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RESEARCH ARTICLE

Induced cytotoxic damage by exposure to gasoline vapors: a study in Sinaloa, Mexico

Carmen Martinez-Valenzuela[1] · Fernanda Balderrama Soto[1] · Stefan M. Waliszewski[2] ·
Enrique Meza[2] · Sandra Gómez Arroyo[3] · Luis Daniel Ortega Martínez[1] ·
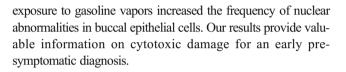Eliakym Arambula Meraz[4] · Mario Caba[2]

Abstract Gasoline is a blend of organic compounds used in internal combustion engines. Gasoline-station attendants are exposed to gasoline vapors, which pose a potentially mutagenic risk. According to the International Agency for Research on Cancer, exposure to gasoline and engine exhaust is possibly carcinogenic to humans. We determined the frequency of micronucleus and other nuclear abnormalities, such as pyknotic nuclei, chromatin condensation, cells with nuclear buds, karyolytic cells, karyorrhexis, and binucleated cells in buccal mucosal smears of 60 gasoline-station attendants and 60 unexposed controls. In addition, we explored if factors such as smoking habits, alcohol consumption, and worked years exert an additional synergistic cytotoxic effect. There were statistically significant higher frequencies ($p < 0.05$) of nuclear abnormalities among exposed attendants compared to the controls. No statistical significant ($p > 0.05$) additional effect of lifestyle habits such as smoking and alcohol consumption or worked years on the cytotoxicity was observed. The results showed that from the beginning exposure to gasoline vapors increased the frequency of nuclear abnormalities in buccal epithelial cells. Our results provide valuable information on cytotoxic damage for an early pre-symptomatic diagnosis.

Keywords Gasoline exposure · Nuclear biomarkers · Cytotoxicity

Introduction

The gasoline used as a fuel for engines in cars is a volatile, flammable liquid and colorless to pale brown or pink in color with a distinctive odor. Gasoline is a mixture of petroleum hydrocarbons containing cycloalkanes with 5 to 18 carbons, olefins (alkenes), and aromatic hydrocarbons, including benzene, toluene, and xylenes (ATSDR 2014). Exposure to gasoline vapor has been shown in several studies to induce elevated frequencies of micronucleated buccal cells (Högstedt et al. 1991; Celik et al. 2003; Benites et al. 2006; Martins et al. 2009; Singaraju et al. 2012) and lymphocytes (Högstedt et al. 1991) in humans, as well as increased frequencies of micronucleated lymphocytes, associated with an increased risk of cancer (Bonassi et al. 2011) along with other diseases (Torres-Bugarín et al. 2015).

Traffic enforcers and gasoline-station attendants are daily exposed to such substances placing them at a greater cytotoxic risk. Epidemiologic methods use several biomarkers to evaluate the risk of deoxyribonucleic acid (DNA) damage originated by exposure to environmental substances (Neumann 2009). One such biomarker is the formation of micronuclei (MN) from chromosome fragments or whole chromosomes that lag behind in anaphase and are excluded from the main nucleus during mitosis (Holland et al. 2008). These acentric fragments or loss of chromosomes generate small nuclei that, when stained, are similar to

✉ Carmen Martinez-Valenzuela
maria.martinezv@udo.mx

[1] Instituto de Investigación en Ambiente y Salud, Universidad de Occidente, Boulevard Macario Gaxiola y Carretera Internacional, Los Mochis, Sinaloa, Mexico

[2] Centro de Investigaciones Biomédicas, Universidad Veracruzana, Xalapa, Ver, Mexico

[3] Centro de Ciencias de la Atmósfera, UNAM, Circuito Exterior Ciudad Universitaria, 04510 Coyoacán, Mexico

