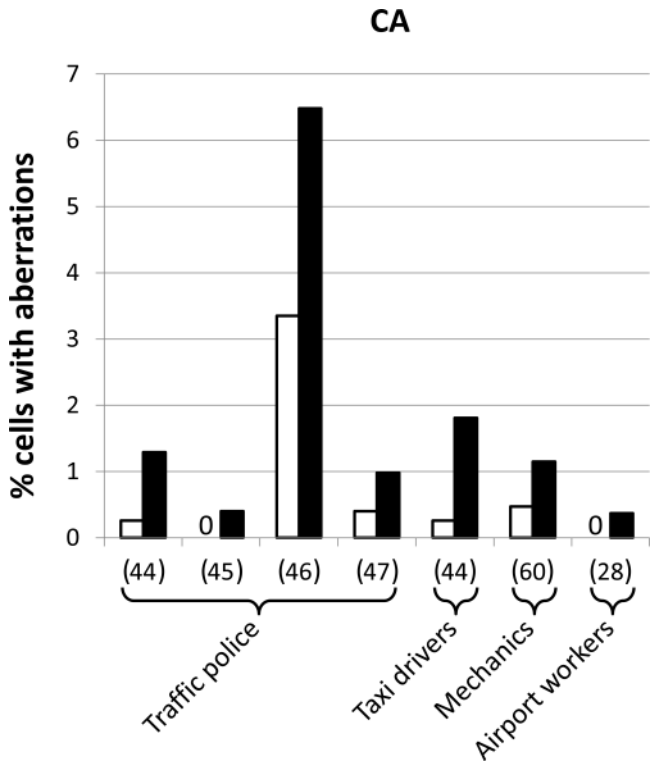

Fig. 4. Data sets from Table V of CA frequencies from control (open bars) and traffic-exposed (solid bars) populations; numbers below the bars are the references; data are expressed as the % of cells with aberrations.

significant between the exposed and control populations, the greatest fold differences between exposed and control were observed among the gate guards and traffic police in China (Figure 3).

A comparison of the CA frequencies among the different exposure groups is shown in Figure 4. Nearly all of the data sets showed a >2-fold difference between the exposed and control groups. The highest frequency of CAs was among traffic police in India (46); however, as with all the other biomarkers, the CA frequencies varied considerably among the various control groups among the studies.

The plots of MN frequencies show data for seven exposure groups, and most showed >2-fold differences between the control and exposed groups (Figure 5). The highest frequencies of MN were found among mechanics in Hungary (8) as well as gas station attendants and traffic police in the Philippines (52). Again, there was considerable variability in the MN frequencies among the control populations, perhaps reflective of both technique as well as the highly variable background exposures among the different studies.

Figure 6 shows the frequencies of SCEs in seven sets of traffic police, which is the largest data set of a single biomarker among a single exposure group that can be compared quantitatively among all the studies reviewed here. With one exception (63), the background frequencies were rather similar among all the data sets. Four of the data sets showed a >2-fold increased frequency in SCEs among the exposed versus control populations (46,55,60,63). The highest SCE frequency was observed among traffic police in India (60).

Exposure assessments

As summarised in Table II, 28 of 63 studies (44%) included exposure assessments involving measurements in blood, urine and/or air, and nearly all of them reported higher levels of exposure among the exposed versus control populations. Measurements of exposure using blood were the least common, whereas measurements in air and urine were more common. The concentration of OHPy was the most used measure of exposure in urine, and the concentration of B[*a*]P or PM was the most frequent measures of exposure in air.

