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Fundamental and Molecular Mechanisms of Mutagenesis

Genetic effects of petroleum fuels: cytogenetic monitoring of gasoline station attendants

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Abstract

Workers in the petroleum distribution trades experience relatively high-level exposures to fuel vapours whose consequences have not been fully elucidated. In this study, the possible relationship between occupational exposure to petroleum fuels and cytogenetic damages in peripheral lymphocytes was investigated. Twenty-three male, non-smoking workers from the area of Rome were enrolled in the study, together with age-paired controls with no occupational exposure to fuels. Peripheral lymphocyte cultures were set up for the analysis of structural chromosome aberrations (CAs), sister chromatid exchanges (SCEs) and micronuclei (MN) in cytokinesis-blocked lymphocytes. Frequencies of CAs, SCEs and MN were compared between exposed and control groups, and evaluated in relation to blood lead level (as an indicator of engine exhausts exposure) for the whole group under study, and to yearly averaged exposure to benzene (8-h time weighted averages, as determined by repeated personal sampling) for fillingstation attendants only. Both CAs and SCEs were slightly increased in station attendants: 1.97 versus 1.46 aberrations per 100 cells, and 4.73 ± 0.15 versus 4.48 ± 0.11 SCEs/cell in exposed and control individuals, respectively. The difference between cumulative CA rates in the exposed and control populations was of borderline statistical significance ($p = 0.066$). However, when the exposed population was dichotomized for benzene exposure, a significant ($p = 0.018$) correlation of CAs with benzene exposure was found. The analysis of SCE data highlighted a significant increase of cells with more than 6 exchanges (HFCs), corresponding to the 75° percentile of the overall distribution, in fillingstation attendants (relative risk (RR) = 1.3, 95% CI = 1.1 – 1.5) in comparison with controls. In the pooled population, the frequency of HFCs showed a statistically significant upward trend at increasing blood lead levels (χ^2 for trend = 27.8, $p < 0.0001$). A complex relationship between SCEs and benzene exposure was observed, with an increased frequency of HFCs in the medium exposure intensity class (RR = 1.5, 95% CI = 1.2–1.7), and no difference for exposure to higher benzene levels (RR = 1.0, 95% CI = 0.9–1.2), compared to reference subjects. Finally, the analysis of MN in both phytohemagglutinin- and pokeweed-stimulated cell cultures did not show significant excess of MN in binucleated lymphocytes of exposed workers with respect to the age-paired controls.

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1. Introduction

Petroleum fuels are complex, highly variable mixtures of aliphatic and aromatic hydrocarbons which contain sizeable amounts of known human and animal carcinogens. Exposure to gasoline vapours is classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans, mainly on the basis of the established carcinogenicity of some component chemicals such as benzene and 1,3-butadiene (International Agency for Research on Cancer, 1989a, b). The current massive use of petroleum fuels in automotive engines, which accounts for most environmental benzene levels (Fishbein, 1988), entails wide human exposure. In particular, workers in the petroleum distribution trades experience relatively high-level exposures to fuel vapours whose health effects have not been fully elucidated. Previous studies suggested either an increase of cytogenetic damages in peripheral lymphocytes of workers in the petrochemical industry (Zhou et al., 1986; Sobti and Bhardwaj, 1993) and in gasoline station attendants (Högstedt et al., 1991; Santos-Mello and Cavalcante, 1992), or no effect at all (Fredga et al., 1979; Khalil et al., 1994). The occurrence of a mixed and variable pattern of exposure in different occupational settings, as well as the lack of quantitative information on exposure levels, prevent any firm conclusion from the above studies on the genetic effect of occupational exposure to fuels. To overcome these limitations, which are common to many biomonitoring studies, the analysis of biomarkers in the exposed populations should be coupled to a rigorous exposure assessment, able to describe the exposure profile of each individual in both qualitative and quantitative terms. In this study, the cytogenetic monitoring of a group of gasoline station attendants from the area of Rome was coupled to a detailed 1-year monitoring of the personal exposure to benzene and alkyl derivatives. The frequency of early markers of genetic effect in the exposed population, i.e. chromosome aberrations (CAs), sister chromatid exchanges (SCEs) and micronuclei (MN) in peripheral lymphocytes, was analyzed in comparison to the results obtained in age-paired control individuals and in relation to the yearly average personal exposure to benzene and to blood lead level, as a proxy measure of exposure to engine exhausts.

