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RESEARCH ARTICLE

Biomonitoring of agricultural workers exposed to pesticide mixtures in Guerrero state, Mexico, with comet assay and micronucleus test

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Abstract The aim of this study was to evaluate the genotoxic effect of pesticides in exfoliated buccal cells of workers occupationally exposed in Guerrero, Mexico, using the comet assay and the micronucleus test. The study compared 111 agricultural workers in three rural communities (Arcelia 62, Ajuchitlan 13, and Tlapehuala 36), with 60 non-exposed individuals. All the participants were males. The presence of DNA damage was investigated in the exfoliated buccal cells of study participants with the comet assay and the micronucleus (MN) test; comet tail length was evaluated in 100 nuclei and 3000 epithelial cells of each individual, respectively; other nuclear anomalies such as nuclear buds, karyolysis, karyorrhexis, and binucleate cells were also evaluated. Study results revealed that the tail migration of DNA and the frequency of MN increased significantly in the exposed group, which also showed nuclear anomalies associated with cytotoxic or genotoxic effect. No positive correlation was noted between exposure time and tail length and micronuclei frequencies. No significant effect on genetic damage was observed as a result of age, smoking, and alcohol consumption. The MN and comet assay in exfoliated buccal cells are useful and minimally invasive methods for monitoring genetic damage in individuals exposed to pesticides. This study provided valuable data for establishing the possible risk to human health associated with pesticide exposure.

Keywords Genotoxicity · Pesticide exposure · Buccal exfoliated cells

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Introduction

In Mexico as well as in other countries, the use of pesticides has increased because agriculture is crucial for food production and socioeconomic development. The Mexican population dedicated to agricultural activity is about eight million individuals (AMIFAC 2015).

The cases of intoxication and death are primarily due to the lack of protective equipment and inadequate handling of pesticides, as well as to the high level of illiteracy and poor education which prevent the agricultural workers from being aware of the risk that the direct and indirect exposure to pesticides constitutes.

Pesticides are one of the major sources of pollution from synthetic products generated as a result of agricultural activity. Some are forbidden or restricted in many countries. Examples of this are insecticides such as organochlorine, endosulfan, organophosphorus, azinphos-methyl, parathion-methyl, methamidophos, omethoate, malathion; carbamates such as aldicarb and carbofuran; fungicides such

as pentachlorophenol; and herbicides such as paraquat and atrazine, among others (Bejarano 2015). The reason for this prohibition is that they are toxic for humans and they affect natural resources, yet in Mexico, there is an indiscriminate use which increases the risk of exposure to them on account of their genotoxic action.

Furthermore, different biomarkers are useful as end points in subjects occupationally exposed to genotoxic agents. The alkaline single-cell gel electrophoresis assay or comet assay has been a useful tool for human biomonitoring studies in the detection of DNA single-strand breaks, alkali-labile sites, and incomplete excision repair events. This assay is a fast and sensitive tool for detecting the damaging effects of different compounds on DNA at an individual cell level, where cells in which DNA is damaged display an increased migration of DNA fragments from the nucleus originating a comet shape, given that during alkali gel electrophoresis the broken DNA strands move towards the anode and form a comet (Singh et al. 1988; Fairbairn et al. 1995). Besides, the displacement of DNA from the nucleus could be used as an indicator of DNA damage (Östling and Johanson 1984). Each damaged cell has the appearance of a comet with a bright head and tail; the undamaged cells appear intact or with complete nuclei and no tail (Möller 2006).

Some studies have employed the comet assay to evaluate the genotoxic effects of pesticides in human populations (Ramírez and Cuenca 2002; Ascarrunz et al. 2006; Castillo-Cadena et al. 2006; Paz-y-Miño et al. 2007; Da Silva et al. 2008; Muñoz Aristizábal 2009; Remor et al. 2009; Simoniello et al. 2010; Peralta et al. 2011; Benedetti et al. 2013; Wilhelm et al. 2015).

The micronuclei (MN) are evidence of chromosomal damage and have been suggested as predictive biomarkers of cancer risk in humans (Bonassi et al. 2005, 2007).

The MN assay carried out in exfoliated buccal cells constitutes a minimally invasive method for monitoring populations exposed to genotoxic agents (Bonassi et al. 2009, 2011). Micronuclei are formed by chromosomal damage in the basal cells of the epithelium; when these cells break up, chromosomal fragments or entire chromosomes that lack an attachment to the spindle apparatus are excluded from the main nuclei in the daughter cells and they appear as Feulgen-specific bodies in the cytoplasm. The MN assay in human exfoliated cells has been widely used to detect the genotoxic effects of environmental mutagens (Gómez-Arroyo et al. 2000; Pastor et al. 2003; Martínez-Valenzuela et al. 2009; Bolognesi et al. 2011; Bonassi et al. 2011).

