Frequency of oral mucosa micronuclei in gas station operators after introducing methanol

Gilka Jorge Figaro Gattás*, Lais de Almeida Cardoso*, Marcília de Araújo Medrado-Faria* and Pedro Henrique Saldanha†

*Department of Legal Medicine, Medical Ethics and Occupational Health, Medical School, University of São Paulo, Brazil; and †Department of Biology, Institute of Biosciences, University of São Paulo

Methanol has been proposed in different countries as an alternative automotive fuel to be used as an additive to, or replacement for, gasoline or ethanol. Utilization of methanol is increasing exposure to low levels of methanol vapors in the environment and more specifically in occupational settings such as gas stations. Pump operators are exposed to relatively high levels of fuel vapors, the consequences of which have not been fully examined. In this study, the micronucleus assay in squamous oral cells was performed on pump operators of 28 gas stations in three different periods in the city of São Paulo, Brazil. The frequency of micronuclei (MN) was evaluated before and 1 year after a mixed fuel called MEG, which contains 33% methanol, 60% ethanol and 7% gasoline, was introduced. The third evaluation, 3 years later, represents a period where the number of cars using alcohol fuel had decreased drastically and the pump operator exposure to MEG became very low. The frequency of MN observed in 76 employees in 1992 (mean = 3.62 ± 0.39) was significantly increased ($P < 0.001$) as compared with 76 operators exposed in 1989 (mean = 1.41 ± 0.26) and 129 exposed in 1995 (mean = 1.20 ± 0.15). These differences were also significant when compared with control groups not exposed professionally to motor fuel. These findings could indicate a mutagenic hazard of the MEG occurring in those with occupational exposure.

Key words: Fuel mutagenicity; gas station operators; methanol fuels; oral micronucleus; oxygenated fuels.

Received 2 June 2000; revised 31 October 2000; accepted 4 December 2000

Introduction

Oil crises all over the world have prompted users to look for alternative sources of energy, mainly for automobiles. Methanol has the potential to become a major automotive fuel, because it is less reactive and releases ozone slowly [1]. Increased exposure to methanol vapor and the consequences of chronic exposure have not been fully investigated [2].

The impact of atmospheric pollutants caused by exhausts from automobiles on human health has been investigated *in vitro* and *in vivo*, including genotoxic effects. Mutagenic and carcinogenic effects of diesel, petroleum vapors [3–5] and fuel agents like benzene and formaldehyde have been reported in humans and/or different biological models [6–14].

Micronuclei (MN) are chromosomal material that originated from acentric fragments of DNA or complete chromosomes that failed in attaching to the mitotic spindle [15]. Therefore, the occurrence of MN constitutes

Correspondence to: Gilka J. F. Gattás, Departamento de Medicina Legal, Ética Médica e Medicina Social e do Trabalho, Faculdade de Medicina, Universidade de São Paulo, Rua Teodoro Sampaio, 115, CEP-05405-000, São Paulo, SP, Brazil. Tel/fax: +55 11 8539677; e-mail: gfgattas@usp.br

a relatively simple and direct assay for screening risks of genetic damage in individuals occupationally exposed to mutagenic agents.

Among the individuals occupationally exposed to mutagenic agents, petrochemical workers [16] and gas station operators are considered particularly because they have to manipulate the fuel and consequently inhale fuel vapors during daily work. Significant increases in structural chromosome aberrations [17–19] and lymphocyte or oral MN have been reported [3,20] in employees who manipulated petroleum fuel derivatives. However, negative results have also been reported [21–25].

In Brazil, the ethyl alcohol alone (hydrated ethanol) extracted from sugar cane has been used as an alternative fuel since the 1970s. However, in November 1991, due to economic reasons, a fuel called MEG, a mixture of 33% methanol, 60% ethanol and 7% gasoline, began to be used in the city of São Paulo instead of hydrated ethanol alone. In Brazil, there are usually no self-service fuel supply pumps and that change would have increased the occupational risks mainly for pump operators who became exposed not only to gasoline with ethanol, but also to the mixture including methanol.

