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Cytogenetic analysis of buccal cells from shoe-workers and pathology and anatomy laboratory workers exposed to *n*-hexane, toluene, methyl ethyl ketone and formaldehyde

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People employed in the shoe manufacture and repair industry are at an increased risk for cancer, the strongest evidence being for nasal cancer and leukaemia. A possible causal role for formaldehyde is likely for cancer of the buccal cavity and nasopharynx. Exfoliated buccal cells are good source of tissue for monitoring human exposure to inhaled and ingested occupational and environmental genotoxicants. To assess the cytogenetic damage related to occupational exposure to airborne chemicals during shoe-making and the processes in pathology and anatomy laboratories, the micronuclei (MN) count per 3000 cells was measured in buccal smears from shoe-workers (group I, $n = 22$) exposed to mainly *n*-hexane, toluene and methyl ethyl ketone (MEK) and from anatomy and pathology staff (group II, $n = 28$) exposed to formaldehyde (FA). Eighteen male university staff were used as controls. The mean time-weighted average (TWA) concentrations of *n*-hexane, toluene and MEK in 10 small shoe workshops were 58.07 p.p.m., 26.62 p.p.m. and 11.39 p.p.m., respectively. The measured air concentrations of FA in the breathing zone of the anatomy and pathology laboratory workers were between 2 and 4 p.p.m. Levels of 2,5-hexadione (2,5-HD) and hippuric acid (HA), metabolic markers of *n*-hexane and toluene exposure, respectively, were significantly higher in the urine of workers in group I than in control subjects ($p < 0.001$ and $p < 0.01$, respectively). The mean (± SD) MN frequencies in buccal mucosa cells from workers in group I, group II and controls were $0.62 \pm 0.45\%$, $0.71 \pm 0.56\%$ and $0.33 \pm 0.30\%$, respectively ($p < 0.05$ and $p < 0.05$ compared with controls for group I and group II, respectively). The effects of smoking, age and duration of exposure on the frequency of micronucleated buccal cells from workers in all three groups studied were also evaluated. Overall, the results suggest that occupational exposure to organic solvents, mainly *n*-hexane, toluene, MEK and FA, may cause cytogenetic damage in buccal cells and that use of exfoliated buccal cells seems to be appropriate to measure exposure to organic solvents.

Keywords: exfoliated buccal cells, micronuclei, shoe-workers, pathology and anatomy laboratory workers.

Introduction

Workers in shoe manufacture are usually exposed to complex mixtures of solvents originating from glues and their diluents. In our previous studies (Burgaz

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et al. 1997), *n*-hexane and toluene were the solvents most frequently found in the glues used in shoe factories, while other solvents such as heptane, acetone and benzene were found in lower concentrations. Benzene concentrations were always less than 1% in the samples analysed. According to the International Agency for Research on Cancer (IARC), people employed in the shoe manufacture and repair industry are at an increased risk of cancer, the strongest evidence being for nasal cancer and leukaemia (IARC 1987).

Formaldehyde (FA) is a widely used industrial and commercial product. It is known that people such as anatomists, embalmers and pathologists have considerable FA exposure. Although the data are controversial, the human cancers considered most likely to be related to FA exposure are those of the buccal cavity and nasopharynx (Blair *et al.* 1990, IARC 1995).

In the light of the fact that 90% of all human cancers arise from epithelial tissues, the micronuclei (MN) assay in exfoliated epithelial cells can be used to study genetic damage, including aneuploidy as well as chromosomal breakage (Stich 1987). Increased MN frequencies have been found in bladder, buccal, nasal, sputum and cervical smear cell preparations from individuals exposed to tobacco smoking, smokeless tobacco, arsenic, chromium, formaldehyde, paints and air pollution (Diaz *et al.* 1990, Sarto *et al.* 1990, Burgaz *et al.* 1995, 2000, Gonsebatt *et al.* 1997, Lehucher-Michel *et al.* 1997, Valverde *et al.* 1997, Salama *et al.* 1999). Since exfoliated buccal cells are a good source of tissue for monitoring human exposure to inhaled and ingested occupational and environmental genotoxicants (Stone *et al.* 1995), and are also capable of metabolizing proximate carcinogens to reactive chemicals (Zhang and Mock 1989), it was of interest to assess cytogenetic damage related to occupational exposure to airborne chemicals during shoe-making and processes in pathology and anatomy laboratories.

