

## Cytogenetic Risk Assessment in Workers of Rubber Industry

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**KEY WORDS** Rubber industry; genotoxic; peripheral blood lymphocytes; chromosomal aberrations; sister-chromatid exchanges; satellite associations.

**ABSTRACT** The genotoxic effect of environmental pollutants generated in a rubber tyre industry was investigated on somatic chromosomes of human lymphocytes of 50 workers exposed for different periods and compared with an equal number of unexposed controls matched in respect of age, sex, social status, period of exposures, smoking habits and drug intake, if any. The mitotic index (MI), chromosomal aberrations (CA), sister chromatid exchanges (SCE) and satellite associations (SA) were analysed. All the parameters showed a significant increase ( $p < 0.01$ ) in the exposed sample compared with controls: viz MI (3.72-6.69), CA (0.92-3.40), SCE, (3.76-10.82) and SA (3.66-11.10). The occurrence of DG type of satellite associations was highest and the 3D type lowest. The frequencies of SCE and CA showed significant elevation ( $p < 0.01$ ) with the duration of exposure. The environmental pollutants generated in rubber industry were thus found to be genotoxic and tobacco smoke was found to enhance the genotoxic effect.

### INTRODUCTION

Detection of hazardous environmental agents and measurement of early biological responses in individuals exposed to such agents are among the major goals of today's preventive toxicology. Cytogenetic techniques used to study chromosomes of somatic cells offer, in theory, an outstanding possibility of detecting genetic damage induced by exogenous agents.

Increasing number of substances used in the synthesis of polymers and in the manufacture of goods are being added to the environment. The rubber industry is known to use a large and expanding array of chemicals some of which are experimentally proven mutagens and carcinogens. Known genotoxic agents have been detected in the effluents from rubber vulcanization viz. thiurams, IPA, benzene, benzo(a) pyrene and nitrosamines (IARC 1982).

Rubber products vulcanized at high temperature and pressure emit biologically active chemical agents. Several of them are known to be suspected mutagens and carcinogens (Falck 1983). In epidemiological studies it has been proven that workers in rubber industry may have an increased cancer risk (Lemen et al. 1990; Matanoski et al.

1990). Derivatives of styrene, butadiene and rubber are carcinogenic in animal studies (Huff et al. 1985; IPCS 1983) and mutagenic *in vitro* and *in vivo* systems (IPCS 1983; Cunningham et al. 1986; Geravasi et al. 1985; Rosenthal 1985; Tice et al. 1987). They induced sister-chromatid exchanges (SCE) and chromosomal aberrations (CA) in cultured chinese hamster ovary (CHO) cells as well as in human lymphocytes (Norppa et al. 1980, 1983; Sasiadek et al. 1991a, b). Whereas several studies showed an increase in the frequencies of CA and SCE in lymphocytes of workers from rubber industry (Anderson et al. 1980; Cammuri et al. 1983; Sorsa et al. 1983) others did not find any increase (Degrassi et al. 1984). To resolve this confusion, workers from a rubber tyre industry were investigated for cytogenetic damage due to work place pollutants.

### MATERIAL AND METHODS

The present investigation included 100 individuals in all, 50 male individuals who worked in tyre industry and were mainly exposed to a variety of chemicals and 50 matched controls mainly office workers, all male individuals, who were not exposed to chemicals emitted from tyre industry. The health and occupational histories of the subjects and controls in respect of age, sex, duration of exposure to pollutants, smoking and drinking habits and drug intake, if any, were recorded on a standard proforma. The data were taken over a period of nine months from January 1998 to September 1998.

The workers did all kinds of jobs from carrying the ingredients to weighing bridge, mixing them and handling, while processing. Thirty five of the workers smoked, while 15 were strictly non-smoker. Their age varied from 20-42 years. The exposure period varied from 2-18 years. Among the control individuals 33 were smokers and 17 non-smokers. Their age varied from 20-39 years. None of the individuals had been exposed to X-rays or had taken antibiotics for three months prior to sampling. The workers did not use any protective devices.