Nearly all the available information about methanol toxicity in humans is concerned with the consequences of acute exposures [26]. Methanol has no mutagenic effects in yeast, bacteria [27], hamsters [28] or mice treated by inhalation [29]. Fisher rats exposed to M85, a mixture containing 85% methanol and 15% gasoline, revealed hematological alterations [30] probably associated with methanol metabolism that results in formaldehyde [31].

The aim of this investigation was to evaluate the frequency of MN in oral squamous cells of gas station attendants working in the city of São Paulo, Brazil, during three different periods: before, during and after the introduction of methanol as an automobile fuel additive.

Materials and methods

Subjects

This investigation was carried out among pump operators of 28 gas stations located in the West Side of the city of São Paulo, Brazil, in three different periods.

The first period corresponds to the evaluation in 1989 when the gas stations had only separate ethanol and gasoline pumps. In this period of the study, 76 subjects with ages ranging from 21 to 59 years (mean ± SE: 33.2 ± 1.0) were investigated. Their duration of employment varied from 8 months to 31 years (mean ± SE: 10.1 ± 0.8 years). The control group consisted of 25 healthy university employees (mean ± SE: 33.6 ± 1.8 years) not actively involved in research occupations.

The second period represents a new survey in the same gas stations ~2 years after the MEG mixture had been introduced to replace ethanol fuel. The age range of the 76 subjects varied between 20 and 57 years (mean ± SE: 37.0 ± 1.1) and employment duration varied from 2 to 30 years (mean ± SE: 11.9 ± 0.8 years). Of this group, only 39 gas station operators had also been examined in 1989. The age of the controls, consisting of another group of 39 healthy university employees, ranged from 22 to 51 years (mean ± SE: 35.6 ± 1.3).

The third period includes a surveyed sample in the same gas station, when gasoline added with 20% ethanol was predominantly used as fuel in São Paulo (1995) and the distribution of MEG fuel was very low. The exposed group in this period included 129 gas station operators with ages ranging from 19 to 70 years (mean ± SE: 37.6 ± 1.0) and employment duration varying from 2 months to 30 years (mean ± SE: 12.4 ± 0.8 years). The control group consisted of 70 civil construction workers, with ages between 18 and 61 years (mean ± SE: 38.6 ± 1.3).

The occurrence of possible confounding issues related to health conditions or lifestyle, including recent diagnostic X-rays, use of medicaments or drugs, tobacco and alcohol intake, were investigated by personal interviews of controls and exposed workers.

The micronucleus assay

The usual method and classification of MN in exfoliated oral cells [32] was used with small modifications. First, the subjects were asked to rinse their mouths with water; then, after gently scraping the right and left cheeks with a cytobrush, the material was submersed in 5 ml of saline solution (0.9% NaCl) and transported immediately to the laboratory (<1 h). After centrifugation (10 min, 1200 r.p.m.), the pellet was fixed in methanol–acetic acid (3:1), twice for 5 min. The cell suspension was dropped on cool glass slides and stained with Feulgen plus fast-green (1%). A cell was considered to contain MN if it contained not more than three cytoplasmic Feulgen staining bodies with a size less than one-third of the main nucleus. The micronucleus analyses were performed in 2000 cells for each individual in a blind fashion.

Statistical methods

MN formation is a rare event exhibiting a Poisson distribution, so the frequencies of oral micronucleated cells were analyzed by non-parametric tests (Kruskal–Wallis, Mann–Whitney, Dunn). Age followed a Gaussian distribution and was analyzed by Student's *t*-test. The critical level for rejection of the null hypothesis was considered to be a *P* value of 5%.