Although there was not always a correlation between the level of exposure and the level of biomarker (Table II), the exposure assessments indicated that there were significant differences in exposure between the selected control and exposed populations. The fact that >80% of the total studies reviewed found higher levels of the biomarker in the exposed versus control populations suggests that the 54% of studies that did not include exposure assessment likely evaluated populations with significant differences in exposure between their control and exposed groups. In general, nearly all of the exposure assessments and biomarkers found differences between the control and exposed populations, suggesting a probable linkage between the class of agent measured by the exposure assessment and the cause of the biomarker studied. PAHs or PAH metabolites were the main class of chemical measured in air and urine, respectively, and this class of compound is recognised as an important component of diesel and automobile exhaust and air pollution in general.

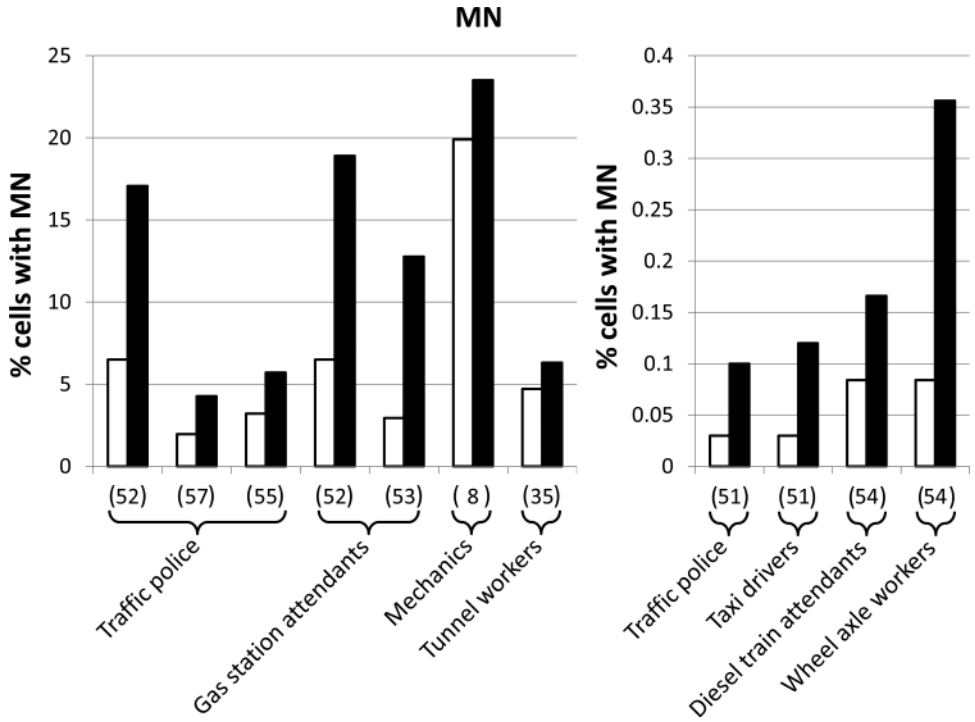

Fig. 5. Data sets from Table VI of MN frequencies from control (open bars) and traffic-exposed (solid bars) populations; numbers below the bars are the references. The data are plotted in two graphs of varying scales to reflect the different range of responses. The data are expressed as % cells with MN.