2. Materials and methods

2.1. Populations

Twenty-three male, non-smoking station attendants from the area of Rome were enrolled in the study. The absence of possible confounders related to health status or life style, such as recent diagnostic X-rays, use of pharmaceutical drugs and high alcohol consumption, was assessed through personal interviews. More than half (14 out of 23) of the workers had been engaged in fuel distribution for more than 20 years. Age-paired, healthy non-smoking blood donors with no occupational exposure to fuels or other chemicals served as controls. Personal exposure to benzene and alkylbenzenes in station attendants was monitored by repeated sampling for one year, with a periodicity scheduled as to compensate for seasonal and daily variations in workload. Breathing-zone air samples were collected during the entire workshift by means of personal pumps with vials of active carbon worn by the attendants. Blood lead level was measured at the time of blood collection on one spot sample per subject by atomic absorption spectrophotometry. The results of the exposure monitoring have been reported in detail elsewhere (Lagorio et al., 1993; Carere et al., 1995).

2.2. Cytogenetic analyses

From each blood sample, 10 cultures were set up using 0.5 ml whole blood cultures in 5 ml RPMI 1640 medium with Hepes and glutamine (Gibco) supplemented with 20% of heat-inactived fetal calf serum and antibiotics.

Four cultures, stimulated with 2% phytohemagglutinin (PHA, Wellcome) and treated with 10^{-5} M colchicine (Sigma Chemical) 3 h before harvesting, were established for the analysis of SCEs and CAs following standard procedures (Degrassi et al., 1984). To three of the four cultures, 9 μg/ml 5-bromodeoxyuridine (BrdU, Sigma) was added. The culture without BrdU was fixed at 48 h for the scoring of CAs. In order to score only first division cells for the incidence of chromosomal aberrations, the M_1, M_2, M_3 ratio was estimated in a parallel culture with BrdU fixed at 48 h. All probands showed less than 10% second mitoses at 48 h. The other two cultures with BrdU were fixed at 72 h for the scoring of SCEs. Preparations were made according to standard procedures (Degrassi et al., 1984).

Six cultures, treated at 44 h with 6 μg/ml cytochalasin B (Sigma Chemical) and harvested at 72 h, were established for the analysis of micronuclei (MN) in binucleated lymphocytes as previously described (Surralés et al., 1992). Cultures for MN analysis were stimulated with either 2% PHA (three cultures) or 100 μg/ml of pokeweed (PKW; Sigma Chemical) (three cultures), in view of published results suggesting that B lymphocytes – stimulated with the mitogen PKW – show an increased frequency of micronuclei in gasoline pump mechanics (Högstedt et al., 1991).

In addition to the above end-points, the proliferation index (PRI) and nuclear division index (NDI) were determined to evaluate the progression of the cell population under study through the mitotic cycle. The proliferation index was calculated on 100 cells/subject from lymphocyte cultures treated with BrdU and harvested at 72 h, as follows: [(no. cells in M1 + (2 × no. cells in M2) + (3 × no. cells in M3)]/100] (Lamberti et al., 1983); the nuclear division index was calculated on 1000 cells per individual in cultures treated with cytochalasin B according to Eastmond and Tucker (1989): [(no. mononucleated + (2 × no. binucleated) + (3 × no. trinucleated) + (4 × no. tetranucleated)]/1000].

For each individual, SCE frequency was determined in at least 75 well-differentiated second metaphases; 200 first metaphases were scored for CAs analysis; MN frequencies were determined in at least 2000 binucleated lymphocytes in PHA-stimulated cultures and in at least 1000 binucleated cells in PKW-stimulated cultures. All slides were blind scored.

2.3. Statistical analyses

The mean values of age, blood lead level, and frequency of micronuclei and sister chromatid exchanges in fillingstation attendants and controls were compared by one-way analysis of variance. The Pearson χ^2 test was used to evaluate the statistical significance of the differences in percentages of chromosomal aberration between exposed and controls.