Short term lymphocyte cultures were established from heparinised blood according to slightly modified method of Moorhead et al.

(1960). Lymphocytes were grown by adding 0.5 ml of blood in 5 ml of RPMI 1640 medium (Hi. Media) containing 20% foetal calf serum, penicillin, streptomycin and 0.1 ml of phytohaemagglutinin (Sigma), Colchicine (10 ug/ml) was added to the cultures two hours prior to harvesting.

For studying chromosomal aberrations lymphocytes were harvested after 48h. Slides were prepared by air drying method and stained with 4% giemsa (E. Merck). As many as 100 good metaphases were screened for individual chromosomal aberrations.

For Sister Chromatid exchanges (SCE), 5 bromo-deoxyuridine (10ug/ml culture, Sigma) was added 24h after setting up of cultures. Harvesting was done after 72h. Slides were prepared by air drying method and stained with Hoechst 33258 (Sigma) and 4% giemsa following the method of Perry and Wolff (1974).

For calculating the frequency of SCE per cell, 25 metaphases were analysed as per standard practice.

For calculating mitotic index (M.I.) 5000 cells per individual were scanned from giemsa stained slides. Mitotic index was calculated using the formula:

$$MI = \frac{\text{No. of dividing cells}}{\text{Total No. of cells screened}} \times 100$$

For evaluating the frequency of satellite associations (SA), 100 good second division metaphases were scanned and the following criteria given by Hansson (1970) were applied.

- (i) The satellite ends of the associating chromosomes had to be directed towards each other with their longitudinal axes meeting between their short arms.
- (ii) The distance between the centromeres of associated chromosomes should not exceed the total length of one 'G' chromosome, its satellite excluded.

For statistical analysis of results, Student's

't'-test was applied (Colton 1980).

## RESULTS

An epidemiological survey showed that nearly all workers complained of headache. Whereas 15 workers out of 50 with long exposure were found to be suffering from various respiratory tract diseases like asthma, irritation and watering of eyes were other symptoms common to all.

The data obtained during the present investigation have been given in tables 1-6. The mean MI in exposed workers (6.69) was significantly higher ( $p < 0.01$ ) than in controls (3.72). The value of MI goes on increasing with duration of exposure and is maximum for 11-15 years of exposure (7.01), then from 16th year onward it started declining (Table 1).

Chromosomal aberrations were found to be elevated in exposed individuals than in controls. The mean percentage of CA in exposed group was 3.20 and that in controls was 0.92 resulting in highly significant values at  $P < 0.01$  (Table 2). Both chromosome type aberrations viz. dicentric, rings, acentric fragments, chromosomal gaps and chromosome breaks and chromatid type aberrations viz. chromatid gaps, chromatid breaks and isochromatid exchanges were encountered in the exposed group. All the values for these aberrations were significant at  $P < 0.01$ . In the control group, chromosome type aberrations, viz. dicentric and acentric fragments were observed. A small number of chromatid type aberrations were also noticed. There was a significant difference in smokers as compared with non-smokers (Table 3). The exposed smokers showed higher values of CA (3.82) as compared with exposed non-smokers (2.40). A similar situation occurred in controls.

Frequencies of SCE in exposed workers and control group have been presented in table 4. The mean number of SCE per cell among exposed

**Table 1: Mitotic Index (MI) in workers engaged in rubber industry and control individuals**

| Duration of exposure (in years) | Number of samples | Number of cells scored | Number of metaphases | MI $\pm$ SD      |
|---------------------------------|-------------------|------------------------|----------------------|------------------|
| Control Individuals             | 50                | 248984                 | 9106                 | 3.72 $\pm$ 0.31  |
| Exposed Individuals             | 50                | 251359                 | 17130                | 6.69 $\pm$ 0.45* |
| 0-5                             | 10                | 50025                  | 3282                 | 6.55 $\pm$ 0.40  |
| 6-10                            | 17                | 85787                  | 5690                 | 6.69 $\pm$ 0.39  |
| 11-15                           | 13                | 65285                  | 4583                 | 7.01 $\pm$ 0.43  |
| 16-20                           | 10                | 50265                  | 3193                 | 6.34 $\pm$ 0.29  |