In order to evaluate the extent of cytogenetic damage in these two groups, we measured the MN frequencies in exfoliated buccal cells from shoe-workers exposed to mainly *n*-hexane, toluene and methyl ethyl ketone (MEK), and from anatomy and pathology staff exposed to FA. Occupational exposure to *n*-hexane and toluene was monitored by analysis of urinary levels of 2,5-hexadione (2,5-HD) (Perbellini *et al.* 1990) and hippuric acid (HA) (Ogata and Taguchi 1987). Environmental concentrations of *n*-hexane, toluene, MEK and FA were also measured.

Materials and methods

Subjects

Group I consisted of 22 workers (all male) employed in a shoe factory in 10 small workshops, where jobs include the use of glues and adhesives containing mainly *n*-hexane, toluene and MEK. Group II consisted of 28 subjects (15 males and 13 females) who were pathologists or staff in pathology or anatomy laboratories. The 18 male volunteer controls were staff at Ankara and Gazi University. None of the referents had been occupationally exposed to potential genotoxic chemicals. A questionnaire including information on age, smoking, drinking and dietary habits, and a brief occupational and medical history, was completed for each subject. Table 1 presents the main characteristics in the exposed and control subjects. Workers and controls reported similar dietary habits, alcohol consumption and use of medication. The frequency of exposure to solvents was between 8 h and 5 days per week in most of the workers. Both exposed and control groups included moderate smokers. The number of heavy smokers (more than 20 cigarettes/day) in group I, group II and controls were two, one and one, respectively.

Environmental monitoring

The concentrations of solvents in the breathing-zone air were measured in the 10 small workshops selected throughout the shift. *n*-Hexane, toluene and MEK concentrations in the air were determined by means of a single beam infrared spectrophotometer (Foxboro Miran IBX - Portable Ambient Air analyser). Each workshop had the same characteristics. For each workshop, vapour concentrations in the workers' breathing area were continuously monitored during the shift (8 h) in order to calculate the time-weighted average (TWA) concentrations of the solvents. Most workshops lacked appropriate local ventilation, and none of the workers wore gloves while using adhesives and solvents.

Analysis of the glues used revealed that they contained mainly *n*-hexane, toluene and MEK, with lower concentrations of acetone, isobutyl alcohol, *n*-butyl alcohol and trichloroethylene. Benzene concentrations were always less than 1% in the samples analysed. Stationary measurements of the FA concentrations in three anatomy and pathology laboratories were determined by means of the Gastec Detector Tube System (model 801, Japan) while laboratory work was taking place. This estimated exposure was not precise enough to be used as an individual marker of exposure.

Biological monitoring

Measurement of urinary 2,5-HD concentration. Urine samples were obtained at the end of the shift and stored at −20C until used. The total 2,5-HD in urine samples was measured according to the method of Perbellini *et al.* (1990). Extraction and gas chromatographic analysis of 2,5-HD in urine samples have been described elsewhere (Burgaz *et al.* 1997). The recovery rate of 2,5-HD was estimated by using three different concentrations of the metabolite: 0.9, 2.9 and 6.8 mg l^{-1}. Three different samples of each concentration were used. The average extraction efficiency of 2,5-HD was $88.9 \pm 12.9\%$. The detection limit for 2,5-HD under the conditions employed was 0.05 mg l^{-1} urine. The 2,5-HD concentration was corrected for the creatinine concentration.

Measurement of urinary HA level. For the analysis of HA, 1 ml of methanol was added to 1 ml of urine. The samples were then centrifuged at 2000 r.p.m. for 5 min and 10 µl of the supernatant obtained was used for high performance liquid chromatography (HPLC) (Hewlett Packard 1050). A Lichrosopher 100RP-18 column (5 µm, 250×4 mm) was used throughout the investigation. For the separation of HA, a mixed solution of KH_2PO_4 (pH 4.93) and CH_3CN (93:7) was used as the mobile phase. The flow rate was 0.8 ml min^{-1} (Ogata and Taguchi 1987). The recovery rate of HA was estimated by using two different concentrations of the metabolite: 0.25 and 0.5 mg ml^{-1}. Three different samples of each concentration were used. The average extraction efficiency of HA was $93.7 \pm 11.6\%$. The detection limit for HA under the conditions employed was 0.05 mg ml^{-1} urine. The HA concentration was corrected for the creatinine concentration.

