Mutagenesis vol. 19 no. 1 pp. 35–41, 2004 DOI: 10.1093/mutage/geh001

Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the Comet and micronucleus assays

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Chromium (Cr) and nickel (Ni) are widely used industrial chemicals. Welders in India are inclined to possible occupational Cr and Ni exposure. The carcinogenic potential of metals is a major issue in defining human health risk from exposure. Hence, in the present investigation, 102 welders and an equal number of control subjects were monitored for DNA damage in blood leucocytes utilizing the Comet assay. The two groups had similar mean ages and smoking prevalences. A few subjects were randomly selected for estimation of Cr and Ni content in whole blood by inductively coupled plasma mass spectrometry. The Comet assay was carried out to quantify basal DNA damage. The mean comet tail length was used to measure DNA damage. Welders had higher Cr and Ni content when compared with controls (Cr, 151.65 versus 17.86 μg/l; Ni 132.39 versus 16.91 μg/l; $P < 0.001$). The results indicated that the welders had a larger mean comet tail length than that of the controls (mean ± SD, 23.05 ± 3.86 versus 8.94 ± 3.16; $P < 0.001$). In addition, the micronucleus test on buccal epithelial cells was carried out in a few randomly selected subjects. Welders showed a significant increase in micronucleated cells compared with controls (1.30 versus 0.32; $P < 0.001$). Analysis of variance revealed that occupational exposure ($P < 0.05$) had a significant effect on DNA mean tail length, whereas smoking and age had no significant effect on DNA damage. The current study suggested that chronic occupational exposure to Cr and Ni during welding could lead to increased levels of DNA damage.

Introduction

Metals are stable and persistent environmental contaminants. Many metals have the potential to cause genetic alterations in the target tissues of exposed humans. Such alterations, if they occur in tumour suppressor genes, may lead to the development of cancer in the target organs (Keshava and Ong, 1999). The heaviest metal exposure occurs in the workplace among occupationally exposed groups. A person spends, on average, one-third of his life at his workplace and therefore the environment in which he works can be a major factor in determining health. Stainless steel welding generates fumes which contain oxides of chromium (Cr) and nickel (Ni), together with a number of other metal oxides. Studies have shown that welding fumes from stainless steel welding are mutagenic (Hedenstedt *et al*., 1977; Maxild *et al*., 1978). Similarly, investigations of welding fumes revealed an increase in the frequency of sister chromatid exchanges (SCEs) and chromosome aberrations (CAs) in cultured Chinese hamster cells (Koshi 1979; Baker *et al*., 1986). However, evidence for the carcinogenicity of welding fumes and gases in humans was evaluated by the International Agency for Research on Cancer (IARC) as limited and in animals as inadequate. Thus welding fumes and gases have been classified by IARC as possibly carcinogenic to humans (International Agency for Research on Cancer, 1990).

Cr and Cr compounds have been tested for genotoxicity in a variety of short-term tests using different end-points (De Flora *et al*., 1990; Manning *et al*., 1994; Stearns *et al*., 2002; Quievryn *et al*., 2003). Moreover, there are reports on positive genotoxic effects in populations exposed to Cr (Sarto *et al*., 1982; Choi *et al*., 1987; Vaglenov *et al*., 1999; Wu *et al*., 2000; Benova *et al*., 2002) as well as negative findings (Nagaya, 1986; Nagaya *et al*., 1989, 1991; Sbrana *et al*., 1990; Gao *et al*., 1994; Zhitkovich *et al*., 1996; Huvinen *et al*., 2002). Workers occupationally exposed to Cr are considered to be at an elevated risk for developing cancer (Langard, 1990; Rosenman and Stanbury, 1996; De Flora, 2000; Gibb *et al*., 2000). However, the bulk of the literature data indicated that only Cr(VI) may pose a carcinogenic risk, and only when inhaled at very high doses in the three occupational settings indicated by the International Agency for Research on Cancer (1990).