Results

Figure 1 shows the distribution of the number of cells with MN and the total number of MN per 2000 cells

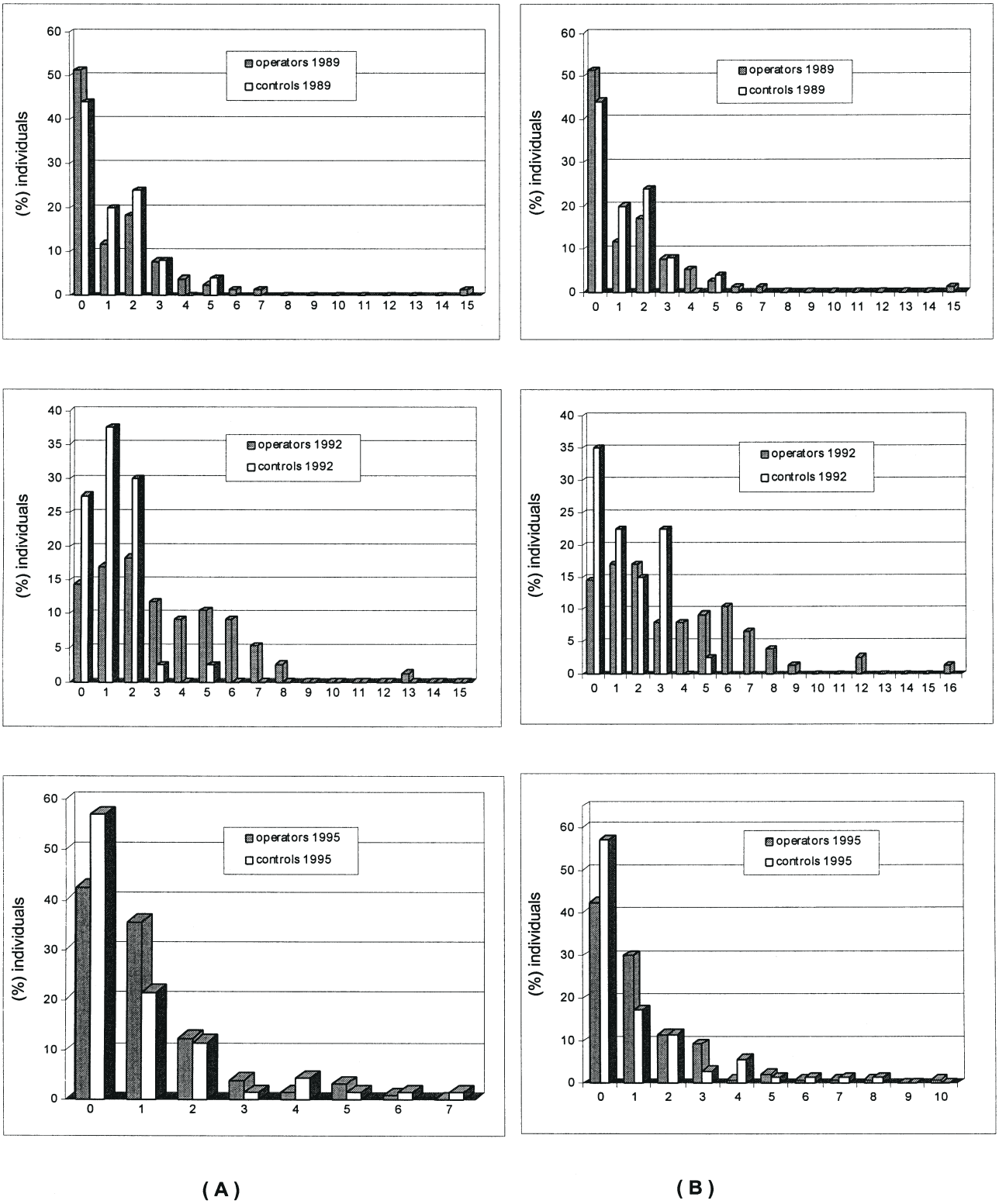

Figure 1. Distribution of the number of cells with MN (**A**) and the number of MN (**B**) per 2000 cells per individual among pump operators and controls in the three surveys (1989, 1992, 1995).

counted among individual pump operators and controls in the three surveys. Both cells with MN and the total number of MN per individual exhibited a highly positively skewed binomial distribution. Since MN formation represents a low-frequency event, its distribution appeared to fit quite well to a Poisson distribution.

