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Short Communication

Genotoxic effects caused by indoor exposure to petroleum derivatives in a fuel quality control laboratory

A.E.O. Araújo, B.P. Mezzomo, Í. Ferrari and C.K. Grisolia

Departamento de Genética e Morfologia,
Instituto de Ciências Biológicas, Universidade de Brasília, Brasília, DF, Brasil

Corresponding author: C.K. Grisolia
E-mail: grisolia@unb.br

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ABSTRACT. We looked for genotoxic effects in laboratory personnel routinely exposed to petroleum derivate compounds in an indoor environment. The exposed group of 21 workers from the Fuel Quality Control Laboratory of the Brazilian Petroleum Agency was matched with a group of 10 people from the staff of the Brazilian Ministry of Health. Chromosome aberrations in peripheral blood lymphocytes, micronuclei in exfoliated cells in the urine and hematological parameters were examined. There was a significantly increased level of chromosome aberrations and micronuclei in the exposed group compared with controls. A high correlation between chromosome aberrations and micronuclei was observed in the exposed group (Spearman rank test, $r = 0.73$, $P = 0.0001$). The hematological parameters in these exposed individuals did not differ from reference values.

Key words: Micronucleus; Chromosome aberration; Biomonitoring; Indoor exposure; Petroleum exposure

INTRODUCTION

Exposure to gasoline vapors is classified by the International Agency for Research on Cancer (IARC, 1989) as possibly carcinogenic to humans based on the established carcinogenicity of some components such as benzene and 1,3-butadiene. Benzene is a genotoxic agent that shows clastogenic and carcinogenic properties, due to benzoquinone, one of its main metabolites, which commonly causes DNA breaks, chromosomal damage and sister chromatid exchanges (Dean, 1985; Sasiadek et al., 1989). Tunka and Egeli (1996) reported an increased frequency of chromosomal aberrations in 58 shoe workers, which was attributed to the high content of benzene and hexane in the glues. Heuser et al. (2005) found an increased level of DNA damage through comet assay in workers employed in footwear manufacture. They were routinely exposed to complex mixtures of solvents. Martino-Roth et al. (2002) found an increased frequency of micronuclei in exfoliated cells of buccal mucosa in car painters from car repair garages, who were exposed to lead, solvents and benzene. Thus, a number of studies have shown increased risk of DNA damage in workers exposed to mono-aromatic organic solvents in indoor environments.

There are many studies on human exposure to petroleum derivate compounds in gas stations in association with genotoxic risk, especially with attendants (Santos-Mello and Cavalcante, 1992; Carere et al., 1995; Bukvic et al., 1998; Çelik et al., 2003; Çelik and Akbas, 2005; Benites et al., 2006). However, there are few studies on genotoxicity risks of exposure to gasoline vapors in laboratory environments. This study was carried out with professionals regularly exposed to petroleum derivatives in a fuel quality control laboratory. We used chromosome aberration on peripheral lymphocytes and micronuclei in urine exfoliated cells to evaluate the genotoxic effects of indoor chronic exposure to organic solvents from petroleum derivate fuels.

MATERIAL AND METHODS

Characterization of sample

The exposed group consisted of 21 workers (15 males and 6 females) from a fuel quality control laboratory of the Brazilian Petroleum Agency. The unexposed group consisted of 10 workers (7 males and 3 females) from the administrative staff of the Brazilian Ministry of Health. Fortunately, all individuals from both groups were non-smokers and not heavy alcohol drinkers. The mean age of both groups was 27 ± 5 years. All individuals answered a questionnaire about their occupational and non-occupational exposure and confounding lifestyle factors. This study was approved by the Research Ethics Committee of the University of Brasília. Hematological analyses were run on an automated analyser ADVIA 1650 (Bayer Diagnostics). A profile of exposed and control groups is shown in Table 1.

Micronucleus in exfoliated cells from urinary bladder

Exfoliated cells of the urinary bladder were obtained after urine centrifugation; two slides were prepared by smearing, fixed with methanol and stained by Giemsa. Two thousand

Table 1. Characterization of exposed and non-exposed groups.

Control group	Gender	Age (years)	Exposed group	Gender	Age (years)	Years of exposure	Working h/week
1	M	22	1	M	25	7	40
2	F	26	2	M	37	5	40
3	F	27	3	F	27	7	40
4	M	27	4	F	24	7	40
5	M	30	5	M	26	7	40
6	F	30	6	M	28	7	40
7	M	35	7	F	28	3	40
8	M	29	8	F	31	11	40
9	M	24	9	M	31	5	40
10	M	27	10	M	29	6	40
			11	M	26	8	40
			12	M	28	5	40
			13	F	21	1	40
			14	F	23	1	16
			15	M	29	2	16
			16	M	35	2	16
			17	M	25	1	16
			18	M	23	2	16
			19	M	34	2	16
			20	M	25	1	16
			21	M	30	1	16

M = Male; F = Female.

non-fragmented cells from each individual were observed for micronucleus scoring. Exfoliated cell preparations contain a large number of degenerated cells. Epithelial cells with intact nuclear structure were differentiated from cells that have undergone karyolysis and karyorrhexis in order to avoid artifacts in identifying micronucleus.