The interrelationships between age and the results for variables under study (indicators of cytogenetic damage, nuclear division index, and proliferation index) were investigated by simple linear correlation analyses. As the frequency of micronuclei was low, the average square root transformation $\frac{1}{2}[\sqrt{y} + \sqrt{y+1}]$ was applied to individual measurements of this end-point, in order to stabilize the variance (Whorton, 1985). The arcsine transformed individual percentages of cells containing chromosomal aberrations ($arctangent[y/\sqrt{1-y^2}]$) were used in these analyses (Yardley-Jones et al., 1990).

Table 1
Relevant characteristics of the studied subjects: filling-station attendants (exposed) and age-matched references (controls)

Variable	Exposed			Controls			p [a]
	No.	Mean (SE)	Range	No.	Mean (SE)	Range	
Age (yrs)	23	45.8 (2.5)	28–64	24	44.2 (2.6)	22–62	0.652
Benzene exposure [b]	21	1.5 (0.7)	0.1–13.1	–	–	–	–
Blood lead level [c]	22	10.7 (0.8)	5.6–19.9	24	8.4 (0.7)	2.8–15.0	0.036
Length of employment (yrs)	23	22.4 (2.3)	3–42	–	–	–	–

[a] p = statistical significance of the difference exposed versus controls, estimated by one-way analysis of variance.
[b] Benzene exposure is average yearly personal exposure to benzene based on 6.5 measurements/subject (mg/m³, 8-h TWAs).
[c] Blood lead level is measured on a spot sample of venous blood per subject (μg/dl).

Relative risks (RRs) of selected outcomes (prevalence of cells with chromosomal aberrations or with high frequency of SCEs) among 'exposed' versus 'references' were estimated as the ratios of the prevalence odds, controlling for potential confounders by logistic regression analysis. Due to the relatively low frequency of the outcomes (CAs and high-frequency cells), the scored metaphases were taken as units of observation. Furthermore, the available quantitative exposure indexes (blood lead and average yearly exposure to benzene) were categorized into low, medium and high levels of exposure. In particular, blood lead concentrations were categorized around the 33° (7.5 μg/dl) and the 66° percentiles (10 μg/dl) of the overall distribution, and the lowest exposure class was taken as reference. As to benzene exposure, controls were taken as reference and fillingstation attendants were categorized around the 50° percentile of the distribution (< 0.2 and ≥ 0.2 mg/m³).

All analyses were carried out with the SPSS/PC statistical package (SPSS/PC, 1990).

3. Results

The characteristics of the exposed and control populations are summarized in Table 1, where mean age and blood lead levels of both study groups are shown, together with the length of employment and time weighted average (TWA) benzene exposure of station attendants. In order to minimize the influence of confounding factors related to personal life style, only non-smokers were enrolled in the study. In addition, quantitative information on alcohol consumption was recorded in order to exclude heavy drinkers, in view of the well-known effect of alcohol abuse on several cytogenetic end-points (Obe and Anderson, 1987). Among the subjects studied, a preliminary inspection of the outcome of cytogenetic analyses in relation to individual daily alcohol intake (0 to 50 g) did not reveal any significant correlation between the parameters (data not shown).

The assessment of the exposure to aromatic hydrocarbons carried out on these workers demonstrated a strict correlation of benzene levels with other aromatic components of gasoline (toluene, xylenes, ethylbenzene), as well as with indirect indexes of workload, such as the amount of fuel sold (Lagorio et al., 1993). Consequently, benzene exposure was regarded as representative of the whole exposure to petroleum fuels for these workers.

Blood lead level was determined for all the subjects studied to provide some information on the personal exposure to vehicle exhausts. The combustion of leaded fuels plays a major role as an emission source of this heavy metal into the environment, especially where unleaded gasoline has no widespread use. In this respect, unleaded fuel accounted for approximately 10% of the gasoline delivered by the attendants enrolled in this study (Lagorio et al., 1993). The comparison of average

Table 2
Correlation coefficients between each cytogenetic end-point (individual means) and the age of the subjects