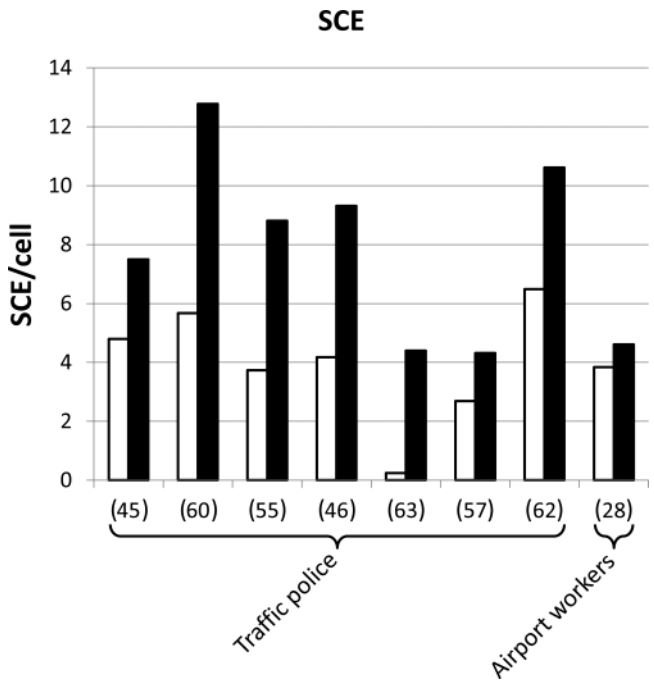

Fig. 6. Data sets from Table VII of SCE frequencies from control (open bars) and traffic-exposed (solid bars) populations; numbers below the bars are the references; the data are expressed as SCE/cell.

Conclusions

Among 63 studies on genotoxicity biomarkers in traffic-exposed subjects, the traditional genotoxicity biomarkers for cytogenetic endpoints (CA, MN and SCE) and for molecular end points for DNA damage (comet assay, urinary OH8dG and ^{32}P-postlabeling) were used for 74 of the 87 assessments reported in these studies. All six biomarkers were equally effective at distinguishing traffic-exposed from control populations, giving >80% positive results among the exposed versus control subjects. In addition, three genomic biomarkers effectively distinguished between these two populations; the assays measured changes in gene expression, leukocyte telomere length and DNA methylation. Thus, these genomic biomarkers hold great promise as genotoxicity biomarkers of exposure to traffic emissions and air pollution in general. Nearly half of all of the studies included exposure assessments involving blood (primarily protein adducts), urine (primarily OHPy) or air (primarily PAHs), and the vast majority of these were able to distinguish the exposed from the control subjects. The numbers of subjects evaluated for genotypic or phenotypic variation were quite limited, and the data provided no consistent evidence regarding the influence of the variants on either the biomarkers or exposure assessments.

All but 3 of the 63 reports were environmental studies that investigated 18 general exposure categories, mostly involving occupations that entailed chronic exposure to traffic emissions. The most studied of these groups were traffic police and automobile/bus mechanics. The studies were performed in 20 countries; however, nearly all of the environmental studies were performed in Asia or Europe, with only one each from Africa, North America and South America. Three studies reported controlled chamber exposures to diesel exhaust or ultrafine carbon particles, all from the USA. Thus, the studies reviewed here provide little information on genotoxicity among traffic-exposed subjects in North and South America or Africa; future studies might focus on these regions of the world.

The majority of the studies reviewed here are relatively new, with 60% (38/63) being published since 2000. Given the changes in traffic fleets throughout the world, future studies might investigate whether such changes would be reflected in

current exposure assessments and biomarkers in some of the same populations studied previously. Several of the biomarkers are associated with increased cancer risk, including CAs (42), MNs (50) and altered telomere length (81). Thus, the data reviewed here strongly suggest that chronic exposure to traffic exhaust is a risk factor for cancer.

These biomarker data support the recent finding by the International Association for Research on Cancer (IARC) that diesel exhaust is a Group 1 (known) human carcinogen and that gasoline exhaust is a Group 2B (possible) human carcinogen (82). In addition, a recent systematic review of 524 articles found that elevated levels of several of the biomarkers reviewed here, including DNA adducts detected by ^{32}P-postlabeling, DNA damage detected by the comet assay, the cytogenetic assays for MN and CAs and assays for DNA methylation, support a causal association between exposure to ambient air pollution and lung cancer (83).

The genotoxicity biomarker data reviewed here provide a mechanistic underpinning for the epidemiological studies showing that working or living near high-traffic roads is associated with increased risk for cancer as well as cardiovascular disease, allergy, asthma and adverse birth outcomes (1). As noted at the beginning of this article, the concerns expressed by mothers worldwide for their children playing in the traffic are magnified by the genotoxicity biomarker results reviewed here.

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