PRINT: ISSN 0972-3757 ONLINE: 2456-6360
IJHG 1(2): 145-150 (2001)
DOI: 10.31901/24566330.2001/01.02.13

Cytogenetical Damage in Petrol Pump Workers

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KEY WORDS Mitotic Index. Chromosomal Aberration. Sister Chromatid Exchange. Lymphocyte. Petrol Pump Workers.

ABSTRACT Cytogenetic studies were carried out on 37 petrol pump workers exposed to benzene, carbon monoxide, chlorobenzene and nitric oxide and 36 control individuals with no known exposure at work. The mean MI in exposed workers (7.86) was found significantly higher than in controls (4.60). CA were observed to be elevated in the exposed group. Frequency of SCE in exposed workers (6.49) was significantly higher than controls (5.62). Exposed group showed about two fold increase in the frequency of SA (13.4) as compared with the controls (7.91). It may be concluded that the petrol pump workers are at a risk to cytogenetic damage at their work places.

INTRODUCTION

Petrochemical is a wide term including a large number of organic chemicals. Both diesel and gasoline engine exhausts are known to contain, in either the particulate or the vapor phase, a variety of mutagenic and carcinogenic agents (IARC 1989). Benzene and toluene are major monocyclic hydrocarbons present in the refinery oils. Petroleum refining products are largely constituted by straight chain alkanes which are a continued source of pollution in various occupational settings. These are found in emission of the diesel operated light duty vehicles. Higher chain alkanes exhibit co-mutagenic and co-carcinogenic properties (Lankas et al. 1978). Nitrated polycyclic aromatic hydrocarbons have also been detected in diesel exhaust emission (Pitts et al. 1982), a predominant mutagen in a sample of the diesel engine exhaust is 1-Nitropyrene (NP) (Rosenkranz 1982). In addition to being a potent bacterial mutagen (Mermelstein et al. 1981), NP is also mutagenic in mammalian cells (Li and Dutcher 1983) and carcinogenic in male rats, both at the site of injection (Ohgaki et al. 1982) and in the mammary tissue (Hirose et al. 1984). NP has been shown to be a lung carcinogen in A/J mice, following intraperitoneal administration (El-Bayoumy et al. 1984).

MATERIAL AND METHODS

Thirty seven petrochemical workers exposed to Benzene 40 mg/m^3, Carbon monoxide 90 mg/m^3, Chlorobenzene 350 mg/m^3 and Nitric Oxide 60 mg/m^3 in the ambient air of petrol pumps and 36 control individuals matched with respect to age, sex and smoking habit and with no known chemical exposure at work and breathing air containing 1 mg/m^3B(a)P_1 1.5 mg/m^3 benzene and 0.02 mg/m^3 H_2S. constituted materials for the present investigation. Only those individuals who had not been on drug treatment or had recent viral infection or X-rays during three months before sampling were selected.

Blood samples were taken using heparinised syringes. Short term lymphocyte cultures were set up within 4 h of sampling following the method of Moorhead et al. (1960). Details of the methods for lymphocyte culture, its harvesting, preparation of slides and estimation of various parameters viz. mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchanges (SCE) and satellite associations (SA) have been described elsewhere (Yadav and Kaushik 1996).

An epidemiological survey was conducted using a proforma especially designed for this purpose. The data obtained was statistically analysed by applying the students 't' test (Colton 1980).

RESULTS

The data obtained during the present investigations have been given in tables 1-6. It is evident from table 1 that mean MI in exposed workers (7.86) was significantly higher ($P<0.1$) than in matched controls (4.60). It was maximum in workers with exposure period of more than five years. Thereafter, there was a gradual decline

Table 1: Mitotic Index (MI) in workers exposed to petrolpump fumes and controls

Duration of exposure in years	*No. of individuals*	*No. of cells screened (n1)*	*No. of metaphases (n2)*	*MI ± SD (n2/n1x100)*
Control	36	176364	8130	4.60 ± 0.65
Exposed	37	180500	14188	7.86* ± 0.65
0-5	12	29952	2642	8.82 ± 0.62
6-10	19	92948	7353	7.91 ± 0.32
11-above	6	57600	4193	7.28 ± 0.13

P < 0.01

of MI with duration of exposure.