\* Significant at  $P < 0.01$

**Table 2: Frequency of chromosomal aberrations in lymphocytes of rubber industry workers and control individuals**

| Group                                        | Exposed<br>Individuals | Control<br>Individuals |
|----------------------------------------------|------------------------|------------------------|
| No. of Individuals                           | 50                     | 50                     |
| No. of Metaphases                            | 5000                   | 5000                   |
| <i>Chromosome Type Aberrations</i>           |                        |                        |
| Dicentrics                                   | 11 (0.22)              | 2 (0.04)               |
| Rings                                        | 2 (0.04)               | -                      |
| Acentric Fragments                           | 7 (0.14)               | 2 (0.04)               |
| Translocations                               | 0 (0.00)               | -                      |
| Chromosome Gaps                              | 5 (0.10)               | 3 (0.06)               |
| Chromosome Breaks                            | 2 (0.04)               | -                      |
| Total (without gaps)                         | 22 (0.44)              | 4 (0.08)               |
| <i>Chromatid Type Aberrations</i>            |                        |                        |
| Gaps                                         | 167 (3.34)             | 73 (1.46)              |
| Breaks                                       | 138 (2.76)             | 42 (0.92)              |
| Isochromatid Exchanges                       | 0                      | -                      |
| Total (without gaps)                         | 138 (2.76)             | 46 (0.92)              |
| Total Chromosomal Aberrations (without gaps) | 160 (3.20)*            | 46 (0.92)              |

Values in parantheses indicate aberrations per 100 metaphases.

\* Significant at  $P < 0.01$

**Table 3: Frequency of chromosomal aberrations (ca) in Rubber industry workers and control individuals**

|                   | Control<br>Individuals |                 | Exposed<br>Individuals |                  |
|-------------------|------------------------|-----------------|------------------------|------------------|
|                   | n                      | Mean $\pm$ S.D. | n                      | Mean $\pm$ S.D.  |
| Total Individuals | 50                     | 0.92 $\pm$ 0.68 | 50                     | 3.40 $\pm$ 1.34* |
| Smokers           | 33                     | 1.06 $\pm$ 0.65 | 35                     | 3.82 $\pm$ 1.08* |
| Non-Smokers       | 17                     | 0.76 $\pm$ 0.72 | 15                     | 2.40 $\pm$ 1.35* |

\* Significant at  $P < 0.01$

n - Number of Samples

**Table 4: Frequency of sister chromatid exchanges (sce) in Rubber industry workers and control individuals**

|                   | Control<br>Individuals |                 | Exposed<br>Individuals |                   |
|-------------------|------------------------|-----------------|------------------------|-------------------|
|                   | n                      | Mean $\pm$ S.D. | n                      | Mean $\pm$ S.D.   |
| Total Individuals | 50                     | 3.76 $\pm$ 0.50 | 50                     | 10.82 $\pm$ 1.09* |
| Smokers           | 33                     | 4.01 $\pm$ 0.38 | 35                     | 11.01 $\pm$ 1.12* |
| Non-Smokers       | 17                     | 3.28 $\pm$ 0.34 | 15                     | 10.31 $\pm$ 0.69* |

\* Significant at  $P < 0.01$

workers is 10.82 and in controls 3.76, the difference being statistically significant ( $P < 0.01$ ). The smokers, of both exposed (11.01) and control group (4.01), showed higher values of SCE compared with non smokers (10.31 and 3.28). Both CA and SCE were positively correlated with du-