[4] Laboratorio de Genética y Biología Molecular, Maestría en Ciencias Biomédicas, Facultad de Ciencias Químico Biológicas, Universidad Autónoma de Sinaloa, Av. de las Américas y Universitarios s/n Ciudad Universitaria, 80010 Culiacán, Sinaloa, Mexico

the main nuclei, but are much smaller; therefore, they are referred to as MN (Ramos et al. 2014). Once formed, MN persist in the cytoplasm of daughter cells. An increased incidence of these micronucleated cells is an indicator of chromosome damage from the recent exposure to genotoxic agents (Kang et al. 2013; Clark et al. 2014; Schreiner et al. 2014). Several reports indicate that the presence of MN in oral mucosa serves as a reliable biomarker to detect cytogenetic damage in human tissues of gas station attendants (Högstedt et al. 1991; Santos-Mello and Cavalcante 1992; Benites et al. 2006).

Buccal cells are the first barrier for the inhalation or ingestion route, and they are capable of metabolizing proximate carcinogens to reactive products and offer a model for mutation research (Kashyap and Reddy 2012). Determination of cytogenetic damage is of major relevance for the assessment of occupational health of workers, a poorly addressed concern in Mexico. With this in mind, we determined the frequency of MN and other nuclear abnormalities in buccal epithelial smears of gasoline-station attendants and unexposed to gasoline vapor individuals from Los Mochis, Sinaloa, Mexico. Additionally, we explored if factors such as smoking habits, alcohol consumption, and worked years have any additional synergistic effect on the occurrence of cells with MN and other nuclear abnormalities in exposed participants.

Material and methods

Subjects

The study groups were randomly formed from the population of the city of Los Mochis, Sinaloa, Mexico, consisting of 60 gasoline-station attendants occupationally exposed to gasoline vapors and 60 unexposed control individuals who were engaged in other tasks and reported no history of occupational exposure to gasoline. All participants, during sampling, answered a questionnaire about age and lifestyle habits, such as smoking and alcohol consumption; additionally, gasoline attendants were asked about worked years to calculate the influence of these factors on nuclear abnormality frequencies.

The Ethics Committee of the *Universidad de Occidente* approved this study and all participants involved gave their informed consent prior to intervention.

Sample collection and staining

Participants were asked to rinse their mouths with water. Thereafter, exfoliated buccal mucosal cells were obtained from the inside of both cheeks by gently scrubbing the mucosa with a sterile wooden spatula. The samples were placed on cleaned and coded slides to avoid bias in counting, air-dried, and fixed with methanol-acetic acid (3:1), then stained using the Feulgen reaction (Martínez-Valenzuela et al. 2009).

Scoring procedure and criteria

Genetic abnormalities were evaluated according to Stich and Rosin (1984), Stich (1987), and Martínez-Valenzuela et al. (2009) procedures. Three thousand differentiated basal cells per subject on coded slides were scored using a light microscope by two observers unaware of the identity of the subjects. The cells were counted and classified according to Tolbert et al. (1992), Thomas et al. (2009), and Bolognesi and Fenech (2013) to determine the frequency of micronuclei and other nuclear abnormalities. Assessed nuclear abnormalities were karyolysis (nucleus depleted of DNA, in which a Feulgen-negative ghost-like image of the nucleus persists), pyknosis (the nucleus is about one-third to two-thirds the size of a nucleus in normal differentiated cells), karyorrhexis (nuclear disintegration related to the loss of nuclear membrane integrity), binucleated cells (contain two nuclei and are most commonly found in cancer cells, they may arise from a variety of causes), nuclear buds (small amounts of genetic material attached to the main cell nucleus and considered as indicators of genotoxic exposure resulting in chromosome aberrations), and chromatin condensation (an indicator of cell apoptosis). Degenerated cells were observed but not scored.