MN frequencies in exfoliated buccal cells. Subjects were asked to rinse their mouths with water and a pre-moistened wooden spatula was used to sample cells from the buccal mucosa. The spatula was then applied to a pre-cleaned microscope slide. Smears were air dried and fixed in 80% methanol. Slides were stained by the Feulgen reaction and then counterstained with Fast Green. A total of 3000 cells per individual were analysed using the criteria previously described (Burgaz *et al.* 2000).

Table 1. General characteristics of exposed and control subjects.

Parameters	Group I	Group II	Controls
n	22	28	18
Age[a]			
Mean ± SD (years)	29.5 ± 8.34	29.68 ± 5.37	31.10 ± 8.77
Range (years)	17–48	23–43	21–52
Smoking habits[b]			
Smokers (*n*)	14	12	7
Non-smokers (*n*)	8	16	11
Duration of exposure			
Mean ± SD (years)	7.68 ± 8.71	4.70 ± 3.33	—
Range (years)	< 1–30	1–13	—

[a] $p > 0.05$ (compared with control group).
[b] χ^2 value for proportions ($p = 0.11$).

There were no significant differences between the controls and groups I and II for age or smoking habits.

Statistical analysis

The mean, standard deviation and standard error were calculated for each biomarker. The significance of the differences between control and exposed endpoint means were analysed using the Student's *t*-test and the Mann–Whitney test. The cytogenetic data were processed using a multifactorial analysis of variance for the simultaneous assessment of the effects of smoking, exposure and gender. In all the analyses, age was used as a covariate. Correlations were evaluated using Spearman's and simple regression tests. Significance levels of 5% or less were considered to be significant. All p values were two-tailed.

Results

The general characteristics of the study population are summarized in table 1. The mean ages of both groups I and II were not significantly different from that of the control subjects ($p > 0.05$). Breathing-zone concentrations of *n*-hexane, toluene, MEK and FA at the workplaces are indicated in table 2. Shoe-workers were exposed to a mixture of *n*-hexane, toluene and MEK, and were exposed to greater concentrations of *n*-hexane than toluene or MEK. Exposure of group II subjects to FA was also evident during laboratory working periods.

Table 3 shows the mean values of urinary 2,5-HD and HA in group I workers. 2,5-HD and HA concentrations were significantly higher in shoe-workers than in controls ($p < 0.001$ and $p < 0.01$, respectively). Urinary 2,5-HD levels were correlated significantly with air *n*-hexane concentrations in each workshop ($r = 0.63$, $p < 0.01$). The correlation coefficient between atmospheric toluene concentration and the *n*-hexane/2,5-HD ratio was also statistically significant ($r = 0.49$, $p < 0.05$). On the other hand, no correlation was found between the atmospheric toluene concentrations in each workshop and urinary HA concentrations.

Table 2. Concentrations of *n*-hexane, toluene, MEK and FA in breathing-zone air in workshops.

	TWA	
	Mean ± SEM (p.p.m.)	Range (p.p.m.)
n-Hexane	58.07 ± 28.09	4.3–300
Toluene	26.62 ± 10.27	5.37–115.2
MEK	11.39 ± 4.36	2.43–47
FA	—	2–4

Table 3. Urinary 2,5-HD and HA concentrations in control and group I subjects.

	Controls ($n = 18$)	Group I ($n = 22$)
2,5-HD concentration		
Mean ± SD (mg g^{-1} creatinine)	1.50 ± 1.76	4.18 ± 3.70[a]
Range (mg g^{-1} creatinine)	ND–6.59	0.75–16.03
HA concentration		
Mean ± SD (g g^{-1} creatinine)	0.90 ± 0.80	2.23 ± 2.04[b]
Range (g g^{-1} creatinine)	0.11–2.86	0.41–5.14

ND, not detected.
[a] $p < 0.001$ (compared with corresponding controls).
[b] $p < 0.01$ (compared with corresponding controls).

Table 4. Results of analysis of MN frequencies in buccal mucosa cells of controls, group I and group II workers according to smoking habits.