Variable [a]	SCE		PRI		MN-PHA		NDI-PHA		MN-PKW		NDI-PKW		CAs(−gaps)	
	No.	r	No.	r	No.	r	No.	r	No.	r	No.	r	No.	r
Age	45	0.30 *	43	0.10	47	0.29 *	47	0.02	29	0.35 *	29	−0.22	47	−0.04
SCE	45	1.00	43	0.14	45	0.25 *	45	0.28 *	27	0.05	27	−0.03	45	−0.14
PRI			43	1.00	43	0.18	43	0.29 *	26	−0.13	26	0.16	43	−0.01
MN-PHA					47	1.00	47	−0.27 *	29	0.59 **	29	−0.47 **	47	−0.05
NDI-PHA							47	1.00	29	−0.40 *	29	0.45 **	47	0.11
MN-PKW									29	1.00	29	−0.18	29	0.01
NDI-PKW											29	1.00	29	0.25
CAs(−gaps)													35	1.00

For this analysis the transformed values of MN (average square root) and of CAs (arcsine) were used (see section Methods).
[a] CAs(−gaps), frequency of chromosomal and chromatid aberrations, excluding gaps; for the other abbreviations used see footnotes of Tables 5 and 7.
* $p < 0.05$, ** $p < 0.01$.

Table 3
Frequency of chromosomal aberrations (CAs) in fillingstation attendants (exposed) and age-matched references (controls)

	Exposed [a]		Controls [b]		p [c]
	No.	%	No.	%	
Gaps	37	0.87	55	1.15	0.161
Chromatid-type aberrations	53	1.25	43	0.95	
Chromatid breaks	2	0.05	1	0.02	0.234
Chromatid exchanges					
Chromosome-type aberrations	21	0.49	16	0.35	
Isochromatid breaks and double minutes	8	0.19	6	0.13	
Dicentrics	84	1.97	66	1.46	0.066
Total (−gaps)					

[a] Exposed = 23 subjects, 4255 metaphases.
[b] Controls = 24 subjects, 4509 metaphases.
[c] p = statistical significance of the difference exposed versus controls, estimated by χ^2 test.

blood lead concentrations in exposed versus control subjects showed significantly higher levels in service station employees ($p = 0.036$, Table 1), in agreement with their expected greater exposure to vehicle exhausts.

A correlation matrix for the biological end-points (SCEs, MN, CAs, PRI, NDIs and age of the study subjects) is shown in Table 2. The correlation coefficients highlight a significant positive relationship between age of the subjects studied and both SCEs and MN rates in peripheral lymphocytes. No correlation was found between rates of CAs and age. The individual frequencies of micronuclei in B and T lymphocytes were strictly intercorrelated, and dis-

Table 4
Distribution of cells with chromosomal aberrations (gaps excluded) in the studied subjects in relation with blood lead level and average yearly exposure to benzene

	CAs			RR [a]	(95% CI [b])	RR [c]	(95% CI [b])
	0	≥ 1	Total				
Blood lead [d] (μg/dl)						1.00	–
< 7.5	2923	55	2978	1.00	–	1.19	0.81–1.76
7.5–9.9	2672	59	2731	1.17	0.81–1.70	0.63	0.40–0.99
≥ 10.0	2822	33	2855	0.62	0.40–0.96		
Total	8417	147	8564				
Benzene exposure [e]						1.00	–
None	4443	66	4509	1.00	–	1.21	0.80–1.82
Low	2019	36	2055	1.20	0.80–1.81	1.67	1.12–2.48
High	1807	43	1850	1.60	1.09–2.36		
Total	8269	145	8414				
χ^2 trend =	5.57	$p = 0.0183$					

[a] RR = crude prevalence odd ratio.
[b] 95% CI = 95% confidence limits of the odd ratio estimate.
[c] RR = prevalence odd ratio adjusted for age and proliferation index score by logistic regression analysis.
[d] The cut-off points correspond to the 33° and 66° percentile of the overall distribution in the studied population.
[e] Occupational exposure: none, references; low, fillingstation attendants with ≤ 0.2 mg/m³ (8-h TWA, average yearly exposure); high, fillingstation attendants with > 0.2 mg/m³.