Table 1. Number of individuals, mean age, working time, cells with MN and number of MN per 2000 cells per individual among gas station operators and controls, at three different periods (1989, 1992, 1995)

Period	*Group*	*n*	*Mean age*	*Working time*	*Total no. of cells*	*Total no. of cells with MN*	*Total no. of MN*	*No. of MN per individual*	*Significance (Mann–Whitney)*
1989	Exposed	76	33.2 ± 1.0	10.1 ± 0.8	152 000	105	107	1.41	
	Controls	25	33.6 ± 1.8	–	50 000	28	28	1.12	$P = 0.95$
1992	Exposed	76	37.0 ± 1.1	11.9 ± 0.8	152 000	235	275	3.62	
	Controls	39	35.6 ± 1.3	–	78 000	47	53	1.35	$P = 0.0001$
1995	Exposed	129	37.6 ± 1.0	12.4 ± 0.8	258 000	129	155	1.20	
	Controls	70	38.6 ± 1.3	–	140 000	64	76	1.08	$P < 0.0001$

Statistical analysis was performed using non-parametric tests of significance.

Because only some of the pump operators were examined in all three periods (1989, 1992, 1995), comparison of their age distribution with that of controls was performed only for the 1995 survey, which includes a larger and prospectively better controlled sample. The age range was very similar between the two groups in the third survey and the small mean difference was not significant, even estimated by Welch's approximated *t*-test ($t = 0.59$; $P = 0.56$), which assumes unequal variances [33].

Table 1 presents the number of individuals, mean age, working time, cells with MN and number of MN per individual among gas station operators and controls in different surveys. Because the total number of MN per individual includes cells with one, two or three MN, it proved to be a more powerful variable in the statistical analysis.

The number of MN per individual in the first survey was 1.12 in the controls and 1.41 in the exposed subjects, which was not a significant difference ($P = 0.95$). However, after the introduction of the MEG mixture, the frequency of MN increased significantly, as indicated by the later results. During the 1992 period, the number of MN rose markedly from 1.35 to 3.62 and during the 1995 period from 1.08 to 1.20, both differences being highly significant ($P = 0.0001$), as evaluated by the Mann–Whitney test (Table 1).

Non-parametric analysis of variance concerning the frequencies of cells with MN and the number of MN per individual in the three different periods was performed using the Kruskal–Wallis test and multiple comparison of the three surveys by means of Dunn's test (Table 2). Results indicated that the temporal heterogeneity was highly significant for both mean frequencies of cells with MN and number of MN per individual. However, mean differences between the 1989 and 1995 periods, as compared by Dunn's multiple comparison test, were not significant.

Table 3 shows comparisons of mean ages, duration of work and numbers of MN for the 39 subjects investigated in two periods (1989 and 1992), and between two sets of

Table 2. Comparisons between the frequency of cells with MN and the number of MN per 2000 cells per individual observed in oral cells of gas station operators in 1989, 1992 and 1995, through Dunn's multiple comparison test

Period	*n*	*Cells with MN (mean ± SE)*	*No. of MN (mean ± SE)*
1989 (A)	76	1.38 ± 0.26	1.41 ± 0.26
1992 (B)	76	3.09 ± 0.29	3.62 ± 0.39
1995 (C)	129	0.98 ± 0.11	1.20 ± 0.15
Significance (Kruskal–Wallis)		$H(\chi^2) = 4631.4$ $P < 0.0001$	$H(\chi^2) = 4589.1$ $P < 0.0001$
Dunn's multiple comparison test			
A × B		$P < 0.001$	$P < 0.001$
A × C		$P > 0.05$	$P > 0.05$
B × C		$P < 0.001$	$P < 0.001$

17 subjects examined in the three different periods (1989, 1992 and 1995). Although both mean age and duration of work increased in subsequent periods, there was not a linear increase in the frequency of MN. For the 39 subjects investigated in two subsequent periods (1989 and 1992), the average number of MN per individual increased from 1.08 to 3.20, a highly significant difference as estimated by Mann–Whitney test (Table 3). However, for the 17 subjects examined in all three subsequent times (1989, 1992 and 1995), the average number of MN per subject increased significantly from 1.18 to 3.47 after methanol introduction (1992), but then decreased to 2.06 in 1995. The ANOVA of the three frequencies through the Kruskal–Wallis test revealed that the heterogeneity did not reach statistical significance (Table 3), probably because of the small sample size.