Statistics

Statistical analyses were conducted using the Minitab statistical software version 12 (Minitab Inc. State College, PA, USA), where continuous variable values of micronucleus and other nuclear abnormalities were expressed by descriptive statistics as arithmetic means ± standard errors of means (SEM). In order to determine the degrees of associations between exposed and control groups, the parametric t test was applied, which compares the equality of two population means, considering $p < 0.05$ as statistically significant for all analyses. To see if the time of exposure expressed as worked years could have an impact on the magnitude of nuclear abnormality frequencies, multivariate factor analysis, which uses factor analysis, like principal components, to summarize the covariance structure data in only two dimensions and which explains the magnitude of association between nuclear abnormality frequencies and worked years was used. However, the factor analysis did not identify the underlying factors to explain the dimensions associated with nuclear abnormalities and worked years. In addition, the exposed attendants were grouped in three tertiles according to worked years: from 1 to 2 years, from 3 to 10 years, and from 11 to 29 years and analyzed by one-way analysis of variance (ANOVA symmetric distribution with Tukey's post hoc analysis). Moreover, to compare differences of biomarker frequencies between exposed attendants and controls, the fold-increase (FI), which reveals the magnitude of differences among nuclear abnormalities observed in exposed workers compared to controls, was

Table 1 Comparison of mean nuclear biomarkers between gasoline-station attendants (exposed $n = 60$) and unexposed controls ($n = 60$)

		Mean ± SEM	*t* test *p* value	Fold-increase (FI)
Micronucleus	Exposed	6.6 ± 0.5	0.001*	3.66
	Controls	1.8 ± 0.2		
Pyknotic nuclei	Exposed	78.4 ± 9.6	0.001*	2.69
	Controls	29.1 ± 3.8		
Chromatin condensation	Exposed	164.1 ± 16.8	0.001*	14.52
	Controls	11.3 ± 1.5		
Nuclear buds	Exposed	6.3 ± 0.8	0.030*	1.70
	Controls	3.7 ± 0.8		
Karyolytic cells	Exposed	4.8 ± 0.6	0.000*	3.0
	Controls	1.6 ± 0.2		
Karyorrhexis	Exposed	3.0 ± 0.4	0.001*	2.0
	Controls	1.5 ± 0.2		
Binucleated cells	Exposed	6.5 ± 0.8	0.001*	2.50
	Controls	2.6 ± 0.7		

Mean corresponds to number of abnormalities counted in 3000 scored cells

SEM standard error of mean

*Statistically significant differences at $p < 0.05$

calculated. The FI permits to correlate the differences among studied groups and reflects the intensity and character of cytotoxicity caused by environmental exposure.

Results

There were no statistically significant differences ($p > 0.05$) between the age of the exposed gasoline attendants and unexposed controls (37.9 vs. 34.9 years). Comparisons among biomarkers studied indicated a statistically significant ($p < 0.05$) higher number of damaged cells among exposed attendants for most nuclear biomarkers (Table 1). To compare the degree of biomarker frequencies and the magnitude of differences between exposed attendants and controls, the FIs were calculated. The highest FI value, 14.52, corresponded to chromatin condensation, followed by cells with micronucleus, 3.66; karyolytic cells, 3.0; pyknotic nuclei, 2.69; binucleated cells, 2.50; karyorrhexis, 2.0; and cells with nuclear buds, 1.70 (Table 1). There were statistically significant differences in all nuclear biomarkers and significantly higher values were observed in exposed gasoline attendants compared to unexposed controls.

Subsequently, we explored the impact of occupational environmental exposure, comparing the cytotoxicity induced by the synergistic effect of lifestyle habits, such as smoking and

Table 2 Comparison of mean nuclear biomarkers among 60 exposed gasoline-station attendants divided into smoking ($n = 16$) and non-smoking ($n = 44$) individuals

		Mean ± SEM	*t* test *p* value	Fold-increase (FI)
Micronucleus	Smoking	5.5 ± 0.9	0.127	0.77
	Non-smoking	7.1 ± 0.6		
Pyknotic nuclei	Smoking	83.3 ± 23.3	0.795	1.08
	Non-smoking	76.6 ± 10.2		
Chromatin condensation	Smoking	210.0 ± 43.8	0.195	1.42
	Non-smoking	147.4 ± 16.1		
Nuclear buds	Smoking	5.8 ± 1.4	0.722	0.91
	Non-smoking	6.4 ± 0.9		
Karyolytic cells	Smoking	4.4 ± 1.0	0.609	0.88
	Non-smoking	5.0 ± 0.6		
Karyorrhexis	Smoking	3.2 ± 0.8	0.806	1.10
	Non-smoking	2.9 ± 0.4		
Binucleated cells	Smoking	3.7 ± 1.0	0.013*	0.49
	Non-smoking	7.5 ± 1.0		