Table 5
Sister chromatid exchanges (SCEs) and proliferation index (PRI) in peripheral lymphocytes of fillingstation attendants (exposed) and age-matched references (controls)

	Exposed			Controls			p [a]
	No.	Mean (SE)	Range	No.	Mean (SE)	Range	
SCEs	22	4.73 (0.15)	3.24–6.29	23	4.48 (0.11)	3.45–5.47	0.181
PRI	21	1.81 (0.03)	1.47–2.02	22	1.88 (0.04)	1.59–2.25	0.106

SCE = frequencies of sister chromatid exchanges per cell, calculated on 87 scored cells per subject on average.
PRI = proliferation index.
[a] p = statistical significance of the difference exposed versus controls, estimated by one-way analysis of variance.

played an inverse relationship with nuclear division indexes. NDI and PRI of PHA-stimulated cultures, both of which measure the progression of cells in the cell cycle in the same cell population, were also significantly correlated.

The analysis of structural chromosome aberrations in PHA-stimulated lymphocytes demonstrated a slight excess of damage in the exposed population with respect to 'unexposed' controls. In both populations, chromatid breaks were the most common aberration found, in agreement with published results for benzene and petroleum exposed workers (Yardley-Jones et al., 1990, Sobti and Bhardwaj, 1993, Tompa et al., 1994) (Table 3). The difference between total aberration rates in exposed and control subjects, although statistically not significant ($p = 0.066$), suggested a possible excess of clastogenic damages in fuel exposed workers. In order to analyze the incidence of structural aberrations with respect to benzene exposure, the exposed population was dichotomized around the 50° percentile and the unexposed subjects were taken as referents. Even though measurements of benzene exposure for the control population were not available, the average benzene exposure of referents may be tentatively estimated from the local environmental benzene levels. A five-year monitoring recently carried out in Rome indicated 0.02 mg/m^3 as the average yearly benzene concentration (Fuselli, 1992). Other significant sources of benzene exposure, such as smoking or professional exposures to fuels, were ruled out at the enrolment phase of the study. Therefore, on the basis of the above figures, the benzene exposure experienced by the 'unexposed' referents should be well below the lowest concentrations measured among servicestation employees (0.1 mg/m^3, see Table 1). The analysis of

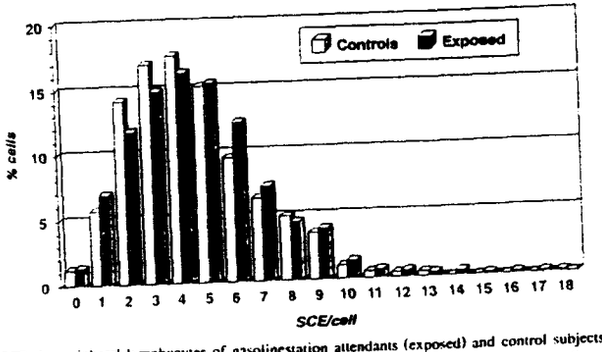

Fig. 1. Distribution of SCEs in peripheral lymphocytes of gasolinestation attendants (exposed) and control subjects. Histograms obtained from the pooled data of all exposed and control individuals. As abscissa, the number of SCEs/cell. As ordinate, the number of cells presenting a given number of SCEs.

the prevalence rate ratios (Table 4) demonstrated a significant overall upward trend in percentages of cells with CAs at increasing levels of benzene exposure (χ^2 for trend = 5.6, $p = 0.02$). Furthermore, fillingstation attendants exposed to average yearly benzene levels above 0.2 mg/m^3 showed a statistically significant increase of CAs in comparison with controls (RR = 1.7, 95% CI = 1.1–2.5). No positive correlation was found between CAs and blood lead levels (Table 4).

The analysis of SCEs demonstrated a significant correlation with the age (Table 2) and a slight excess of exchanges in the exposed individuals (Table 5). The difference of the average individual SCE rates in exposed and control populations (4.73 ± 0.15 versus 4.48 ± 0.11 SCEs/cell) did not attain statistical significance ($p = 0.181$). However, the comparison of the distribution of SCEs per cell in the exposed and control populations indicated a possible excess of cells with high number of exchanges in the exposed population (Fig. 1), which was further investigated. To this aim, the frequency of cells with ≥ 6 exchanges (corresponding to the 75° percentile of the overall distribution) was evaluated in both study groups. This cut-off was preferred to the frequently used 95° percentile to define high-frequency cells (HFC), in order to handle a larger number of cases and to increase the statistical power of the analyses. The results obtained demonstrate a significant excess of HFCs among exposed individuals (Table 6). Within the whole studied population, a strict correlation ($p < 0.0001$) was observed between the frequency of HFCs and lead levels, even when taking into account the effect of age and PRI on SCE rates by logistic regression analysis (Table 6). No clear association was found between HFCs and benzene