	Controls	Group I[a]	Group II
All			
n	18	21	28
MN frequency			
Mean ± SD (%)	0.33 ± 0.30	0.62 ± 0.45	0.71 ± 0.56
Range (%)	0.00–1.00	0.00–1.67	0.00–2.00
Smokers			
n	7	13	12
MN frequency			
Mean ± SD (%)	0.33 ± 0.19	0.69 ± 0.52	0.89 ± 0.52
Range (%)	0.00–0.67	0.00–1.67	0.33–2.00
Non-smokers			
n	11	8	16
MN frequency			
Mean ± SD (%)	0.33 ± 0.37	0.50 ± 0.31	0.58 ± 0.57
Range (%)	0.00–1.00	0.00–1.00	0.00–1.67
Number of cells analysed	54×10^3	63×10^3	84×10^3

[a] Buccal cells from one individual were not available.

Table 5. Results of multifactorial analysis of variance for buccal MN frequencies in workers exposed to *n*-hexane, toluene, MEK and FA.

Source of variation	Mean square	F ratio	*p* value
Group I			
Covariate: age	0.269	0.411	0.526
Factors			
Exposure	1.041	7.078	0.013[a]
Smoking status	5.165E-03	0.035	0.853
Group II			
Covariate: age	1.165E-02	0.042	0.838
Factors			
Exposure	1.388	5.060	0.031[a]
Smoking status	3.852E-04	0.001	0.970
Gender	0.134	0.489	0.489

[a] $p < 0.05$.

The results of the cytogenetic analyses of buccal smears from workers in groups I and II and from control subjects are presented in table 4. Analysis of variance (table 5) indicated that only occupational exposure, but not smoking habits and gender, was associated with an increased MN frequency in groups I and II ($p < 0.05$). Duration of exposure was significantly asssociated with the MN frequency in group I ($r = 0.48$, $p < 0.05$).

Discussion

Workers in shoe manufacture

In a our previous study (Burgaz *et al.* 1997), shoe-workers were exposed to mainly *n*-hexane, toluene, and, at lower concentrations, heptane, acetone and benzene. Our results show that *n*-hexane continues to be the principal component,

along with toluene and MEK, in the shoe workshops in this study. In the present study, *n*-hexane concentrations in the air of the workshops ranged from 4.3 p.p.m. to 300 p.p.m. TWA values of *n*-hexane in these workshops were above the current occupational exposure limit, i.e. 50 p.p.m., as recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) (ACGIH 1999). However, exposure levels for toluene and MEK were well below the TWA values (i.e. 50 p.p.m. and 200 p.p.m., respectively), except for one workshop. Benzene concentrations were not measured in the present study. In our previous study, the benzene concentration in breathing-zone air was 0.65 p.p.m. (Burgaz *et al.* 1997). In Turkey, the Ministry of Labour regulates the use of benzene in the glues and adhesives used in the shoe manufacturing processes.

The concentrations of 2,5-HD and HA in the urine of shoe-workers were significantly elevated, confirming exposure to *n*-hexane and toluene. However, these levels remained lower than tentative maximum permissible values for occupational settings (i.e. 5 mg g^{-1} creatinine and 2.5 g g^{-1} creatinine, respectively) (ACGIH 1999). Although a definite effect of other solvents on the concentration of 2,5-HD was not suggested, we investigated the correlation between the atmospheric *n*-hexane/urinary 2,5-HD ratio and environmental levels of toluene at the workplaces to get a rough idea of the possible influence of toluene on the biotransformation of 2,5-HD. In our study, a significant correlation between the atmospheric toluene concentration and the *n*-hexane/ 2,5-HD ratio was obtained, and this may indicate that co-exposure to toluene might cause some degree of metabolic interference in workers. We are also aware that possible metabolic and toxicological interferences of MEK with *n*-hexane, kinetic factors, metabolic clearance, alveolar ventilation, genetic differences as well as previous exposure may contribute to inter- and intra-individual variability in urinary 2,5-HD levels (Takeuchi *et al.* 1993, Ichihara *et al.* 1998). No correlation was found between atmospheric toluene concentrations in each workshop and urinary HA concentrations. This may be due to the presence of HA endogenously in all individuals and the greater inter-individual differences in HA excretion, which is influenced by a number of factors such as diet, medical treatment, alcohol consumption, etc.