Stepwise regression analysis was performed by considering anamnestic data (independent variables) such as previous diseases, number of miscarriages related to operators' partners, use of medicaments and X-rays in the 6 months before examination, tea and coffee drinking, alcohol intake, smoking and use of psychotropic drugs as semi-quantitative parameters. The results of stepwise multiple regression analysis of the number of cells with MN and the number of MN per 2000 cells per individual

Table 3. Comparisons between mean age, working time, cells with MN and number of MN per 2000 cells per individual among samples of 39 gas station operators investigated in two periods (1989, 1992) and among 17 subjects investigated in three different periods (1989, 1992, 1995)

Period	*Mean age*	*Duration of work*	*Cells with MN*	*Total no. of MN*	*No. of MN per individual*	*Significance*
1989	34.5 ± 1.4	10.9 ± 1.2	42	42	1.08[a]	Mann–Whitney:
1992	36.3 ± 1.5	12.9 ± 1.2	123	125	3.20[a]	$P = 0.0001$
1989	35.5 ± 2.1	11.5 ± 1.3	20	20	1.18[b]	Kruskal–Wallis:
1992	37.5 ± 2.1	13.5 ± 1.3	56	59	3.47[b]	$P = 0.06$
1995	40.6 ± 2.1	16.6 ± 1.3	31	35	2.06[b]	

[a]78 000 cells analyzed.

[b]34 000 cells analyzed.

Table 4. Multiple regression analysis of number of cells with MN (CMN) and total number of MN (TMN) per 2000 cells per individual upon statistically significant independent variables, estimated from the 1995 operator sample

Y (mean ± SD)	*X*	*b*	S_b	*t*	*P*	r^2
CMN (0.97 ± 1.24)	Miscarriages	0.442	0.217	4.133	0.044	0.032
TMN (1.20 ± 1.68)	Age	0.032	0.013	6.425	0.012	0.049
	Tea	32.044	12.960	6.114	0.015	0.046
			$a = 0.187 \pm 1.625$; $F = 5.316$; $P = 0.006$			

Y = dependent variable; X = independent variable; $b + S_b$ = coefficient of regression ± SE; t = t-test significance; r^2 = coefficient of determination; P = probability; a = y intercept; F = variance ratio significance.

upon statistically significant independent variables estimated from operator data are presented in Table 4.

The number of cells with MN per individual showed a low but statistically significant regression upon the frequency of miscarriages that occurred among the operators' partners. Square root transformation, a tentative approach for normalizing dependent variables, produced the same statistical results. Also, the number of cells with MN showed a positive regression with the frequency of miscarriages among operators' wives, and the number of MN per individual exhibited a positive regression with age and tea-drinking confirmed by square root transformation. ANOVA of multiple regression was highly significant ($P = 0.006$).

Discussion

Oxygenated fuels such as ethanol and methanol, with a higher oxygen content than conventional gasoline and diesel fuel, have been used in some countries not only for economic reasons but also to improve air quality. This alternative fuel releases lower rates of carbon monoxide and hydrocarbons, and should contribute to decreased ozone levels [1].

The frequency of oral MN among pump operators increased after the introduction of methanol (MEG mixture) into hydrated ethanol fuel (Tables 1 and 2). The results reported for the third period of investigation were similar to those observed before the introduction of methanol-based fuel (Dunn's test in Table 2). As expected, mean age and duration of work, i.e. exposure to the fuel, increased accordingly during the three periods; this finding may explain the small mean difference between 1989 and 1995 samples (Table 3). These changes also need to be interpreted with reference to the decrease in the number of cars fueled by alcohol in the city of São Paulo. From 1980 to 1990, the national fleet of cars produced by the Brazilian industry included 90% alcohol fuel motor, but in 1995 there were only ~1.5 million cars (~30%) moved by alcohol and for this reason MEG fuel distribution was drastically reduced.