Table 6
Distribution of cells with high SCE frequency (> 75° percentile of the overall distribution) in the studied subjects in relation with exposure status, blood lead level, and average yearly exposure to benzene

Exposure	SCE/cell			RR [a]	(95% CI [b])	RR [c]	(95% CI [b])
	< 6	≥ 6	Total				
Controls	1518	577	2095	1.00	–	1.00	–
Exposed	1227	575	1802	1.23	(1.07–1.42)	1.30	(1.12–1.51)
Total	2745	1152	3897				
Blood lead [d] (μg/dl)							
< 7.5	1052	356	1408	1.00	–	1.00	–
7.5–9.9	833	347	1180	1.23	(1.03–1.46)	1.08	(0.90–1.30)
≥ 10.0	789	420	1209	1.57	(1.33–1.86)	1.40	(1.17–1.67)
Total	2674	1123	3797				
χ^2 trend =	27.79	$p < 0.0001$					
Benzene exposure [e]							
None	1518	577	2095	1.00	–	1.00	–
Low	542	300	842	1.46	(1.23–1.73)	1.56	(1.30–1.86)
High	618	242	860	1.03	(0.86–1.23)	1.03	(0.85–1.24)
Total	2678	1119	3797				
χ^2 trend =	1.58	$p = 0.2087$					

[a] RR = crude prevalence odd ratio.
[b] 95% CI = 95% confidence limits of the odd ratio estimate.
[c] RR = prevalence odd ratio adjusted for age and proliferation index score by logistic regression analysis.
[d] The cutoff points correspond to the 33° and 66° percentile of the overall distribution in the studied population.
[e] Occupational exposure: none, references; low, fillingstation attendants with ≤ 0.2 mg/m^3 (8-h TWA, average yearly exposure); high, fillingstation attendants with > 0.2 mg/m^3.

Table 7
Micronuclei (MN) and nuclear division index (NDI) in phytohemagglutinin- and pokeweed-stimulated peripheral lymphocyte cultures of fillingstation attendants (exposed) and age-matched references (controls)

	Exposed			Controls			p [a]
	No.	Mean (SE)	Range	No.	Mean (SE)	Range	
MN-PHA [b]	23	5.40 (0.70)	1.5–15.3	24	5.50 (0.60)	1.5–13.0	0.916
NDI-PHA [c]	23	1.92 (0.08)	1.3–2.6	24	1.93 (0.05)	1.6–2.5	0.928
MN-PKW [d]	12	9.60 (1.50)	2.7–18.0	17	7.7 (0.90)	3.0–17.3	0.264
NDI-PKW [e]	12	1.83 (0.07)	1.4–2.2	17	1.70 (0.04)	1.5–2.0	0.127

[a] p = statistical significance of the difference exposed versus controls, estimated by one-way analysis of variance.
[b] MN-PHA = micronuclei per 1000 binucleated cells, from phytohemagglutinin (PHA)-stimulated cultures; average sample size 2128 scored cells/subject.
[c] NDI-PHA = nuclear division index of PHA stimulated cultures.
[d] MN-PKW = micronuclei per 1000 binucleated cells, from pokeweed (PKW)-stimulated cultures; average sample size 1370 scored cells/subject.
[e] NDI-PKW = nuclear division index of PKW-stimulated cultures.

exposure. Among station attendants, the incidence of HFCs was increased in the group with lower benzene exposures and decreased in workers with the highest exposure levels (Table 6).