The genotoxic effects of Ni and Ni compounds have been demonstrated in various short-term *in vitro* (Hong *et al*., 1997; Lei *et al*., 2001; Blasiak *et al*., 2002) and *in vivo* (Dhir *et al*., 1991; Saplokoglu *et al*., 1997) tests. Investigations that reported the genotoxic effect of Ni in occupationally exposed humans have revealed both positive (Senft *et al*., 1992; Perminova *et al*., 1997, 2001a,b,c) and negative results (Kiilunen *et al*., 1997). In addition, epidemiological studies have implicated Ni in the causation of human cancers (Doll *et al*., 1977; International Agency for Research on Cancer, 1990; Costa *et al*., 2001).

Investigations that have examined genotoxicity in workers occupationally exposed to both Cr and Ni are few (Deng *et al*., 1988; Gennart *et al*., 1993; Lai *et al*., 1998; Burgaz *et al*., 2002). Studies on the evaluation of genetic damage in welders exposed to Cr and Ni are scanty and have yielded conflicting results (Husgafvel-Pursiainen *et al*., 1982; Littorin *et al*., 1983; Koshi, 1984; Elias *et al*., 1989; Popp *et al*., 1991; Knudsen *et al*., 1992; Costa *et al*., 1993; Jelmert *et al*., 1994; Werfel *et al*., 1998). The lung cancer mortality among stainless steel welders was found to be higher than the general population in a large European cohort (International Agency for Research on Cancer, 1990). However, some studies have found no association between welding and the subsequent development of cancer (Simonato *et al*., 1991; Moulin *et al*., 1997).

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In spite of the genotoxicity of Ni and Cr in some populations, some of the biomonitoring studies carried out have shown negative results. The inconsistent genotoxicity data could be due to differences in levels of exposure, in the protective measures employed or in the end-points utilized. Therefore, results from one investigation in a specific occupational setting cannot be used to judge the genotoxic potential in another occupational setting. Hence, there is a need to evaluate different populations and to analyse different genotoxic parameters.

Early identification of hazards is crucial to reduce exposure and carcinogenic risk. A survey of the literature has revealed that no investigation has been conducted on Indian welders using the Comet assay and the micronucleus test (MN test). Therefore, the aim of our study was to investigate the genotoxic effects associated with occupational exposure to Cr and Ni by analysing DNA damage in blood leukocytes of welders in India, using the alkaline single cell gel electrophoresis (SCGE) assay, also known as the Comet assay. The SCGE assay has been found to be a very sensitive method for measuring DNA damage (Singh *et al*., 1988; Tice *et al*., 1995). It is a quick, reliable and fairly inexpensive way of measuring DNA damage. It has a further advantage that the observations are made at the single cell level. Moreover, it is an invaluable tool for investigating DNA damage in human populations (Collins *et al*., 1997). To substantiate our results and to provide a cytogenetic parameter, the MN test was also carried out. This test allows the detection of both clastogenic and aneugenic agents (Schmid 1975) and it has been adopted for exfoliated cells (Stich *et al*., 1982). The use of the MN test in exfoliated cells has substantially increased as it is considered a useful biomarker of genotoxic effects in populations exposed to genotoxicants (Salama *et al*., 1999).

The occupational load of each welder may, however, result from increased uptake due to an increased workload. Biomonitoring of concentrations of Cr and Ni in blood is therefore used in exposure assessment in several studies of steel welding (Angerer and Lehnert 1990). Hence, in the current investigation Cr and Ni levels in whole blood were quantified using inductively coupled plasma mass spectrometry (ICP-MS). Several reports have appeared in the literature on the use of ICP-MS in the analysis of body fluids, such as blood (Schutz *et al*., 1996; Bergdahl *et al*., 1997). The influence of confounding factors like age, smoking, duration of exposure and metal content on the differences in DNA damage was also analysed.