In the field of cytogenetics, Stich and Rosin (1983) were the first to propose the adaptation of the MN test for use in exfoliated cells as a measure of chromosome damage in epithelial tissues. Whereas lymphocytes must be stimulated, epithelial cells do not need to be; MN in exfoliated cells reflect genotoxic events that occurred in the dividing basal layer 1–3 weeks earlier (Stich 1987). Genotoxic changes in buccal, nasal, bronchial, oesophageal, cervical, breast duct and other types of epithelia have been reported (Fenech *et al.* 1999). An assay in exfoliated cells holds promise as a site-specific biomarker of exposure to genotoxins and for cancer risk. Casartelli *et al.* (2000) have recently demonstrated that MN frequencies increased from normal mucosa to pre-neoplastic lesions to carcinomas. However, additional studies are needed to validate the induction of MN in epithelial tissues as a biomarker of cancer risk (Fenech *et al.* 1999).

Few studies have addressed the cytogenetic changes in exfoliated buccal cells of individuals exposed to organic solvents such as toluene, xylene and benzene (Diaz *et al.* 1990, Surralles *et al.* 1997). In our study we found a significant increase (about two-fold) in MN frequencies in buccal cells from shoe-workers.

Diaz *et al.* (1990) have observed significantly increased levels of MN in oral mucosa cells in Cuban paint industry workers exposed to mainly xylene and toluene. Surralles *et al.* (1997) have suggested that benzene exposure levels of ~1 p.p.m. do not induce statistically significant MN frequencies in buccal cells or lymphocytes. There is almost no evidence for the genotoxicity of toluene *in vitro*. In addition, toluene has not led to unequivocal positive findings in genotoxicity assays *in vivo* (McGregor 1994). Funes-Cravioto *et al.* (1977) and Pelclova *et al.* (1990, 2000) obtained significant increases in chromosomal aberrations in the peripheral lymphocytes of printers exposed to toluene, where benzene exposure could be ruled out. On the other hand, other studies (Maki-Pakkanen *et al.* 1980, Richer *et al.* 1993, Pitarque *et al.* 1999) did not find any cytogenetic alterations in surrogate tissues from workers exposed to toluene. Positive findings were reported for DNA damage in other studies where benzene contamination was also present (Nise *et al.* 1991, Popp *et al.* 1992).

n-Hexane was reported to yield negative results in the *Salmonella*/microsomal assay (Mortelmans *et al.* 1986). In DNA damage assays, n- hexane was found not to induce DNA damage in either *Escherichia coli* or *Bacillus subtilis* (McCarroll *et al.* 1981a, b). In contrast, Mayer and Goin (1994) demonstrated that *n*-hexane and 2-hexanone, alone or in combination, induced only marginally positive chromosome loss, whereas the metabolite and presumed proximal genetically active agent 2,5-HD was strongly positive when tested alone and in combination in *Saccharomyces cerevisiae*. However, no human subjects have been examined after exposure to this chemical. Further research on surrogate tissues as well as on exfoliated epithelial cells is needed to gain insight into the possible genotoxicity induced by *n*-hexane.

Overall, our data suggest that genotoxicity may occur in shoe-workers exposed to a mixture of organic solvents, even at exposures lower than the threshold limit values (TLVs), and that buccal mucosal cells seem to be more sensitive to the induction of cytogenetic damage by organic solvents than peripheral lymphocytes (Narod *et al.* 1988, McGregor 1994, Holz *et al.* 1995, Lemasters *et al.* 1999). However, we are aware that shoe-workers in the present study were simultaneously exposed to several organic solvents, mainly *n*-hexane, toluene and MEK, and it is therefore difficult to assess which one plays the major role.

Workers in pathology and anatomy laboratories

In this study we investigated the frequencies of MN in exfoliated buccal mucosa cells of pathology and anatomy laboratory workers. Exposure of workers to FA was assessed from direct measurements of FA levels in the air of the pathology and anatomy Laboratories, which were never higher than 2 p.p.m. and 4 p.p.m., respectively. The FA levels measured in our study were as high as or higher than the short-term exposure limit (STEL) of 2 p.p.m. It is well known that persons such as anatomists, embalmers and pathologists have considerable FA exposure. Excess risks have been reported for leukaemia and cancers of the nasal cavity, nasopharynx, lung and brain associated with FA exposure. Although some of the data are controversial, the human cancers most likely to be related to FA exposure are cancers of the buccal cavity and nasopharynx (IARC 1995).