Mean corresponds to number of abnormalities counted in 3000 scored cells

SEM standard error of mean

*Statistically significant differences at $p < 0.05$

Table 3 Comparison of nuclear biomarkers among 44 non-smoking exposed gasoline-station attendants and 45 non-smoking unexposed controls

		Mean ± SEM	t test p value	Fold-increase (FI)
Micronucleus	Exposed	7.1 ± 0.6	0.002*	4.17
	Controls	1.7 ± 0.2		
Pyknotic nuclei	Exposed	76.6 ± 10.2	0.021*	2.47
	Controls	31.0 ± 4.7		
Chromatin condensation	Exposed	147.4 ± 10.2	0.000*	12.38
	Controls	11.9 ± 1.9		
Nuclear buds	Exposed	6.4 ± 0.9	0.040*	1.52
	Controls	4.2 ± 1.0		
Karyolytic cells	Exposed	5.0 ± 0.7	0.143	3.57
	Controls	1.4 ± 0.2		
Karyorrhexis	Exposed	2.9 ± 0.4	0.204	2.07
	Controls	1.4 ± 0.2		
Binucleated cells	Exposed	7.5 ± 1.0	0.048*	2.42
	Controls	3.1 ± 1.0		

Mean corresponds to number of abnormalities counted in 3000 scored cells

SEM standard error of mean

*Statistically significant differences at $p < 0.05$

alcohol consumption; results are shown in Tables 2, 3, 4, and 5. Regarding smoking, gasoline-station attendants with a smoking habit as compared to non-smoking did not reveal a statistically significant influence ($p > 0.05$) of smoking on the frequency of cytotoxic damage on the monitored cells (Table 2). The absence of smoking was compared between 44 non-smoking gasoline attendants and 45 non-smoking controls; results are shown in Table 3. The results revealed significantly higher nuclear abnormality frequencies expressed by chromatin condensation frequencies (FI = 12.38) and other abnormalities in exposed attendants, indicating accelerated apoptosis processes in this group by exposure to gasoline vapors. The results indicate statistically significant higher values ($p < 0.05$) for all nuclear abnormalities, as well as higher FI, in

exposed attendants compared to unexposed controls (Table 4). The higher abnormality frequencies were due to occupational exposure to gasoline vapors. Comparisons suggest that alcohol consumption did not exert a significant additional effect on the magnitude of nuclear abnormalities observed among gasoline-station attendants, because the primary damage was caused by inhalation of gasoline vapors. When only alcohol consumption and lack of alcohol consumption among gasoline attendants were compared, no significant differences ($p > 0.05$) were noted in most biomarkers, but we found higher binucleated cell frequencies ($p < 0.05$) in non-alcohol consumers (Table 5). These results indicate that alcohol per se had no effect on the increased nuclear abnormality frequency values found among exposed gasoline attendants.

Table 4 Comparison of mean nuclear biomarkers between 37 exposed gasoline-station attendant's alcohol consumers and 38 unexposed alcohol consumer controls

		Mean ± SEM	t test p value	Fold-increase (FI)
Micronucleus	Exposed	6.1 ± 0.6	0.000*	2.90
	Controls	2.1 ± 0.2		
Pyknotic nuclei	Exposed	73.0 ± 10.0	0.000*	2.81
	Controls	26.0 ± 4.3		
Chromatin condensation	Exposed	154.7 ± 19.7	0.000*	14.32
	Controls	10.8 ± 1.6		
Nuclear buds	Exposed	6.7 ± 1.1	0.023*	1.61
	Controls	3.3 ± 0.9		
Karyolytic cells	Exposed	5.0 ± 0.7	0.000*	3.12
	Controls	1.6 ± 0.3		
Karyorrhexis	Exposed	2.7 ± 0.5	0.022*	1.80
	Controls	1.5 ± 0.2		
Binucleated cells	Exposed	5.1 ± 0.9	0.086	1.76
	Controls	2.9 ± 0.8		