Materials and methods

Subjects

The study involved 204 male subjects divided into two groups. The first group consisted of 102 welders employed in welding plants located at Hyderabad, India. The welders had varying durations of exposure (1–24 years) and they were in the age group 18–50 years. All the welders were engaged in shielded manual metal arc welding. Welders were working with consumable stainless steel electrodes usually containing ~20% chromium with 10% nickel. The second group, comprising 102 subjects, was the control group. It was selected from the general population with no history of occupational exposure to welding fumes or any known physical or chemical agent in the workplace, but belonged to the same age group and socio-economic status as the welders. The selection criteria for the subjects were based on a questionnaire. The questionnaire was intended to elicit information on the subject's age, smoking habits, alcohol consumption, duration of exposure and medicine usage. We ensured that the welders and the controls did not markedly differ from each other except for occupational exposure. We also ensured that all the subjects had not been taking any medicines nor had they been exposed to any kind of radiation for 12 months before sampling. The subjects who smoked >5 cigarettes/day at least for 1 year were considered as smokers in both groups. All subjects were informed of the objective of the study and gave their consent. To avoid possible bias, the samples were coded. The institutional ethical committee approved the research procedures used in this study

Analysis of chromium and nickel content

Fifty-one welders and 49 controls were selected randomly from the total number of subjects for blood sampling for metal analysis. Blood was sampled during the morning hours on Thursday (the 4th day of the working week). For estimating Cr and Ni content, an ultra mass 700 inductively coupled plasma mass spectrometer (Varian, Australia) was utilised (Lutz *et al*., 1991). Whole blood (5 ml) was collected from all the selected subjects by a trained technician using sterilized non-heparinized syringes. The blood was homogenized for 10 min by mechanical shaking. Aliquots (0.5 ml) were diluted 1:9 with a solution of 4.5 ml of deionized water containing ammonia (0.07 M), Triton X-100 (500 mg/l) and Na_2-EDTA (500 mg/l). The sample digests were filtered with Wattman paper several times to obtain a clear solution. The diluted digests were measured directly by IC-PMS and concentrations of Cr and Ni were quantified as micrograms per liter (μg/l).

Comet assay

For the study of DNA damage, blood samples were collected from all the welders and control subjects. A total of 40 μl of blood sample was taken from a finger prick of each subject into a heparinized glass capillary for the Comet assay, which was carried out according to Singh *et al*. (1988) with slight modifications. The samples were transported on ice to the laboratory and were processed within 2 h. Cell viability determined by the trypan blue exclusion technique (Pool Zobel *et al*., 1994) ranged from 90 to 95% (data not shown). An aliquot of 40 μl of whole blood was used to quantitate basal DNA damage. Slides were prepared in duplicate per person. Fully frosted microscopic slides were covered with 140 μl of 0.75% regular melting point agarose (40–42°C). After application of a coverslip the slides were allowed to gel at 4°C for 10 min. Meanwhile, 20 μl of whole blood was then added to 110 μl of 0.5% low melting point agarose (37°C). After carefully removing the coverslips a second layer of 110 μl of sample mixture was pipetted out on the pre-coated slides and allowed to solidify at 4°C for 10 min. The coverslips were removed and a third layer of 110 μl of low melting point agarose was pipetted out on the slides and allowed to gel at 4°C for 10 min. The slides (without coverslips) were immersed in freshly prepared, cold lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris–HCl, pH 10, 1% sodium *N*-lauroyl sarcosinate, 1% Triton X-100 and 10% DMSO, DMSO added just before use) and refrigerated overnight. Slides were then placed in alkaline electrophoresis solution (300 mM NaOH and 1 mM EDTA, pH 13) for 20 min to allow unwinding of the DNA to occur. Electrophoresis was conducted for 25 min at 25 V (0.66 V/cm) adjusted to 300 mA by raising or lowering the buffer level in the tank. Slides were then drained, placed on a tray and washed slowly with three changes of 5 min each of neutralization buffer (0.4 M Tris–HCl, pH 7.5). DNA was precipitated and slides were dehydrated in absolute methanol for 10 min and were left at room temperature to dry. The whole procedure was carried out in dim light to minimize artefactual DNA damage.