**Table 5: Frequency of ca and sce in rubber industry workers with duration of exposure**

| Duration<br>of Exposure<br>(In years) | Number of<br>Individuals | CA<br>Mean $\pm$ SD | SCE<br>Mean $\pm$ SD |
|---------------------------------------|--------------------------|---------------------|----------------------|
| 0-5                                   | 10                       | 3.10 $\pm$ 1.37     | 9.58 $\pm$ 0.38      |
| 6-10                                  | 17                       | 3.29 $\pm$ 1.36     | 10.46 $\pm$ 0.56     |
| 11-15                                 | 13                       | 3.53 $\pm$ 1.27     | 11.08 $\pm$ 0.71     |
| 16-20                                 | 10                       | 3.70 $\pm$ 1.26     | 12.26 $\pm$ 0.64     |

**Table 6: Frequency of satellite associations (Sa) observed in rubber industry workers and control individuals**

|                                | Number of Individuals  |                        |
|--------------------------------|------------------------|------------------------|
|                                | Control<br>Individuals | Exposed<br>Individuals |
| Total Cells Scanned            | 50                     | 50                     |
| Type of Satellite Associations | 5000                   | 5000                   |
| DD                             | 40 (0.80)              | 164 (3.28)             |
| DG                             | 92 (1.84)              | 219 (4.38)             |
| GG                             | 29 (0.58)              | 73 (1.46)              |
| 2DG                            | 8 (0.16)               | 37 (0.74)              |
| 2GD                            | 5 (0.10)               | 37 (0.74)              |
| 2G2D                           | 5 (0.10)               | 16 (0.32)              |
| 3D                             | 4 (0.08)               | 9 (0.18)               |
| Total                          | 183 (3.66)             | 555 (11.10)            |
| Associations Per Cell          | 3.66 $\pm$ 1.79        | 11.10 $\pm$ 2.7*       |

Figures in parentheses indicate the satellite associations per 100 metaphases

\*Significant at  $P < 0.01$

ration of exposure (Table 5).

Table 6 shows the frequency of SA in exposed and control individuals. The types of SA observed were DD, DG, GG, 2DG, 2GD, 2D2G and 3D. The exposed group showed an almost three fold increase (11.10) compared with controls (3.66) which was statistically significant at ( $P < 0.01$ ). DG type association were found to be maximum (4.38) while 3D type association showed the lowest occurrence (0.08).

## DISCUSSION

Vapours that are generated during the curing of rubber tyres may cause certain forms of pulmonary diseases (Phillips 1997). Headache and irritation in eyes and upper respiratory tract, dermatitis, vertigo, drowsiness and loss of consciousness have also been reported in rubber industry workers (Delzell and Monson 1981; Plunkett 1987). The present findings very well fit in these observations.

Mitotic index was found to be higher in the

exposed workers as compared with the controls. The value of MI showed elevation upto 15 years. After that it declined. The maximum effect of chemicals of rubber industry was found in workers exposed for a period of 11-15 years. The decrease in MI after this period may be due to accumulation of chemicals in cells which start destroying them resulting in the decrease in MI. Similar results were shown in the  $\text{SO}_2$  (Yadav and Kaushik 1996),  $\text{NO}_x$  (Yadav and Seth 1998a) and PAH (Yadav and Seth 1998b) exposed workers upto 5 years and in those exposed to  $\text{NH}_3$  (Yadav and Kaushik 1997) for over 10 years. Rupa et al. (1989) observed a decrease in MI in smokers exposed to pesticides for longer period.

There was a significant increase in total CA in the exposed group as compared with the controls. Chromatid gaps were not taken into account because their significance in cytological monitoring of populations is still a matter of discussion (Brogger 1982). The total chromatid aberrations were more than the total chromosome aberrations in both exposed workers and controls. A similar elevation in CA in the lymphocytes of workers from rubber industry was reported earlier (Anderson et al. 1980; Sorsa et al. 1983; Sasiadek 1992). However, Degraasi et al. (1984) could not observe increased level of CA in exposed sample. A significant elevation in the frequency of CA in peripheral blood lymphocytes among persons belonging to a given group may indicate an increased cancer risk (Forni 1966; Awa 1983). Significantly higher frequencies of CA induced by Styrene were also found in cultured chinese hamster ovary (CHO) cells as well as in human lymphocytes (Norppa et al. 1980, 1983).