Mean corresponds to number of abnormalities counted in 3000 scored cells

SEM standard error of mean

*Statistically significant differences at $p < 0.05$

Table 5 Comparison of mean nuclear biomarkers in exposed gasoline-station attendants between 37 alcohol consumers and 23 non-alcohol consumers

	Alcohol	Mean ± SEM	t test p value	Fold-increase (FI)
Micronucleus	Yes	6.1 ± 0.6	0.189	0.82
	No	7.4 ± 0.9		
Pyknotic nuclei	Yes	71.1 ± 9.9	0.703	0.79
	No	90.2 ± 19.5		
Chromatin condensation	Yes	150.8 ± 19.6	0.395	0.81
	No	185.4 ± 30.5		
Nuclear buds	Yes	6.7 ± 1.1	0.557	1.17
	No	5.7 ± 1.1		
Karyolytic cells	Yes	4.9 ± 0.7	0.698	1.06
	No	4.6 ± 1.0		
Karyorrhexis	Yes	2.7 ± 0.4	0.291	0.73
	No	3.7 ± 0.7		
Binucleated cells	Yes	4.9 ± 0.8	0.046*	0.54
	No	9.1 ± 1.5		

Mean corresponds to number of abnormalities counted in 3000 scored cells

SEM standard error of mean

*Statistically significant differences at $p < 0.05$

Table 6 summarizes the FIs for all the analyzed nuclear materials affected by gasoline exposure. The highest frequencies correspond to chromatin condensation, pyknotic nuclei, binucleated cells, and micronucleus. Nuclear buds and karyorrhexis were the nuclear abnormalities with the lowest FIs in exfoliated buccal cells.

Finally, we explored the effect of worked years by applying multivariate factor analysis, summarizing the data of covariance in a two-dimension structure, and calculating the magnitude of association between nuclear abnormality frequencies and worked years. As a measure of internal consistency, Cronbach's alpha (α) was calculated to assess internal consistency and to determine how highly these items are correlated and how well they predict each other. The obtained results for worked years were low: micronucleus, $\alpha = 0.019$; pyknosis, $\alpha = -0.045$; chromatin condensation, $\alpha = -0.042$; nuclear buds, $\alpha = -0.842$; karyolysis, $\alpha = -0.274$; karyorrhexis, $\alpha = -0.005$; binucleated cells, $\alpha = -0.101$, suggesting that the items have little in common and are not good measures of the single construct. Moreover, the polynomial regression analysis, which relates nuclear abnormalities to worked years, reveals higher p values expressing the worked years as a not significant predictor for nuclear abnormalities and lower R^2 values, which indicates a lower variability in the response of this model and demonstrates that the data are not evenly spread about the regression line, implying lack of fit.

Therefore, to assess the possible particular differences among exposed gasoline attendants, the participants were grouped in three tertiles from 1 to 2, 3 to 10, and 11 to 29 years (Table 7). As a result of gasoline exposure, all nuclear biomarkers showed higher frequencies from the beginning of exposure to gasoline vapors and thereafter; the biomarkers

Table 6 Comparison of nuclear biomarker fold-increases between exposed gasoline-station attendants and unexposed controls

	Exposed/controls	Exposed smoking/exposed non-smoking	Exposed non-smoking/controls non-smoking	Exposed alcohol consumers/controls alcohol consumer	Exposed alcohol consumers/exposed non-consumer alcohol
Micronucleus	3.66	0.77	4.17	2.90	0.82
Pyknotic nuclei	2.69	1.08	2.47	2.81	0.79
Chromatin condensation	14.52	1.42	12.38	14.32	0.81
Nuclear buds	1.70	0.91	1.52	1.61	1.17
Karyolytic cells	3.0	0.88	3.57	3.12	1.06
Karyorrhexis	2.0	1.10	2.07	1.80	0.73
Binucleated cells	2.50	0.49	2.42	1.76	0.54