All the slides were then stained with 50 μl of ethidium bromide (20 μg/ml) and viewed under a fluorescence microscope. Analysis was performed using a 40× objective with a Leica optiphase microscope equipped with a 515–560 nm excitation filter and a 590 nm barrier filter. A total of 50 individual cells were screened per sample (25 cells from each slide). An undamaged cell resembles an intact nucleus without a tail and a damaged cell has the appearance of a comet. The length of the DNA migrated in the comet tail, which is an estimate of DNA damage, was measured using an ocular micrometer. Quantification of the DNA damage for each cell was calculated as: comet tail length (μm) = (maximum total length) – (head diameter).

Micronucleus assay (epithelial cells)

The MN test was carried out on buccal epithelial cells of 58 welders and 53 controls, selected randomly from the total number of subjects, according to the method of Rajeswari *et al*. (2000). Oral buccal cells were obtained by gently scraping both cheeks with a wooden applicator. The cells were then smeared on a pre-cleaned slide, fixed in methanol and stained with 2% Giemsa. A total of 2000 cells per individual were analysed and the percentage of cells with micronuclei was calculated.

Statistical analysis

The samples were coded at the time of preparation and scoring. They were decoded before statistical analysis for comparison. Mean and standard deviation (SD) were calculated for each biomarker. The significance of the differences between control and welder end-point means were analysed using Student's *t*-test, whereas simple and multiple linear regression analyses were performed to assess the association between end-points and the independent

variables. All calculations were performed using the MINITAB Release 11.21 Software package for windows. Mean values and standard deviations were computed for the scores and the statistical significance ($P < 0.05$) of effects (exposure, smoking and age) was determined using analysis of variance (ANOVA).

Results

The distribution of subjects with respect to age, smoking, alcohol consumption and years of exposure is given in Table I. The two groups studied had similar demographic characteristics.

Chromium and nickel concentrations in whole blood

The heavy metals Cr and Ni were estimated in the whole blood of 51 welders and 49 controls by ICP-MS. Welders showed significantly higher Cr and Ni concentrations when compared with controls as analysed by ANOVA (Cr, 151.65 versus 17.86 μg/l; Ni, 132.39 versus 16.91 μg/l; $P < 0.001$).

DNA damage

Basal DNA damage (BDD) was studied in a total of 204 subjects using the Comet assay. The results of DNA damage are given in Tables I–III. In welders a significant increase in DNA mean tail length indicating BDD was observed when compared with controls as analysed by ANOVA (23.05 versus 8.94 μm; $P < 0.001$) (Table II). In welders, no significant difference was observed between smokers and non-smokers or between alcohol drinkers and never drinkers in relation to DNA migration ($P > 0.05$) (Table III). However, a significant difference between smokers and non-smokers among the control group was observed. Similarly, there was a marginally significant ($P = 0.047$) effect of alcohol in the control group. DNA damage was higher in subjects with a longer duration of work ($P < 0.05$) (Table III). Simple and multiple linear regression analyses between the different independent variables showed that the levels of Cr and Ni correlated positively with the DNA damage (Table V).

Micronucleus frequency in buccal cells

The frequency of micronuclei (MN) was studied in 58 welders and in 53 controls. Welders revealed a significant induction of MN when compared with controls (1.30 versus 0.32; $P < 0.001$) (Table II). An age-dependent increase in MN frequency was observed in welders (1.51 versus 0.97; $P = 0.007$) (Table IV). Welders showed an increased MN frequency with an increase in duration of work (1.60 versus 0.82, $P = 0.0001$) (Table IV). A significant correlation was observed between MN induction and chromium levels in welders, although not with nickel content (Table V).

ANOVA between and within the two groups (controls and welders) for the categories DNA damage, micronucleus, chromium and nickel concentrations showed significance ($P = 0.001$).

Discussion

The wide use of welding rods containing Cr and Ni in industrial settings has elicited concern over the safety of workers and surrounding populations. There is no study available on the biomonitoring of Indian welders. The current investigation reports genotoxicity in welders from Hyderabad, India. The health concerns raised by welders were welder's flash, sore eyes, headaches, nose bleeds and discharges from the nose. Most welders expressed concern regarding excessive smoke levels in the workplace and inadequate ventilation. None of the welders wore protective gear.