Age proved to be significantly related to dependent variables, as estimated through regression analysis of data from gas station operators (Table 4). This finding is expected since mutagenicity could increase with age [34].

The positive regression of the number of cells with MN upon miscarriages observed among operators' wives was an unexpected result and suggested that methanol could affect the germinal cells of operators. Square root transformation of the dependent variables confirmed the significant results (Table 4). In fact, an increased risk of spontaneous abortion for women working in petrochemical complexes has been associated with exposure to benzene and gasoline [35].

Similarly, it is difficult to envisage how the constituents of tea, e.g. caffeine, could increase the number of oral

MN. Nevertheless, the results deserve further investigation. Other variables and intervening factors, such as smoking and alcohol consumption, had no influence on the frequency of MN as analyzed by regression analysis.

The gas station operators in this study were exposed to methanol vapors and cutaneous contact when handling the fuel. Although some personal protection, such as the use of gloves, masks and rubber boots, was considered mandatory at the beginning of their employment, these rules were not followed after a few months by the gas station employees. Prolonged methanol skin contact, after working with methanol-soaked clothes, can produce systemic effects [36]. The subjects were also exposed to benzene when handling gasoline. It was not possible to disentangle the effects of gasoline, ethanol and MEG exposure, but gasoline was always present in the occupational area. Gasoline is a complex mixture of different hydrocarbons, some additives, and probably 3–5% benzene. The carcinogenic action of benzene in gasoline has consistently been demonstrated [37] and its mutagenic effect is even detected at low levels [13], but other components in the fuel may additively or synergistically contribute to the observed effects to some extent.

It should also be considered that in the MEG mixture the ethanol increases cellular membrane permeability, while methanol itself is rapidly absorbed. The mixture may, therefore, contribute in different ways to the increased MN frequencies. In fact, occupational exposure to gasoline in gas stations was reported to increase the frequency and the size of lymphocyte MN [20]. Also, the particulates from gasoline-powered engines have a direct effect on markers of chromosome damage, such as sister chromatid exchange, aneuploidy, polyploidy and *in vitro* cell transformations [8] probably related to benzene [20]. Solvents have free passage across cellular membranes and can interfere with cell proliferation and function [38], as well as with normal cell absorption of metabolic substances [39]. There is also evidence that dermal absorption of methanol is enhanced by gasoline–methanol mixtures [36].

In conclusion, the present results indicate a mutagenic risk caused by occupational exposure to MEG in gas station operators during the period when the fuel was predominantly used. The effect could be related to methanol and possibly also synergistically associated with other components of gasoline. While MN formation results from chromosome aberrations, its increased incidence may indicate prospective biological consequences such as cancer [40], fetal wastage and genetic abnormalities, so occupational exposure of gas station operators must be considered to be important. However, the precise impact of the genotoxic harm from occupational exposure to motor fuel including MEG mixture or oxygenated fuel remains to be clarified by means of direct methods, such as analysis of long-term DNA adducts produced by those fuels during DNA repair [41].

Acknowledgements

The authors are grateful to the Sugar and Alcohol Industry Association of São Paulo for financing part of this study, the Occupational Health Service of Oscar Freire Institute and to R. C. Estanqueiro, J. A. Soares-Vieira and E. Döppenschimitt for technical assistance. This study was also partially financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and LIM-HC-FMUSP.