Chromosomal aberrations were found to be elevated in the exposed group. Frequencies of aberrations in benzene exposed workers are presented in table 2. Aberrations of all types viz., dicentrics, rings, acentric fragments, translocations, chromatid gaps, breaks and isochromatid exchanges, were significantly higher in exposed sample than the controls. Frequency of total CA was 2.54% in exposed workers, whereas the background frequency was 0.72%, the difference being statistically significant (P<0.05). The total chromatid type aberrations were more than total chromosome type aberrations in both exposed workers and controls.

Frequency of SCE (Table 3) in exposed workers (6.49) was significantly higher than controls (5.62). Exposed smokers showed higher SCE frequency (7.03) than controls (6.01). Similarly frequency of SCE was significantly more in exposed non- smokers (6.29) than non-smokers among control sample (5.49). Frequencies of SCE in exposed workers with duration of exposure are presented in table 4. It was highest (7.39) in exposed workers with an exposure period of 11-20 yrs. With duration of exposure there is increase in value of mean SCE.

Frequencies of SA in both exposed and control groups are given in table 5. Exposed group showed about two fold increase (13.4) as compared to controls (7.91). The difference was statistically significant (P<0.05). DG type associations were found to be maximum (5.94), while 3 D type associations showed the lowest occurrence (0.24). Almost all the petrochemical workers complained of irritation in eyes and annoyed behaviour. Those who joined freshly suffered some gastrointestinal disorders, periodic allergic colds and bronchial disorders.

Table 2: Frequency of chromosomal aberrations in workers exposed to petrolpump fumes and in controls

Group	*Control*	*Exposed*	*'t'*
No. of Persons	36	37	
No. of metaphases	3590	3700	
Chromosome Type Aberrations			
Dicentrics	1 (0.02)	1 (0.02)	
Rings	0	0	
Acentric fragments	1 (0.02)	3 (0.08)	
Total	2 (0.05)	4 (0.10)	P < 0.05
Chromatid Type Aberrations			
Gaps	42 (1.16)	96 (2.59)	
Breaks	24 (0.6)	89 (2.40)	
Isochomatid Aberrations	0	1 (0.02)	
Total	24 (0.06)	90 (2.43)	
Total CAs [except gaps]	26 (0.72)	94 (2.54)	P < 0.01

Figure in brackets indicate aberration per hundred metaphases

Table 3: Frequency of SCEs in workers exposed to petrolpump fumes and in controls

Parameters	*n*	*Exposed group mean SCE ± SEM*	*n*	*Control group mean SCE ± SEM*	*`t'*
Total SCEs	37	6.49 ± 0.09	36	5.62 ± 0.09	P < 0.01 (t= 3.32)
Smokers	10	7.03 ± 0.16	9	6.01 ± 0.16	P < 0.05
Non-smokers	27	6.29 ± 0.08	27	5.49 ± 0.09	P < 0.01

n= No. of individuals

Table 4: Frequency of SCEs in workers with duration of exposure to petrolpump fumes

Duration of expsoure (Yrs)	*No. of individuals*	*Mean SCEs ± SD*	*No of smokers*
0-5	12	5.86 ± 0.19	8
6-10	19	6.60 ± 0.31	2
11-20	6	7.39 ± 0.37	0

Table 5: Frequency of satellite association patterns observed in petrol pump workers and controls

n	*Total*	*Type of Satellite Association*								*Association*
	cell scanned	*DD*	*DG*	*GG*	*2DG*	*2GD*	*2G-2D*	*3D*	*Total*	*per cell + S.E.M.*
E-37	3700	90 (2.43)	220 (5.94)	100 (2.7)	30 (0.81)	31 (0.83)	19 (0.51)	9 (0.24)	499	13.4 ± 0.75
C-36	3600	33 (0.91)	142 (3.94)	34 (0.94)	31 (0.86)	19 (0.52)	20 (0.55)	7 (0.19)	285	7.91 ± 0.44