Table 7 Comparison of mean nuclear biomarker frequencies from exposed gasoline-station attendants divided in tertiles according to the worked years

Variable, tertiles	Mean ± SEM	ANOVA p value
Micronucleus 1	6.91 ± 0.98	0.90
Micronucleus 2	6.62 ± 0.76	
Micronucleus 3	6.28 ± 0.91	
Pyknotic nuclei 1	101.1 ± 24.6	0.38
Pyknotic nuclei 2	67.8 ± 11.6	
Pyknotic nuclei 3	75.3 ± 17.7	
Chromatin condensation 1	195.7 ± 46.5	0.497
Chromatin condensation 2	161.5 ± 19.9	
Chromatin condensation 3	141.7 ± 28.1	
Nuclear buds 1	7.2 ± 1.4	0.450
Nuclear buds 2	6.3 ± 1.2	
Nuclear buds 3	4.4 ± 1.1	
Karyolytic cells 1	5.1 ± 1.1	0.802
Karyolytic cells 2	5.1 ± 0.9	
Karyolytic cells 3	4.2 ± 1.1	
Karyorrhexis 1	4.0 ± 1.1	0.272
Karyorrhexis 2	2.5 ± 0.4	
Karyorrhexis 3	2.7 ± 0.5	
Binucleated cells 1	6.8 ± 1.6	0.901
Binucleated cells 2	6.8 ± 1.5	
Binucleated cells 3	6.0 ± 1.0	

Mean corresponds to number of abnormalities counted in 3000 scored cells. Worked years are from 1 to 2 years (*1*), from 3 to 10 years (*2*), and from 11 to 29 years (*3*)

SEM standard error of mean

*Statistically significant differences at $p < 0.05$

showed a decreasing tendency in the subsequent years without statistically significant ($p > 0.05$) changes among mean values expressed by the ANOVA test.

Discussion

Our results indicate that epithelial cells of the oral mucosa from gasoline-station attendants depict cytotoxic damage, originated principally by occupational exposure to gasoline vapors. Exposed attendants had a higher mean of damaged cells compared to non-exposed individuals, as revealed by chromatin condensation (164.1 vs. 11.3) and MN (6.6 vs. 1.8). The mean MN in controls was 1.8 per 3000 cells (0.6/1000 cells), which is within the normal range for the human oral epithelium (0.5–2.5 MNC/1000) (Holland et al. 2008; Ceppi et al. 2011). However, this frequency can increase due to several factors and the individual variability among subjects was rather large (Ceppi et al. 2011). MN increases (12-fold) due to formaldehyde exposure (Suruda et al. 1993) and 4.34-fold increases in petrol station attendants (Sellappa et al. 2010)

have been reported. Our results agree with the latter two studies and those of others regarding diesel fuel and vehicle exhaust gas exposures (Celik and Akbas 2005; Benites et al. 2006; Hallare et al. 2009; Martins et al. 2009; Sellappa et al. 2010). The increase in MN is considered to be a predictor of an elevated risk for cancer (Knudsen and Hansen 2007; Ceppi et al. 2011). In general, our results indicate that exposure to gasoline vapors increases the frequency of nuclear abnormalities such as chromatin condensation, micronucleus, karyolysis, karyorrhexis, and binucleated cells. Additionally, we found that the mean fold-increase (FI) was a good measure to express the cytotoxic damage magnitude (Hallare et al. 2009; Sellappa et al. 2010; De Marini 2013; Martínez-Valenzuela et al. 2015). We observed higher values of FIs in exposed attendants; it is to be noted that chromatin condensation (14.52) and MN (3.66) had the highest values.