References

1. Marshall E. Gasoline: the unclean fuel. *Science* 1989; **246:** 199–201.
2. Kavet R, Nauss KM. The toxicity of inhaled methanol vapors. *Crit Rev Toxicol* 1990; **21:** 21–50.
3. Hogstedt B, Gullberg B, Mark-Vendel E, Mitelman F, Skerfving S. Micronuclei and chromosomal aberrations in bone marrow cells and lymphocytes of humans exposed mainly to petroleum vapors. *Hereditas* 1981; **94:** 179–187.
4. Rosenkranz HS. Diesel emissions revisited: is the carcinogenicity due to a genotoxic mechanism? *Mutat Res* 1987; **182:** 1–4.
5. Crebelli R, Conti L, Crochi B, Carere A, Bertoli C, Giacomo ND. The effect of fuel composition on the mutagenicity of diesel engine exhaust. *Mutat Res* 1995; **346:** 167–172.
6. Forni A, Cappelini A, Pacifico E, Vigliani EC. Chromosome changes and their evolution in subjects with past exposure to benzene. *Arch Environ Health* 1971; **23:** 385–391.
7. Picciano D. Cytogenetic study of workers exposed to benzene. *Environ Res* 1979; **19:** 33–38.
8. Hadnagy W, Seemayer NH. Cytotoxic and genotoxic effects of extract of particulate emission from a gasoline-powered engine. *Environ Mol Mutagen* 1988; **12:** 385–396.
9. Kerns WD, Pavkov KL, Donofrio DJ, Gralla EJ, Swenberg JA. Carcinogenicity of formaldehyde in rats and mice after long-term inhalation exposure. *Cancer Res* 1983; **43:** 4382–4392.
10. Natarajan AT, Darroudi F, Bussman CJM, Kesteren-van LAC. Evaluation of the mutagenicity of formaldehyde in mammalian cytogenetic assays *in vivo* and *in vitro*. *Mutat Res* 1983; **122:** 355–360.
11. Ma T, Harris MM. Review of the genotoxicity of formaldehyde. *Mutat Res* 1988; **196:** 37–59.
12. Titenko-Holland N, Levine AJ, Smith MT, *et al*. Quantification of epithelial cell micronuclei by fluorescence *in situ* hybridization (FISH) in mortuary science students exposed to formaldehyde. *Mutat Res* 1996; **371:** 237–248.
13. Nilsson RI, Nordlinger RG, Tagesson C, Walles S, Jarvholm BG. Genotoxic effects in workers exposed to low levels of benzene from gasoline. *Am J Ind Med* 1996; **30:** 317–324.
14. Lewis RJ, Schnatter AR, Katz AM, *et al*. Updated mortality among diverse operating segments of a petroleum company. *Occup Environ Med* 2000; **57:** 595–604.