The aim of the present study was to evaluate the genotoxic effect produced by pesticide mixtures, using the comet assay and the micronucleus test in exfoliated buccal cells of workers occupationally exposed in the area of Arcelia, Ajuchitlan, and Tlapehuala located in a region called Tierra Caliente, Guerrero, in the Mexican Republic.

Material and methods

Study populations

Guerrero state is situated in the southwest of the Mexican Republic. The study was made in 111 agricultural workers exposed to pesticides, living and working in Arcelia, Ajuchitlan, and Tlapehuala located in a region called Tierra Caliente, Guerrero, in the Mexican Republic. This area has a warm climate most of the year, with temperatures higher than 40 °C and on some occasions up to 50 °C. During the time in which this study was made, the temperatures registered were 45 °C. The main economic activity in this area is agriculture, concentrated primarily on the production of corn, sorghum, watermelon, melon, mango, banana, sesame, tomato, chilli, lemon, papaya, and potherb. The non-exposed group, which comprised 60 individuals from the same locations, was selected based on their socioeconomic characteristics that were similar to those of the exposed group; they were not in direct contact with pesticides or any particular environmental agent. The main activity of the control group consists in administrative and commercial functions and office work, and they were recruited after being persuaded to take part in our study. All the participants were males.

This study was performed in accordance with the principles stated in the Declaration of Helsinki. All participants were informed regarding the study and they accepted the idea of taking part in it. They subsequently signed the document in which they agreed to complete a standardized questionnaire with personal data related to age; time of exposure, habits such as smoking and alcohol consumption, drugs, and diets; the type of work performed; and protective measures used. The questionnaire also included a history of recent illness and medical treatment, as well as of their knowledge about the pesticides used in these agricultural areas. The extent of their consumption was mainly organophosphorus (40 %) and carbamates (35 %). Due to the fact that a quantitative estimation of pesticide exposure is difficult to handle, duration of use was substituted for exposure given the difficulty in making a quantitative evaluation of the exposure because it refers mainly to pesticide mixtures. Considering that buccal cells turn over every 7–21 days, the samples were taken out in these intervals following the exposure as has been suggested by Holland et al. (2008). No subject in either the exposed or the non-exposed groups smokes more than five cigarettes per day, drinks more than two beers per day, or is a weekend alcoholic.

Chemicals and reagents

The chemicals used were the following: low melting agarose, normal melting agarose; Tris, dimethylsulfoxide (DMSO), Triton X-100, disodium ethylenediaminetetraacetic acid (EDTA), ethanol, ethidium bromide, and Schiff's reagent, all

purchased from Sigma-Aldrich, St. Luis, MO, USA. Sodium chloride (NaCl), sodium hydroxide (NaOH), acetic acid, and chlorine acid (HCl) were acquired from J.T. Baker, Phillipsburg, NJ, USA.

Comet assay in buccal exfoliated cells

The comet assay was carried out in the buccal epithelial cells. The alkaline comet assay was performed according to the procedure described previously (Singh et al. 1988; Tice et al. 2000; Speit and Hartmann 2006) with some modifications. The subjects were asked to rinse their mouth with water and the buccal cells were collected with a small sterile spoon and resuspended in 1 mL of physiological solution at 37 °C, then centrifuged at 1000 rpm for 10 min. The supernatant was decanted and 50 μL of the cell pellet was resuspended in 50 μL of low melting point agarose (1 % in phosphate buffer). The sample was carefully stirred, dropped on a slide, covered with a coverslip precoated with normal agarose (1 % in phosphate buffer), and kept on ice during the polymerization of each gel layer. Two slides were made per donor. The coverslip was carefully removed and the slides were then immersed in a tank filled with a freshly made lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10), 10 % DMSO, and 1 % Triton X-100 for 24 h at 4 °C.