Finally, the analysis of micronuclei in cytokinesis-blocked lymphocyte cultures did not show significant increases of micronuclei in the exposed individuals (Table 7). However, in both studied groups a higher frequency of micronuclei was recorded in pokeweed-stimulated cells with respect to phytohemagglutinin-stimulated cultures. This result is in agreement with previous studies (Högstedt et al., 1988b; Högstedt et al., 1991; Slavutsky and Knuutila, 1989) which reported higher rates of micronucleated cells in cultures stimulated with pokeweed. In agreement with literature data (see e.g. Hando et al., 1994), micronuclei were significantly related to the age (and to the NDI) in both populations. NDIs were similar in exposed and controls, but significantly greater ($p < 0.02$, t test) in PHA-stimulated cultures with respect to PKW-stimulated ones, indicating a faster proliferation of T lymphocytes in vitro.

4. Discussion

A preliminary survey of the mortality of fillingstation attendants from the Latium region highlighted possible increased risks of different neoplasms, concentrated among workers employed in small stations characterized by high sales of fuel per employee (Lagorio et al., 1994). In this study, we have undertaken a detailed cytogenetic investigation on a subgroup of the above cohort, with the aim of investigating the possible relationship between personal exposure to aromatic hydrocarbons and some early indicators of genetic effects.

Published data on occupationally exposed individuals settle to about 1–10 ppm as the lowest benzene concentration able to produce cytogenetic damages in peripheral lymphocytes detectable with standard protocol and techniques (Dean, 1985, Tompa et al., 1994). According to this finding, the results reported in this paper indicate that the low occupational exposure to benzene (1.58 mg/m^3, or 0.5 ppm, on average) experienced by the service-station attendants enrolled in the study does not result in an overt excess of chromosomal damages in comparison with age-paired unexposed individuals. However, a more detailed analysis of the data highlighted a significant correlation of CA frequencies with intensity of benzene exposure, suggesting that also low-level exposure to petroleum fuels may result in an increase of genetic damages in peripheral lymphocytes of exposed people. It is conceivable that the greater analytical power of the newly developed molecular cytogenetic techniques may help clarify this point.

In agreement with other investigations (Sarto et al., 1985), SCE frequencies (average value per individual) were significantly related with age, pointing to the requirement for controlling this confounding factor in biomonitoring studies. The results obtained also show a slight increase of SCE rates in fillingstation attendants. The simple comparison of the average values in exposed and control populations does not reveal significant differences. This approach, however, may be inadequate in population studies, because it does not take into account the possible existence of cellular subpopulations with greater sensitivity to genotoxic agents. To account for this possibility, several statistical approaches have been developed which consider the distribution of SCEs in the whole cell populations investigated (exposed and controls) (Carrano and Moore, 1982). We have found an excess prevalence of cells with high number of SCEs (above the 75° percentile) in service-station employees. The frequency of HFCs in the exposed individuals showed a complex relationship with benzene exposure, with lower values at the highest dose. Interestingly, some published data also report a decrease in the frequency of SCEs with high exposure to benzene (Sarto et al., 1984, Watanabe et al., 1980), possibly related to a persistent adverse effect on DNA replication (Dean, 1985; Watanabe et al., 1980). Furthermore, SCE frequencies showed a significant upward trend with increasing blood lead concentrations. Literature data on the genetic effects of occupational or environmental exposure to lead mainly reported structural chromosomal aberrations rather than SCEs in peripheral lymphocytes of subjects with blood lead levels above 20 μg/dl (Winder and Bonin, 1993). Therefore, a direct effect of this metal on the SCE rates is most unlikely. However, blood lead can be regarded also as an individual marker of exposure to vehicle exhausts. In this framework, our findings may point to the possibility of genotoxic effects resulting from exposure to air pollutants, detectable even among residentially exposed individuals. This result adds to previous investigations which demonstrate an increased frequency of genetic damages in relation to the environmental exposure to genotoxic pollutants (Motykiewicz et al., 1992; Perera et al., 1992).

In conclusion, the findings of this study are compatible with a detectable genotoxic effect from exposure to gasoline vapours and vehicle exhausts. Taking into account the prognostic significance of some cytogenetic end-points for cancer development (Hagmar et al., 1994; Högstedt et al., 1988a; Bonassi et al., 1995), it can be envisaged that the cytogenetic surveillance of populations living in polluted areas or professionally exposed to petroleum fuels may provide relevant information for the evaluation of long-term risks.

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