15. Heddle JA, Hite M, Kirkhart B, *et al*. The induction of micronuclei as a measure of genotoxicity. *Mutat Res* 1983; **123:** 61–118.
16. Zhou X, Li L, Cui M, Yu R, Li L, Yan Z. Cytogenetic monitoring of petrochemical workers. *Mutat Res* 1986; **175:** 237–242.
17. Sarto FI, Cominato AM, Pinton PG, *et al*. A cytogenetic study on workers exposed to low concentrations of benzene. *Carcinogenesis* 1984; **5:** 827–832.
18. Santos-Mello R, Cavalcante B. Cytogenetic studies on gas station attendants. *Mutat Res* 1992; **280:** 285–290.
19. Carere A, Antoccia A, Crebelli R, *et al.* Genetic effects of petroleum fuels: cytogenetic monitoring of gasoline station attendants. *Mutat Res* 1995; **332:** 17–26.
20. Högstedt B, Holmén A, Karlsson A, Raihle G, Nillius K, Vestlund K. Gasoline pump mechanics had increased frequencies and sizes of micronuclei in lymphocytes stimulated by pokeweed mitogen. *Mutat Res* 1991; **263:** 51–55.
21. Fredga K, Reitalu J, Berlin M. Chromosome studies in workers exposed to benzene. In: Berg K, ed. *Genetic Damage in Man Caused by Environmental Agents*. New York: Academic Press, 1979; 187–203.
22. Khalil AM, Qassem W, Kamal OM. No significant increase in sister-chromatid exchanges in cultured blood lymphocytes from workers in large oil refinery. *Mutat Res* 1994; **312:** 187–191.
23. Pitarque M, Carbonell E, Lapeña N, *et al*. No increase in micronuclei frequency in cultured blood lymphocytes from a group of filling station attendants. *Mutat Res* 1996; **367:** 161–167.
24. Surrallés J, Autio K, Nylund L, *et al*. Molecular cytogenetic analysis of buccal cells and lymphocytes from benzene-exposed workers. *Carcinogenesis* 1997; **18:** 817–823.
25. Torres-Bugarín O, Anda-Casillas AD, Ramirez-Munhõz MP, Sánchez-Corona J, Cantú JM, Zúñiga G. Determination of diesel genotoxicity in firebreathers by micronuclei and nuclear abnormalities in buccal mucosa. *Mutat Res* 1998; **413:** 277–281.
26. James CR. The toxic effects of organic solvents. In: Williams PL, Burson JL, eds. *Industrial Toxicology: Safety and Health Applications in the Workplace*. New York: Van Nostrand Reinhold, 1985; 230–259.
27. Abbondandolo A, Bonatti S, Corsi C, *et al.* The use of organic solvents in mutagenicity testing. *Mutat Res* 1980; **79:** 141–150.
28. Obe G, Ristow H. Mutagenic, cancerogenic and teratogenic effects of alcohol. *Mutat Res* 1979; **65:** 229–259.
29. Campbell JA, Howard DR, Backer LC, Allen JW. Evidence that methanol inhalation does not induce chromosome damage in mice. *Mutat Res* 1991; **260:** 257–264.
30. Maejima K, Suzuki T, Niwa K, *et al*. Toxicity to rats of methanol-fueled engine exhaust inhaled continuously for 28 days. *J Toxicol Environ Health* 1992; **37:** 293–312.
31. Lee EW, Garner CD, Terzo TS. Animal model for the study of methanol toxicity: comparison of folate-reduced rat responses with published monkey data. *J Toxicol Environ Health* 1994; **41:** 71–82.
32. Sarto F, Finotto S, Giacomelli L, Mazzotti D, Tomanim R, Levis AG. The micronucleus assay in exfoliated cells of the human buccal mucosa. *Mutagenesis* 1987; **2:** 11–17.
33. Zar JH. *Biostatistical Analysis*, 3rd edn. Upper Saddle River, New Jersey: Prentice Hall, 1996.
34. Bolognesi A, Abbondandolo A, Barale R, *et al*. Age-related increase of baseline frequencies of sister chromatid exchanges, chromosome aberrations, and micronuclei in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* 1997; **6:** 249–256.
35. Xu X, Cho S, Sammel M, *et al*. Association of petrochemical exposure with spontaneous abortion. *Occup Environ Med* 1998; **55:** 31–36.
36. Agency for Toxic Substances and Disease Registry. Monograph on methanol toxicity. *Am Fam Physician* 1993; **47:** 163–171.
37. American Conference of Government Industrial Hygienists, Threshold Limit Value Committee. Notice of intended changes—benzene. *Appl Occup Environ Hyg* 1990; **5:** 453–463.
38. Friday KE, Howard GA. Ethanol inhibits human bone cell proliferation and function *in vitro*. *Metabolism* 1991; **40:** 562–565.
39. Topinka J, Binková B, Srám RJ, Fojtiková I. DNA-repair capacity and lipid peroxidation in chronic alcoholics. *Mutat Res* 1991; **263:** 133–136.
40. Hagmar L, Bonassi S, Strömberg U, *et al*. Cancer predictive value of cytogenetic markers used in occupational health surveillance programs: a report from an ongoing study by the European Study Group on Cytogenetic Biomarkers and Health. *Mutat Res* 1998; **405:** 171–178.
41. Pfohl-Leszkowicz A, Weber-Lotfi F, Masfaraud JF, *et al*. DNA adducts detection: some applications in monitoring exposure to environmental genotoxic carcinogens. In: Phillips DH, Castegnaro M, Bartsch H, eds. *Postlabelling Methods for Detection of DNA Adducts*. IARC Scientific Publications No. 142, Lyon, 1993; 373–378.