After lysis immersion, the slides were transferred to a horizontal electrophoresis chamber (Owl A5, Lab System Inc) filled with a freshly made electrophoresis buffer solution (300 mM NaOH and 1 mM Na_2EDTA, pH 13); the DNA was allowed to unwind for 20 min. Electrophoresis was performed for 20 min at 25 V and 300 mA. After electrophoresis, the slides were rinsed three times in a freshly prepared neutralization buffer (0.4 M Tris, pH 7.5), fixed in absolute methanol for 5 min, and stained with 75 μL of ethidium bromide solution (20 μg/mL) for 10 min. The whole process was done under yellow light to prevent the occurrence of additional DNA damage. Two slides were prepared for each participant, 50 cells per slide were analyzed in an epifluorescence Axiostar Plus Carl Zeiss microscope, equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. To visualize DNA damage, we examined slides at ×400 magnification using a micrometric eyepiece/objective (1 unit= 2.41 μm at ×40 magnification). The parameter scored to determine DNA damage was the comet tail length (DNA distance of migration) in the image and it was estimated in micrometers (from the nuclear region to the end of the tail) in 100 consecutive nuclei. All the slides were coded before scoring so as to avoid bias.

Micronucleus test in buccal exfoliated cells

The subjects were asked to rinse their mouth with water and a wooden spatula was used to obtain the sample cells from the buccal mucosa. The sample was then applied to a clean microscope slide. Smears were air-dried and fixed in methanol-acetic acid (3:1). The cell smears were stained using the Feulgen reaction technique described by Stich and Rosin (1984) and Stich (1987). The technique was modified as follows: smears were pretreated with 1 N HCl for 10 min at room temperature, placed for 10 min in 1 N HCl at 60 °C, rinsed in distilled water, put in Schiff's reagent for 90 min, and washed with running tap water. The criteria followed for estimating the frequency of micronucleated cells were according to Stich and Rosin (1984) and Thomas et al. (2009). Three thousand epithelial cells were screened for each individual to determine the MN frequency and other nuclear anomalies such as nuclear buds (NB), karyolysis (KL), karyorrhexis (KR), and binucleate cells (BN), all of which were classified according to Tolbert et al. (1992) and Holland et al. (2008). All the slides were also coded before scoring so as to avoid bias.

Statistical analysis

The comet assay data were analyzed using a Kruskal-Wallis non-parametric test. In the case of micronuclei, analysis was carried out through the ANOVA test. When significant F values were found ($P<0.0001$), the Tukey-Kramer multiple comparison test was used to identify groups showing significant differences at $P<0.001$. The Spearman rank order correlations were applied between age, exposure time to pesticides, as well as between comet tail length and the micronuclei frequency.

Results

The individuals working in an open field and exposed to pesticides (Table 1) in Arcelia, Ajuchitlan, and Tlapehuala, Guerrero, used no protective measures. Table 2 shows the average age of both the exposed and non-exposed groups; it indicates the pesticide exposure time and the type of exposure in the three locations and shows the mean comet tail length for exfoliated buccal cells in the exposed group. Significant differences were observed when the three exposed groups were compared with the non-exposed group using the Kruskal-Wallis non-parametric test ($P<0.001$). Moreover, when Arcelia and Ajuchitlan were compared in relation to exposure time and comet tail length, no statistical differences were found between the two rural communities, but both differed significantly when compared with Tlapehuala. However, no correlation was noted between exposure time of the pesticide mixture and the comet tail length in the whole population.

Table 3 shows the frequencies of micronuclei in the exposed and non-exposed groups. The difference was significant for the ANOVA ($P<0.0001$) and the Tukey-Kramer multiple

Table 1 Pesticides commonly used in Guerrero state by the exposed group

Organochlorines	Organophosphorus	Carbamates	Pyrethroids	Others
Insecticides				
Endosulfan (II)	Parathion-methyl (Ia)	Aldicarb (Ia)	Cypermethrin (III)[b]	
	Azinphos-methyl (Ib)	Carbofuran (Ib)	γ-Cyhalothrin (II)	
	Methamidophos (Ib)	Lannate (Ib)		
	Omethoate (Ib)	Vydate (Ib)		
	Phoxim (II)	Propoxur (II)[a]		
	Diazinon (II)	Pirimicarb (II)[a]		
	Dimethoate (II)[b]			
	Malathion (III)			
Herbicides				
				Paraquat (II)
				2,4,D (III)
				Atrazine (U)
				Glyphosate (U)
				Dicamba (III)
				Picloram (U)
				Fluazifop-p-butyl (III)
				Nicosulfuron (U)
Fungicides				
Pentachlorophenol (Ib)[a]		Mancozeb (U)[b]		Thiabendazole (U)[c]
		Propineb (U)		Benomyl (U)[b]
				Captan (U)

WHO (2010) hazard classification: Ia=extremely hazardous, Ib=highly hazardous, II=moderately hazardous, III=slightly hazardous, U=unlikely to present acute hazard in normal use

[a] IARC (2006) classification: probable human carcinogen

[b] IARC (2006) classification: possible human carcinogen

[c] IARC (2006) classification: likely to be carcinogenic to human at high doses; not likely to be carcinogenic to human at low doses

comparisons test ($P<0.001$). No correlation was observed between the time of exposure and the micronuclei frequency.