Where a = Significant ($p<0.05$)
E = Exposed
C = Control
Figures in brackets indicate satellite associations per 100 metaphases

DISCUSSION

Mitotic index is known to respond to various pollutants present in petrol-pump fumes. During the present investigation, a significant increase in MI values of workers exposed to petrol- pump fumes as compared to controls was observed. The trend continued for exposure up to 5 years. Thereafter the MI declined. This could be due to the fact that accumulated benzene and other petrol-pump fumes in exposed individuals might have started destroying lymphocytes resulting in decrease in MI. Motykiewicz et al. (1991) reported that besides having mutagenic activity, organic extracts of air borne pollutants from Silesia region were found to influence mitotic division *in vitro* as indicated by mitotic arrest.

Au et al. (1991) observed that in mice, chromosome aberration frequencies were lower in high dose group of a chemical mixture of benzene, chloroprene, epichlorohydrin and xylene than those in medium and low dose groups. Yardley et al. (1990) and Nise et al. (1991) found increase in chromosomal aberrations (particularly chromatid deletion and gaps) in metaphase chromosome preparations of human population exposed to benzene. Tompa et al. (1994) also found that benzene is capable of inducing chromosomal aberrations, thus providing evidence for clastogenic effects of benzene. Chromosomal damage has been reported in animals exposed to diesel exhaust. A clear dose response relationship for induction of chromosome aberrations was noted in the chinese hamster ovary cells (CHO) exposed to a potent diesel exhaust particle extract (Li et al. 1983). Mark-Vendel et al. (1981) and Hogstedt et al. (1981), observed a higher frequency of structural chromosome aberrations in individuals exposed to petroleum vapors. Zhou et al. (1986) also observed a significant increase in chromosome aberrations in petrochemical workers. Hasegawa et al. (1988) examined the effects of diesel exhaust from engine in V79 cells. They observed that diesel exhaust particulates from the light duty engines induced a significant number of chromosome aberrations and sister chromatid exchanges as compared to the heavy duty engine exhaust. Bauchinger et al. (1972) reported no increase in frequency of the chromosome aberrations in their cytogenetic monitoring of the policemen exposed to the vehicular exhaust. But the studies of Chandersekaran and Lakshamanperumalswamy (1988) and Wagida and Abdel (1988) did show that there was a significant increase in the frequencies of both the chromosome aberrations and sister chromatid exchanges in traffic policemen exposed to the vehicular exhaust. Batish (1993) reported that mean frequency of gaps was maximum in personnel exposed to petrol exhaust, whereas mean frequency of breaks was maximum in persons exposed to diesel exhaust. However, in the present study some workers were exposed to diesel as well as petrol fumes. Gaps, breaks, acentric fragments, translocations, rings, dicentrics and isochromatid exchanges all were found to be maximum in exposed workers as compared to controls.

Edward and Priestly (1993) assessed occupational exposure in petroleum workers employed in suburban petroleum retail outlets and found that there was significant increase of sister chromatid exchange with combined exposure to petrol and cigarette smoking, but not with

either factor alone. Berale et al. (1993) undertook a multidisciplinary study on population exposed to vehicle exhaust. Results showed probable difference between exposed and control individual in terms of SCE frequencies.

Khalil et al. (1994) found a nonsignificant elevation of SCE frequency in workers exposed to petrochemicals and reported that SCE frequencies were influenced neither by age nor by smoking. However, Yadav and Thakur (2000a,b) reported that Hookah and Bidi smoke increase all the parameters.

Perera (1982) reported that exposure of the Syrian hamsters to a high level of diesel exhaust particulates for several months increased the frequency of sister chromatid exchanges in primary cultures of lungs from the exposed animals. Intratracheal instillation of diesel exhaust particulates also caused more sister chromatid exchanges. Li et al. (1983) observed an increase in the sister chromatid exchange frequency in the Chinese hamster ovary cells exposed in vitro to diesel exhaust particulates.