In relation to lifestyle habits, such as smoking and alcohol consumption, we did not find a synergistic effect of these agents on the frequency of most nuclear abnormalities. Tobacco smoke is known for containing numerous genotoxic chemicals, although the literature is contradictory. It has been reported that using tobacco increases the risk of having a high frequency of micronuclei in buccal mucosa cells (Gabriel et al. 2006). However, in agreement with our observations, several authors found that the occurrence of cells with micronucleus was not significantly associated with smoking habit status in individuals that were occupationally exposed to fuel derivatives (Benites et al. 2006; Roma-Torres et al. 2006; Hallare et al. 2009; Martins et al. 2009; Demircigil et al. 2014).

Alcohol is also described as a genotoxic substance (García et al. 2012). However, in our study, its consumption was not significantly related to the increase in frequency of nuclear abnormalities in the buccal epithelium of gasoline attendants. Our results agree with those of Benites et al. (2006) and Swick et al. (2014), who observed a small but not significant increase of micronuclei, binucleated cells, and cells with nuclear buds, among exposed individuals with an alcohol consumption habit. We did not find an influence or synergistic effects of lifestyle habits, and our results suggest that the significant increase of nuclear abnormality frequencies among gasoline attendants was principally due to the constant exposure to chemicals volatilized at the gasoline station.

The multivariate analysis of nuclear abnormality frequencies in relation to worked years showed no statistically significant differences in the magnitude of the cytotoxic damage and in nuclear abnormality frequencies; these data agree with other studies done in gasoline-station attendants (Benites et al. 2006; Hallare et al. 2009). In contrast, Fenech (2002) and Mrdjanović et al. (2014) showed that attendants with a longer occupational exposure had a significantly higher incidence of micronuclei and other nuclear abnormalities, but the difference of these workers consists in that they were exposed to petrol derivative chemicals in a petrol refinery. The kinetics of

replication and half-life of buccal cells may affect micronucleus expression, but these differences become unimportant in chronic exposure, a phenomenon that was observed in our studies when analyzing nuclear abnormality frequencies during worked years, where the increase was expressed at the beginning of gasoline exposure. The posterior steady state is caused because chronic exposure leads to a steady-state elevated expression level of nuclear abnormalities, regardless of division rate if the period of exposure exceeds the time frame for one nuclear division (Fenech 2002).

In summary, the present results indicate a significant association between the occupational exposure to gasoline vapors and the occurrence of nuclear abnormalities in buccal epithelial cells from the gasoline attendants in Sinaloa, Mexico. We consider that biomonitoring studies of nuclear damage are extremely important to detect early potential health concerns and should be an issue of utter relevance for public health institutions.

This study addresses a concern that has not been covered in Mexico, that is, the damage induced by constant exposure to gasoline vapors as occurs with gasoline-station attendants, and provides important information that can be compared to results obtained in other countries. We also emphasize the importance of the biomarker used in this study, that is, MN as a scarcely invasive technique but of great sensitivity to detect damage in the cells at an early stage when faced with diseases as severe as cancer.

Conclusions

Nuclear biomarker studies used to analyze the buccal cells and nuclear abnormalities are instrumental to assess the potential alterations in cellular kinetics, metabolism, structural profile of the buccal mucosa, as well as genomic instability events. We obtained valuable information to detect potentially degenerative diseases during early pre-symptomatic diagnoses. The used MN assay plays an important role in identifying individuals with a higher health risk. Gasoline-station attendants depict an increased frequency of cells with nuclear abnormalities, a phenomenon that probably was caused by the cytotoxic effect of gasoline vapors, to which they are exposed. The DNA damage biomarkers in the studied population confirm the extensive evidence that links the frequency of nuclear abnormalities to environmental and occupational exposures to genotoxic agents, such as gasoline. Our results, which revealed divergence in the effects of occupational exposure on nuclear abnormalities, may provide a starting point for defining assessment groups of persons with a cumulative health risk, as expressed by the magnitude of nuclear abnormalities observed since the early contact with the volatile compounds contained in gasoline.

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