The analysis of exfoliated cells of the buccal mucosa also provides evidence of other nuclear anomalies such as the following: binucleate cells (presence of two nuclei within a cell), condensed chromatin (chromatin appears aggregated), nuclear buds (nuclei appear cinched with a Feulgen-negative band), pycnosis (shrunken nuclei), karyorrhexis (disintegrated

Table 2 Number of individuals, age, exposure duration, type of exposure, and comet tail length in exfoliated buccal cells of agricultural workers exposed to pesticide and in non-exposed individuals of the three communities of Guerrero state

Group	Number	Age, years (mean±SE)	Exposure time, years (mean±SE)	Comet tail length, μm (mean±SE)	Type of exposure	
					Workers exposed during one annual cycle (%)	Workers exposed during two annual cycles (%)
Non-exposed	60	37.55±0.2		106.08±2.6		
Arcelia	62	51.16±2.9	15.66±1.3	222.23±8.1*	65	35
Ajuchitlan	13	52.69±4.2	17.50±3.4	194.30±16.4*	69	31
Tlapehuala	36	33.39±3.5	9.94±2.0	155.78±6.7*	64	36
Whole exposed population	111	45.75±3.5	14.36±4.8	190.77±10.4*	66	34

No subject in both the exposed and the non-exposed groups smokes more than five cigarettes per day

*Significant differences among exposed and non-exposed groups were obtained with Kruskal-Wallis non-parametric test Kw=109.909 (corrected for ties) $P<0.001$

Table 3 Frequencies of MN and nuclear anomalies in 3000 cells of the exfoliated buccal cells of agricultural workers exposed to pesticides and non-exposed subjects of the three communities of Guerrero state

	Arcelia	Ajuchitlan	Tlapehuala	Whole exposed population	Non-exposed
MN	1.66±0.20*	3.23±0.65*	2.11±0.31*	2.33±0.39*	0.88±0.56
Binucleate	0.83±0.14*	0.55±0.30*	0.4 1±0.20*	0.60±0.21*	0.09±0.02
Pycnosis	1.13±0.15*	0.54±0.27	1.86±0.29*	1.17±0.24*	0.60±0.14
Con. chromatin	0.27±0.06*	0.54±0.26*	0.06±0.14	0.29±0.15*	0.08±0.04
Nuclear buds	0.29±0.07*	0	0.25±0.09*	0.18±0.05	0.16±0.07
Karyolysis	0.02±0.02	0	0.28±0.11	0.10±0.04	0.24±0.09
Karyorrhexis	0.02±0.01	0	0	0.006±0.003	0.08±0.15

Values obtained as a mean±SE of ‰ of MN and nuclear anomalies in 111 exposed individuals and 60 non-exposed

*Significant differences among exposed and non-exposed were obtained by analysis of variance $F=151.70$, $P<0.0001$, and therefore, the Tukey-Kramer multiple comparisons test was applied $P<0.001$

nuclei), and karyolysis (nuclear dissolution, in which a Feulgen-negative, ghost-like image of the nucleus remains). The binucleate cells were significant in the three locations, and no correlation was found with the exposure time. Pycnosis and nuclear buds were only observed in Arcelia and Tlapehuala, and condensed chromatin was found in Arcelia and Ajuchitlan. Karyolysis and karyorrhexis were not significant in any of the three sites (Table 3).

No significant effect on genetic damage was observed as a result of smoking and alcohol consumption. The same behavior was noted for other confounding factors such as drugs, diet, and others. Age is another factor that did not influence MN frequency.

Sixty-five percent of the agricultural workers of Arcelia, 69 % of Ajuchitlan, and 64 % of Tlapehuala mentioned that they were constantly exposed during one annual cycle (4 to 6 months in a year), and 35, 31, and 36 % indicated two annual cycles (8 to 10 months in a year), respectively; none used personal protective equipment. The individual activity of the workers is diverse: most of them apply, handle, and prepare the pesticide mixture as well as cultivate the land; 16 % keep the pesticides inside their house, 11 % outside their house, and 73 % in sites far from their house. Some of them said they used the same clothes for several days, but the majority mentioned that they wore clean clothes after handling pesticides and they washed their hands before eating.