With the duration of exposure the age of individual also increases. The age of an individual, therefore, can bring about a great variation in the frequency of chromosomal aberrations following exposure of cells *in vivo* or *in vitro* to a variety of environmental agents (Bochkov and Pilosoa 1968; Lerda and Rizzi 1992; Maki-Paakkanen et al. 1991; Prasad et al. 1986).

The frequency of CA in the exposed smokers was found higher as compared with the control smokers in our study. Similar results were shown by Sasiadek (1992), in workers from rubber industry, Yadav and Kaushik (1996, 1997) in  $\text{SO}_2$  and  $\text{NH}_3$  exposed workers and Yadav and Seth (1998a, b) in  $\text{NO}_x$  and PAH exposures. On the contrary Degraasi et al. (1984) showed that CA frequencies were not co-related with smoking habits in rubber industry workers.

The frequency of SCE in exposed workers of tyre industry was higher than in controls. The SCE frequency showed a positive correlation with the period of exposure. Several studies had earlier depicted an increase in the frequency of SCE with increase in duration of exposure in lymphocytes of workers from rubber industry (Camurri et al. 1983; Sasiadek et al. 1991a,b; Sorsa et al. 1983). Both among exposed workers and controls tobacco smokers had higher SCE frequency. This is similar to the results of Sorsa et al. (1982a, b); Yadav and Seth (1998a, b) and Yadav and Kaushik (1996, 1997). Sasiadek (1992) found that SCE in exposed smokers showed an increase by 4 SCE per cell, whereas during the present investigation the effect observed was about 1 SCE per cell as has been also reported by Kelsey et al. (1991).

During the present investigation almost three fold increase was observed in the frequency of SA per cell. The difference is significant and may lead to increased probability of chromosomal translocations. The frequency of DG-Type associations was highest, whereas the frequency of 3D-type associations was lowest. Similar results were reported earlier for workers exposed to  $\text{SO}_2$  (Yadav and Kaushik 1996);  $\text{NH}_3$  (Yadav and Kaushik 1997);  $\text{NO}_x$  (Yadav and Seth 1998a) and PAH (Yadav and Seth 1998b) and for the grape garden workers exposed to pesticides (Rita et al. 1987). Hansson (1970) suggested that the tendency of SA is genetically controlled and specific environments may influence the frequency of SA.

The contradictory findings concerning workers in the rubber industry are most probably explainable by differences in the exposure situations and job categories. The positive findings reported relate to mixing and weighing operations in the rubber industry (Sorsa et al. 1980), while the negative results concern vulcanizers of rubber (Degraasi et al. 1984). Comparisons between discrepant results, especially "borderline results", obtained in otherwise well-documented studies are thus largely related to the fact that no two human exposure situations are alike (Sorsa and Yager 1987).

In the working environment of rubber industry, workers are exposed to several hundred different chemicals (Holmberg 1977). Some of them being known or suspected carcinogens and mutagens. Rubber workers exhibited significantly higher mutagenic activity in their urine than the occupationally unexposed controls (Falck et al. 1980). Chemicals such as TMTD (Tetramethyl

thiuram disulfide), Ziram (Zinc dimethyldithio carbamate) and TETD (tetraethyl thiuram disulfide) have been found to be mutagenic in *E. coli* (Hedenstedt et al. 1979) and cell cultures (Hinderer et al. 1983), *Aspergillus nidulans* (Zdzienicka et al. 1981) and mice (Cilieviei et al. 1983).

Considerable difficulties have been met in the research for specific carcinogenic agents, because of both the organisation of working process and the complex interaction between the several hundred chemicals used, which may result in the formation of new carcinogenic materials (IARC 1982). The analysis of SCE and CA from the samples of two rubber plants revealed a slight increase in SCE frequencies which was not paralleled by an increase in the aberration rate (Sorsa et al. 1982b, 1983).

The positive results obtained in various cytogenetic tests during present investigation prove the genotoxic potential of various chemicals produced in the processing of rubber in the Tyre industry. The synergistic effect of tobacco smoke has been also noticed.

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