It is not possible to measure the concentration of hexavalent chromium in biological material because its oxidizing properties mean that it readily reacts with a number of substances present in the human body. In this situation the observation that only hexavalent chromium is able to pass cell membranes is of great value. In this manner chromate ions also enter erythrocytes. They are reduced there and bound to constituents of the cell. In contrast, trivalent chromium ions do not succeed in passing cell membranes (Gray and Sterling, 1950). These differences between tri- and hexavalent chromium ions offer the possibility of obtaining a specific measure of internal chromate exposure by determining the chromium concentration in blood (Lewalter *et al*., 1985; Wiegand *et al*., 1985). This property of chromate ions is especially valuable for the biological monitoring of exposed workers. Indeed, our results indicate that the Cr concentration in blood seems to be a suitable parameter of chromate exposure.

The absorption of Cr and Ni quantified in whole blood samples of welders in the present study was found to be significantly higher than in the control population. Similarly, the average blood and plasma Ni concentration was elevated among electrolysis workers (Hogetveit *et al*., 1978; Kiilunen *et al*., 1997). In this study, welders showed 8- to 9-fold higher Cr levels when compared with controls. Likewise, 6- to 7-fold higher amounts of Cr in whole blood of exposed subjects were reported in comparison with controls (Huang *et al*., 1999). Metal analysis revealed a significant correlation with the frequency of DNA single-strand breaks in the present investigation. Similarly, the alkaline filter elution rate of DNA from lymphocytes revealed a significant relationship between the frequency of DNA single-strand breaks and the concentration of Cr in urine (Popp *et al*., 1991). A recent study indicated that chronic occupational exposure to trivalent Cr can lead to a detectable increase in lymphocyte DNA damage which

Table I. General information of controls and welders

	Controls (n =102)	Welders (n =102)	t, χ^2	P
Age (years) (mean ± SD)	33.52±6.04	35.00±6.13	$t = 1.73$	0.084
Smoking: n (%)				
Yes	57 (55.9%)	67 (65.7%)	$\chi^2 = 2.06$	0.152
No	45 (44.1%)	35 (34.3%)		
Alcohol drinking: n (%)				
Yes	51 (50.0%)	57 (55.9%)	$\chi^2 = 0.708$	0.400
No	51 (50.0%)	45 (44.1%)		

Values were compared by Student's t-test and the χ^2 test.

Table II. Inter-group comparison of mean DNA damage (comet tail length in μm) and micronucleus frequency in controls and welders

Group	Basal DNA damage (μm)			Micronucleus frequency (%)		
	n	Mean ± SD	Range	*n*	Mean ± SD	Range
Control	102	8.94 ± 3.16	4.14–17.10	53	0.32 ± 0.22	0.00–0.80
Welders	102	23.05 ± 3.86[a]	17.24–35.62	58	1.30 ± 0.66[a]	0.12–2.89

[a]$P < 0.001$.

Table III. DNA mean tail length (μm) by smoking habit, alcohol, exposure and age in controls and welders

Parameter	Control			Welders		
	n	Mean ± SD	Range	*n*	Mean ± SD	Range
Smoking						
Yes	57	10.67 ± 2.62	4.80–17.10	67	23.31 ± 4.23	17.24–35.62
No	45	6.77 ± 2.34	4.14–12.42	35	22.56 ± 3.02	18.06–28.80
		$F = 60.53$ $P = 0.0001$			$F = 0.87$ $P = 0.353$	
Alcohol						
Yes	51	9.57 ± 2.89	4.32–16.56	57	23.45 ± 4.10	18.06–35.62
No	51	8.33 ± 3.32	4.14–17.1	45	22.55 ± 3.50	17.24–31.34
		$F = 4.04$ $P = 0.047$			$F = 1.38$ $P = 0.242$	
Years of exposure						
⩾10				58	24.22 ± 4.19	18.06–35.62
<10				44	21.51 ± 2.72	17.24–28.90
					$F = 13.93$ $P = 0.001$	
Age (years)						
⩾35	44	11.11 ± 2.46	5.76–17.1	52	23.71 ± 4.23	18.06–35.62
<35	58	7.31 ± 2.60	4.14–14.94	50	22.37 ± 3.34	17.24–31.34
		$F = 55.79$ $P = 0.0001$			$F = 3.12$ $P = 0.081$	