Chromosome aberration in peripheral lymphocytes

Venous blood was drawn into heparinized tubes. Lymphocyte cultures were set up by adding 0.5 mL whole blood to 4.5 mL RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum (Gibco), 2% phytohemaglutinin, 1.5% penicillin-streptomycin (5000 IU-5000 µg/mL; Sigma). Cultures were grown at 37°C for 48 h. Slides were prepared according to standard methods and stained by Giemsa. A total of 100 well-spread metaphases containing 46 ± 1 chromosomes for each individual were examined on coded slides. Chromosome and chromatid type aberrations were recorded. Blood and urine samples were collected at the same time at the end of one week.

Statistical analyses for both assays were carried out through the Mann-Whitney U-test with significance ($P < 0.05$). The correlation between chromosome aberration and micronucleus was analyzed through the Spearman rank test. Data were analyzed using the SIGMASTAT 3.1 package.

RESULTS

Our results showed increases in the number of chromosomal aberrations in peripheral lymphocytes with a mean of 3.19 ± 0.82 in the exposed group and a mean of 0.10 ± 0.10 in the control group, as well as in the number of micronuclei in the exfoliated cells from the urinary bladder (mean 3.28 ± 0.90) in the exposed group, compared with the respective control group (mean 0.40 ± 0.22) (Figure 1). The most frequent chromosome damage found in the exposed

group was chromatid break. A high correlation was observed between chromosomal aberrations and micronuclei in the exposed group (Spearman rank, r = 0.73, P = 0.0001). No relationship between exposure-time and chromosome damage was observed. All individuals were also submitted to a hematological examination. Results of hematological evaluation showed no differences in relation to reference values (Table 2).

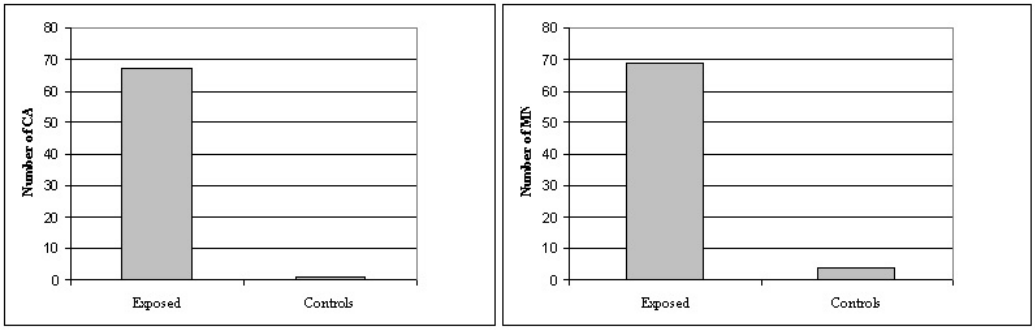

Figure 1. Differences between the exposed group and the respective control in relation to chromosomal aberration (CA) in peripheral lymphocytes and micronuclei (MN) in urine exfoliated cells. Y-axis shows the number of CA and MN found in both groups, respectively.

Table 2. Main values of the hematological evaluations in both groups and reference values.

	Erythrocytes x 10^9/mm^3	Leukocytes/mm^3	Lymphocytes/mm^3	Platelets/mm^3
Exposed	5.01 ± 0.46	6467 ± 1264	2289 ± 599	237,000 ± 51,000
Control	5.72 ± 0.39	6450 ± 1258	2612 ± 481	216,000 ± 64,000
Reference	4.0 to 6.1	3700 to 11000	740 to 5500	140,000 to 45,0000

DISCUSSION

Exfoliated cells hold strong potential as a tool for biomonitoring human populations exposed to genotoxic agents because they can be easily collected from the mouth, nose, and bladder by noninvasive procedures. In addition, more than 90% of cancers arise in epithelial tissues; in many cases, these tissues are the actual targets of carcinogens, as indicated by the sites of cancers related to the exposures (Fenech et al., 1999). Increases in the micronucleus frequency in exfoliated cells were also observed as a result of exposure to pesticides, neoplastic drugs, radiotherapy, smoking, and arsenic in drinking water (Fenech et al., 1999). Chromosome aberrations in human lymphocytes has been established as a sensitive method for the detection of genotoxicity caused by environmental mutagens in populations exposed to genotoxicants, through direct contact with ingested or inhaled compounds (Salama et al., 1999).

Chronic exposure to the volatile fraction of petrol means exposure to benzene, toluene and xylene. Toluene and xylene have not been proven to be genotoxic or carcinogenic for humans or laboratory animals (WHO, 1986, 1997). Exposure to benzene induces hemato-lymphoid toxicity, including pancytopenia, aplastic anemia, myelodysplastic and myeloid leukemia (Infante and White, 1983). The ultimate metabolite of benzene, 1,2,4-benzenetriol, causes DNA

damage resulting in sister-chromatid exchanges, micronuclei and chromosomal aberrations in cultured human lymphocytes (Andersson and Hellman, 2007). Many studies have shown that benzene exposure induces DNA damage through classical cytological tests (Barreto et al., 2009). Therefore, from the findings presented in this study, benzene and its metabolites can be responsible for the increased frequency of micronucleus and chromosome aberrations in the occupationally exposed group. In conclusion, although no hematological disturbance was found in the exposed group, indoor exposure to petroleum derivates revealed genotoxicity.

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