Furthermore, the data provided by the agricultural workers from the questionnaire in relation with spontaneous abortions in their wives revealed that in Arcelia 22 wives presented 32 cases of spontaneous abortions as well as 3 deaths in premature infants and 2 deaths at the moment of giving birth to offspring. These represent 40.3 % of the total population of the community. In Ajuchitlan, wives of 10 participants presented 13 spontaneous abortions and 5 deaths after giving birth to offspring, which represent 76.92 % of the total population of this community; in Tlapehuala, 4 spontaneous abortions in 3 wives represent 8.33 % of the total population of this community. In all cases, the individuals worked during two annual agriculture cycles.

Discussion

A careful medical examination revealed that some of the pesticide-exposed workers showed acute intoxication and occasional headache, skin and nasal mucosa irritations, as well as nausea when they were in contact with the pesticides. The results of this study indicated that the occupational exposure to a pesticide mixture caused a significant increase in the level of DNA damage. Exposed workers presented significantly longer comet tail lengths in exfoliated buccal cells than the non-exposed individuals. Our results agree with those of other authors who have used the comet assay in human lymphocytes to evaluate DNA damage in the biomonitoring of populations occupationally exposed to pesticides and it has demonstrated positive results (Garaj-Vrhovac and Zeljezic 2000, 2001; Zeljezic and Garaj-Vrhovac 2001; Ünderğer and Başaran 2002; Grover et al. 2003; Ascarrunz et al. 2006; Castillo-Cadena et al. 2006; Remor et al. 2009; Paiva et al. 2011; Wilhelm et al. 2015).

The evaluation of agricultural workers exposed to a complex mixture of pesticides, using the comet assay in exfoliated buccal cells, evidences that DNA damage increased in the individuals occupationally in contact with these substances in the three areas studied, in relation with the non-exposed individuals. The DNA damage revealed by the alkaline version probably originated from DNA single-strand breaks (SSB), alkali-labile sites (ALS), DNA-DNA/DNA-protein crosslink, and SSB associated with incomplete excision repair sites (Tice et al. 2000) induced by pesticide mixtures used in Guerrero state, as has been suggested by Garaj-Vrhovac and Zeljezic (2000) and Zeljezic and Garaj-Vrhovac (2001) in individuals exposed to mixtures of atrazine, alachlor, cyanazine, 2,4-dichlorophenoxyacetic acid, and malathion.

Further evidence was found by Grover et al. (2003) in pesticide production workers exposed simultaneously to acephate, chlorpyrifos, phorate, fenvalerate, cypermethrin, monocrotophos, dimethoate, and carbendazim and by Remor et al. (2009) in subjects exposed to mancozeb, atrazine glyphosate, paraquat, methamidophos, and other similar pesticides. Some of these are also used by the agricultural workers in our study. Besides, other mechanisms such as oxidative stress may also be involved in comet induction as has been mentioned by Muniz et al. (2008) and Kisby et al. (2009) in agricultural workers exposed to the organophosphate pesticide azinphos-methyl, which is used together with other organophosphates in Guerrero state.

On the whole, population correlation was not observed between the comet tail length and exposure time in our results. However, considering that the relative amount of DNA that migrates provides a simple way to measure DNA damage in the cell, it is important to mention that the two communities with higher exposure time (Arcelia 15.66 years and Ajuchitlan 17.50 years) were compared in relation to exposure time and comet tail length. No statistical differences were found between the two rural communities, but both differed significantly when compared with Tlapehuala which presented lower tail length suggesting that tail length is a good indicator of DNA damage produced by pesticides, that is, the higher exposure time to these compounds increases DNA migration. In this sense, Tice et al. (2000) mentioned that migration length is generally believed to be related directly to fragment size and would be expected to be proportional to the level of SSB and ALS.

In our study, the exfoliated buccal cell analysis of the workers exposed to pesticides exhibited results that showed a significant increase in the MN with respect to the non-exposed individuals. These results are in accord with those of other studies in buccal epithelial cells that have reported positive results (Gómez-Arroyo et al. 2000; Sailaja et al. 2006; Ergene et al. 2007; Bortoli de Moura et al. 2009; Martínez-Valenzuela et al. 2009), although no correlation was found in any of these studies between the exposure time and the micronuclei frequency as occurred in our study.