Table IV. Micronucleus frequency with respect to smoking habit, work duration and age in controls and welders

Parameter	Controls ($n = 53$) (mean ± SD)	Welders ($n = 58$) (mean ± SD)
Smoking		
Yes	0.47 ± 0.22 (23)[a]	1.26 ± 0.69 (38)
No	0.20 ± 0.13 (30)	1.39 ± 0.62 (20)
	$F = 29.73$ $P = 0.0001$	$F = 0.47$ $P = 0.497$
Years of exposure		
⩾10		1.60 ± 0.63 (36)
<10		0.82 ± 0.36 (22)
		$F = 28.12$ $P = 0.0001$
Age (years)		
⩾35	0.48 ± 0.23 (22)	1.51 ± 0.68 (32)
<35	0.21 ± 0.13 (31)	0.97 ± 0.55 (26)
	$F = 28.72$ $P = 0.0001$	$F = 7.72$ $P = 0.007$

[a]Figures in parentheses indicate the number of individuals in each group.

correlates with significant exposure of cells to the metal (Medeiros *et al*., 2003).

Welders were investigated for genotoxic effects in the current study using the Comet assay and the MN test. The Comet assay is increasingly being used to monitor genotoxic effects in occupationally exposed humans (Kassie *et al*., 2000). In the present study, a significant increase in BDD was observed in welders when compared with controls by the Comet assay. These results indicate that the level of exposure in the workplace is sufficiently high and also highlights the sensitivity of the assay used. Studies of welders utilizing the Comet assay are lacking. However, elevated levels of DNA protein cross-links were observed in a study of welders (Costa *et al*., 1993). A statistically significant enhancement in the frequency of protein cross-linking and DNA strand breaks was noticed in the blood lymphocytes of welders (Popp *et al*., 1991). Similarly, welders showed a significantly higher level of DNA single-strand breaks with the alkaline filter elution method (Werfel *et al*., 1998). Our results are in line with these studies.

Smoking did not have a significant effect on DNA damage in the present study. The lack of an influence of smoking on the Comet assay results of this study is supported by a report on lead-exposed workers in which smoking did not significantly affect the Comet assay values (Fracasso *et al*., 2002). In the current study no significant effect of alcohol on DNA damage was observed in welders. The results indicated a significant increase in mean BDD in the welders with increasing duration of work in the polluted environment. In a study of lead-exposed workers, DNA damage increased significantly with increase in years of exposure (Danadevi *et al*., 2003). A significant effect of age on DNA damage was not observed in welders. Many studies have shown that the development of lung cancer in chromate workers usually starts at a relatively old age, but one

Table V. Simple and multiple linear regression analysis of end-points

Welders	r	r^2	R^2	P
DNA mean tail length versus years of exposure	0.322	0.104		0.001
DNA mean tail length versus age	0.197	0.039		0.047
DNA mean tail length versus age and years of exposure			0.108	0.004
Micronucleus versus years of exposure	0.616	0.380		0.0001
Micronucleus versus age	0.438	0.191		0.001
Micronucleus versus age and years of exposure			0.381	0.0001
DNA mean tail length versus chromium content	0.653	0.426		0.001
DNA mean tail length versus nickel content	0.410	0.168		0.004
Micronucleus versus chromium content	0.379	0.143		0.039
Micronucleus versus nickel content	0.046	0.002		0.817
Chromium content versus years of exposure	0.529	0.280		0.0001
Nickel content versus years of exposure	0.344	0.118		0.018

r, simple correlation coefficient; r^2 = simple determination coefficient; R^2 = multiple determination coefficient.

study reported a patient who developed cancer at 37 years of age and who had 15 years of occupational exposure to chromate. Thus the period of exposure to the carcinogen is the factor that should be considered, rather than the age of the worker (Ewis *et al*., 2001).