Previous studies of subjects exposed to FA using cytogenetic endpoints in peripheral lymphocytes, such as chromosome aberrations, sister chromatid ex-

changes, MN and DNA–protein crosslinks, have yielded conflicting results (Thompson *et al.* 1984, Shaham *et al.* 1997, He *et al.* 1998). These findings are consistent with the knowledge that FA is highly reactive and affects only directly exposed cells (Heck *et al.* 1990).

In the present study we have applied the MN test to exfoliated buccal cells of FA-exposed workers in order to detect possible genotoxic effects. The application of the MN test to human exfoliated cells provides information on the degree of cytogenetic damage in tissues that are possible targets of human carcinogens and from which carcinomas will develop (Stich 1987). Exfoliated buccal cells have been used for monitoring exposure to FA, arsenic, smoking, radiation, antineoplastic drugs and urban pollution (Sarto *et al.* 1987, Titenko-Holland *et al.* 1996, Gonsebatt *et al.* 1997, Valverde *et al.* 1997, Burgaz *et al.* 1999, Karahalil *et al.* 1999).

We found a significant increase (two-fold) in the MN frequency in buccal cells of workers. In our study smoking habits did not significantly influence the MN frequency. Some studies have reported a significant increase in the frequency of MN in the buccal cells of cigarette smokers compared with control groups (Sarto *et al.* 1987, Piyathilake *et al.* 1995). On the other hand, other studies did not show any significant increase in MN frequency in smokers (Sarto *et al.* 1990, Machado-Santelli *et al.* 1994, Gonsebatt *et al.* 1997, Karahalil *et al.* 1999). Gender was also not a confounding factor in the present study. This observation is consistent with the results of other authors (Surralles *et al.* 1997, Casartelli *et al.* 2000).

To date, four studies have evaluated the cytogenetic alterations in the epithelial buccal mucosa cells from individuals exposed to various levels of FA. Norppa *et al.* (1993) reported an increased MN frequency in the buccal cells of workers in a plywood plant and fibre glass factory, while no change was observed in lymphocytes. Suruda *et al.* (1993) demonstrated that low level exposure to FA (0.33 p.p.m.) was associated with cytogenetic changes in the epithelial cells of the buccal cavity and in the blood lymphocytes of male students participating in an embalming course. Titenko-Holland *et al.* (1996) found a 3.3-fold increase in MN frequency in buccal cells from mortuary science students exposed to FA, whereas in nasal cells there was none. Ying *et al.* (1997) showed significant increases (1.5-fold) in the MN frequencies in the buccal and nasal cells but not in the lymphocytes of anatomy class students exposed to FA (0.5 p.p.m.). Our findings are in accordance with these results. Further, an increased MN frequency was recently found in these subjects in exfoliated nasal cells (Burgaz *et al.* 2001).

Keeping in mind the limitations of any comparison of composition of cohorts, the number of scorable buccal cells, and FA exposure levels and exposure frequency among studies, the available data appear to suggest that an increase in MN frequency in exfoliated buccal cells is inducible at FA-exposure levels lower than the TWA of 0.75 p.p.m. (Suruda *et al.* 1993, Ying *et al.* 1997).

Our data demonstrate that occupational exposure to FA is occurring in these workers, and that, after controlling for possible confounding factors, this FA exposure may contribute to the observed genotoxic damage in their exfoliated buccal cells. Buccal mucosa and nasal cells exposed through respiration are important targets of FA-induced genotoxic effects, in agreement with earlier human and animal studies (Heck *et al.* 1990, IARC 1995, Ying *et al.* 1997, Burgaz *et al.* 2001).

Conclusion

The results of this study indicate that occupational exposure to organic solvents, mainly *n*-hexane, toluene, MEK and FA, may cause cytogenetic damage in exfoliated buccal cells, and so the use of buccal cells seems to be appropriate for measuring exposure to organic solvents. However, in interpreting the present results, it has to be remembered that few studies have been performed on the cytogenetic damage induced by organic solvents in exfoliated buccal cells and the sensitivity of this test has not been proven (Salama *et al*. 1999). Thus it will be of great value to assess whether the biomarker effects of organic solvents in exfoliated buccal cells are consistently greater than those in blood cells.

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