Koracic et al. (1995) found a significant increase in dicentric chromosomes and SCE in persons exposed to less than 50mg/m^3 of benzene. In the present study frequency of SCE in persons exposed to benzene and other pollutants showed higher SCE frequency than control smokers and control non-smokers respectively. The induction of chromosomal aberrations and SCE cannot be attributed solely to benzene and some aromatic hydrocarbons. Because, the attendant absorbs both the fumes emitted by the fuel which contain genotoxic substances such as benzene, and the engine emission products of the vehicles constantly stopping to refuel at the station. Emissions resulting from the internal combustion of automobile engines consist of thousands of separate compounds. These emission products also present cytotoxic and genotoxic properties, as demonstrated by Hadnagy and Seemayer (1988). According to Santos-Mello and Cavalcante (1992), alcohol is added to gasoline in Brazil, with a consequent greater vapor pressure of the resulting mixture (gasoline + ethanol) than of alcohol or gasoline used individually (Furey and Jackson 1977; Mukerjee 1990). With this type of fuel there is reduction in hydrocarbon and carbon monoxide emission in comparison to gasoline. Santos-Mello and Cavalcante (1992) however, noted an increase in aldehyde production, particularly formaldehyde . Some of these aldehydes may also have a genotoxic effect (Auerbach et al. 1977; Restani and Galli 1991) and contribute to the increased production of carcinogenicity (IARC, 1987).

Trezepiziur et al. (1995) observed that chromosomes 21-22, which are smaller than chromosomes 13-15, possess more extended nucleolar organizer regions and consequently acrocentric chromosomes 21-22 enter into associations more frequently than chromosomes of group 13-15. Nilsson et al. (1975) also reported increased associated tendency of chromosome 14 and 21 in culture of control individuals. Yadav and Kaushik (1996, 1977) found a two-fold increase in the frequency of SA in SO_2 and NH_3 exposed workers respectively and concluded that this significant difference in frequency of SA may lead to increased probability of chromosomal translocations. During the present investigation exposed group showed about two-fold increase in the frequency of SA as compared to controls. DG type association was found to be maximum while 3D type association showed the lowest frequency (Table 5). This seems to be a necessary corollary to the observations of Trezepiziur et al. (1995) and Nilsson et al. (1975) (vide supra).

Frequencies of aneuploid cells are shown in table 6. Although exposed group showed slightly higher percentage (2.57%) of aneuploid cells than the control group (2.25%), yet the data is not statistically significant. This is in line with the observation of Santos-Mello and Cavalcante (1992), who reported aneuploidy in persons exposed to automotive vehicle emission. Their results were also not significant.

Table 6: Aneuploidy among petropump workers and controls

Group	*n*	*TC*	*Aneuploid Cells*
Control	36	3600	80 (2.25%)
Exposed	37	3699	95 (2.57%)

ns= nonsignificant
TC= Total No. of Cell screened
Figure in brackets indicate aneuploidy per 100 metaphases

Mulvihill (1975) suggested that an increase in the frequency of chromosome aberrations is associated with an increased risk of developing malignant processes, including leukemias. Awa (1983) postulated a positive correlation between the risk of cancer and genetic disease in the population and the levels of cytogenetic effects. Studies by Au et al. (1990) strengthened the hypothesis that chromosome aberrations are initial events in carcinogenesis. Thus, an elevation in the frequency of chromosome aberrations may indicate exposure to a given agent at an undesirable level of health risk. Considering, significant increase in CA and SCE in workers exposed to petrol-pump fumes during the present investigations, it may be concluded that these workers represent a risk group that should be carefully monitored.

ACKNOWLEDGEMENTS

We are thankful to the subjects who voluntarily provided blood samples, to the authorities of Kurukshetra University for providing laboratory facilities, to U.G.C., New Delhi for awarding a fellowship to NS, to Dr. A.S. Yadav, and A.K. Chhillar for varied help.

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