This possibility is based on the fact that some individuals who work less time with pesticides are exposed during two annual cycles and many of them apply, handle, prepare, and cultivate the land whereas others keep the pesticides inside their houses, so it is difficult to have a clear idea of their exposure.

The micronucleus test in epithelial cells of the buccal mucosa allowed us to conclude that the exposure to pesticides significantly increases genetic damage. This implies that the tissue was damaged at the chromosomal level and that it underwent chromosome breakage and/or mitotic spindle alterations, along with other nuclear abnormalities such as pycnosis, karyolysis, karyorrhexis, and nuclear buds.

Besides the MN, different types of nuclear degenerative changes have been suggested, and we have used them as biomarkers of genotoxicity, including pycnosis, chromatin condensation, and karyorrhexis related with cytotoxicity (necrosis and keratinization); karyolysis, which is associated with cell toxicity, was also used. In Guerrero, as well as in other agricultural regions in the world, profound changes are required in the adequate use of pesticides so as to decrease the possible risk to health associated with the exposure to these substances.

Due to the fact that none of the subjects in our study smoked more than five cigarettes and drank more than two beers per day or drank in excess once a week, they were considered as non-smokers and non-alcohol drinkers, both in the exposed and the non-exposed groups. Besides, significant differences were found neither in comet tail length or in micronuclei frequency nor in the smoking and alcohol consumption, findings which are in agreement with results found in other Mexican populations exposed to pesticides (Gómez-Arroyo et al. 2000; Martínez-Valenzuela et al. 2009).

The agricultural workers referred to in the present study were exposed to complex mixtures of pesticides whose active ingredients are mainly organophosphorus and carbamates. According to the WHO (2010) classification, some of these mixtures include two “extremely hazardous” compounds (parathion-methyl and aldicarb) and six “highly hazardous” compounds (azinphos-methyl, methamidophos, omethoate, carbofuran, Lannate, and Vydate). And according to the IARC (2006), three probably human carcinogens (pentachlorophenol, propoxur, and pirimicarb), four possibly human carcinogens (dimethoate, mancozeb, cypermethrin, and benomyl), and thiabendazole are likely to be carcinogenic to humans at high doses, yet they are not likely to be carcinogenic to humans at low doses. Parathion-methyl and aldicarb as well as azinphos-methyl, Lannate, and Vydate are also used in Sinaloa, Mexico (Martínez-Valenzuela et al. 2009), which indicates that the individuals are exposed to the same dangerous compounds.

Although the main objective of this study was not spontaneous abortions, it is important to mention that such abortions reported in some of the wives of the exposed individuals can be explained as a result of pesticide contamination; in other words, when the husband returns home with his clothes impregnated with these substances, he brings pesticides to the pregnant wife through the contaminated clothing or when he keeps the pesticides inside his house.

Some studies on pesticides have described the increase of miscarriages and/or fetal loss in the spouses of pesticide applicators (Garry et al. 2002). Petrelli et al. (2000) determined the paternal association with the pesticide exposure and the increased risk of spontaneous abortion in the wives. Arbuckle et al. (2001) observed that the wives of male pesticide applicators have an increased risk of spontaneous abortion when the husband has been exposed to herbicides such as phenoxy

acetic acids, triazines, glyphosate, and captan, as well as to insecticides as organophosphorus and carbamates, some of which are also used by the agricultural workers in the three sites of Guerrero state. Reports have also described that pesticides can affect the male reproductive system (Tas et al. 1996) and that male exposure may cause a variety of adverse consequences in the offspring of unexposed females (Petrelli et al. 2000), a lack of fertility (Koifman et al. 2002), and congenital malformations (Rojas et al. 2000). It is advisable that further epidemiologic research be made on the reproductive toxicity of pesticide use in this region.

The MN and comet assay in exfoliated buccal cells are useful and minimally invasive methods for monitoring genetic damage in individuals exposed to pesticides. The present study provided valuable data for establishing the possible risk to human health associated with pesticide exposure.

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Compliance with ethical standards This study was performed in accordance with the principles stated in the Declaration of Helsinki. All participants were informed regarding the study and they accepted the idea of taking part in it. They subsequently signed the document in which they agreed to complete a standardized questionnaire with personal data related to age; time of exposure, habits such as smoking and alcohol consumption, drugs, and diets; the type of work performed; and protective measures used.

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