The MN test in buccal epithelial cells has been increasingly accepted as a reliable biomarker of genotoxicity in occupationally exposed groups (Karahalil *et al*., 1999). The present investigation suggests that welders under their particular conditions of exposure reveal clear evidence of genotoxic activity in buccal epithelial cells when evaluated by the MN test. Previous investigations reporting genotoxic effects in welders using the MN test in epithelial cells are scanty. Benova *et al*. (2002) found double the frequency of buccal MN in Cr platers when compared with control persons. On the other hand, electroplaters showed no significant increase in MN in buccal or nasal mucosa (Sarto *et al*., 1990). Nevertheless, studies on welders with other genetic end-points have been reported. A significant increase in CAs in peripheral blood lymphocytes of welders was revealed by a few studies (Koshi *et al*., 1984; Elias *et al*., 1989; Knudsen *et al*., 1992; Jelmert *et al*., 1994). Likewise, investigations have shown an enhancement in the frequencies of SCEs in lymphocytes of welders (Koshi *et al*., 1984; Werfel *et al*., 1998). The above reports are consistent with our results. In contrast, a study has demonstrated no significant difference in frequency of CAs and SCEs in peripheral blood lymphocytes of welders (Husgafvel-Pursiainen *et al*., 1982). Similarly, no difference in SCEs was found in lymphocytes of welders and controls (Popp *et al*., 1991; Jelmert *et al*., 1994). Likewise, an investigation has shown a lack of genotoxic effect in blood lymphocytes of welders using CAs, SCEs and the MN test (Littorin *et al*., 1983).

In the welders of the present study, blood Cr levels correlated significantly with MN induction. Similarly, a good relationship was found between the amount of Cr present in the air and erythrocytes and the frequency of MN (Vaglenov *et al*., 1999). In the current study, no significant effect of smoking on MN induction in exposed groups was observed. Interestingly, similar results were obtained by other researchers (Littorin *et al*., 1983; Popp *et al*., 1991; Jelmert *et al*., 1994). However, some investigations were inconsistent with our data on the effects of smoking (Husgafvel-Pursiainen *et al*., 1982; Elias *et al*., 1989; Knudsen *et al*., 1992). Welders in this study showed a significant correlation between occupational exposure and MN frequency. Similarly, there was a significant correlation between length of employment as a welder and the frequency of CAs (Elias *et al*., 1989). In the present study, age had an influence on the MN frequency in welders. Likewise, a biomonitoring study of genotoxic exposure among welders found that CAs and SCEs increased significantly with age (Knudsen *et al*., 1992).

Although the MN test and Comet assay represent different end-points, the results obtained from both were very similar. However, the results of this study are not enough to establish any causal connection, although there is experimental evidence that supports the genotoxicity of Cr and Ni. Also, the possibility of unrecognized confounding factors is inevitable in studies such as this.

However, the best remedy for occupational exposure is prevention. Workers in many occupational settings are exposed to certain genotoxic agents. These workers may not be aware that they have been exposed to genotoxic agents nor do they know the type and amount of agent to which they have been exposed. Therefore, there is a need to educate those who work with heavy metals about the potential hazard of occupational exposure and the importance of using protective measures.

Acknowledgements

We are grateful to Dr Y.R.Ahuja (Head, Genetics Unit, Mahavir Hospital) for his guidance throughout this study. We are thankful to Dr K.V.Rao for his suggestions regarding statistics. The authors express their sincere thanks to Dr K.V.Raghavan (Director, Indian Institute of Chemical Technology, Hyderabad) for providing facilities and his encouragement during the study. IICT Communication no. 030407.

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Received on May 19, 2003; revised on October 20, 2003; accepted on October 23, 2003