



(RESEARCH ARTICLE)



Genotoxic damage evaluation in agricultural workers by exposure to pesticides in Vicente Guerrero-Tlaxcala, Mexico

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Abstract

Pesticides are one of the main inputs in agricultural activities; however, their exposure has been linked to the generation of various health effects. Today, many studies show the ability of pesticides to induce genotoxic damage. The aim of this study was to evaluate the level of genotoxic damage of agricultural workers from Vicente Guerrero-Tlaxcala community, Mexico, exposed to pesticides. The study was composed by 50 agricultural workers from the Vicente Guerrero community and 16 non-exposed individuals. To assess genotoxic damage, the micronucleus (MN) test was applied to exfoliated buccal cell samples by analyzing their frequency and that of other nuclear abnormalities. The agricultural workers showed a frequency of micronucleated cells ($P < 0,05$) and nuclear outbreaks ($P < 0,05$) higher than the control group. The types of pesticides most referred to by the producers were herbicides, belonging to the chemical groups chlorophenoxy and triazine. The exposure time is more than 6 years in more than half of the workers, while the use of personal protective equipment is low. The detected anomalies did not show statistical associations with age, diseases, alcohol, and tobacco consumption. The study revealed that the significant increase in the frequency of MN observed in the exposed group is induced by exposure to pesticides. The application of biomarkers of genotoxicity represents a useful tool to estimate the genetic risk of a population when exposed to a complex mixture of chemicals.

Keywords: Pesticides; Micronuclei; Agriculture workers; Mexico

1. Introduction

Pesticides are a heterogeneous category of chemicals specifically intended to control pests, weeds or plant diseases. The increase in its consumption and variety corresponds to the demands of the population and agricultural production, however, its nature is toxic and its exposure implies possible adverse effects on health [1].

Agricultural workers are exposed to pesticides mainly during the preparation and application of the solutions they will spray; absorbed when inhaled or by direct contact with the skin, increasing the chances of adverse effects due to intensive use, misuse or lack of control measures. Neurological, reproductive or developmental effects and cancer have been related to exposure to pesticides, showing a significant correlation in the presence of degenerative diseases such as Alzheimer's, Parkinson's, Amyotrophic Lateral Sclerosis, leukemia and Non-Hogkind's Lymphoma [2, 3].

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The evaluation of transient and permanent genotoxic responses to mutagenic and carcinogens through biomarkers has made it possible to estimate the genetic risk in human populations. In recent years, the incidence of cytogenetic damage from exposure to pesticides has been published as the frequency of chromosomal aberrations (CA), sister chromatid exchange (SCE) and micronuclei (MN) or the breaks of a single DNA strand with EC in agricultural workers, flower growers, applicators, women packing and factory workers [4].

The micronucleus assay is an effect biomarker used to evaluate chromosomal damage in exposed populations, since its collection is by non-invasive procedures, it is easily and quickly widely used. Micronuclei are formed by chromosomal damage in the basal cells of the epithelium during the metaphase-anaphase transition in mitosis and can be complete chromosomes lagged by damage to mitotic use (aneuploidogenic effect) or fragments of chromosomes without a centromere (clastogenic damage). These fragments form their own membranes and appear as Feulgen bodies-specific by lagging behind and excluding themselves from the main nuclei in daughter cells [5,6]. It is these cells that later mature and exfoliate, thus allowing evaluation of DNA damage.

In Mexico, pesticides and other agrochemicals are the second most valuable input for agricultural production, several studies report exposure to complex mixtures of insecticides, herbicides and fungicides, many of which have been classified as possible carcinogens, highly dangerous and probably teratogenic according to IARC, WHO and CICOPLAFEST [7]. That is why the objective of this study is to evaluate the level of genetic damage through the MN test, determining the risk factors for exposure to pesticides in agricultural workers in the community of Vicente Guerrero-Tlaxcala, Mexico.

2. Material and methods

2.1. Study area and sample selection

The study was carried out with farmers from the Vicente Guerrero community, located in the central Mexican highlands between parallels 19° 22' and 19° 30' north latitude and the meridians 98° 20' and 98° 31' longitude west, altitude between 2,400 and 2,900 meters above sea level, in the municipality of Vicente Guerrero-Tlaxcala, Mexico. The temperature ranges between 12 °C and 14 °C, and it has a temperate subhumid climate and vegetation represented mainly by *Pinus* and *Quercus* forests.

A total of 50 farmers were contacted for the research, who were interested in participating voluntarily. A previously validated survey with a reliability test of 0.92 was applied by applying Cronbach's Alpha statistical test for the response scale. The information collected included sociodemographic data (age, sex, level of education), habits of alcohol and tobacco consumption, as well as their medical history, productive activity (type of crops, pesticides used, protective equipment, hygiene practices) and the presence of representative symptoms of acute poisoning from such exposure.

In addition, a reference group was formed, made up of 16 individuals from different places with different demographic characteristics, with activities that have no relationship whatsoever with exposure to pesticides or other substances that could alter the test performed.

2.2. Micronucleus assay

The epithelial cell samples were collected with the help of a tongue depressor by scraping the oral cavity, spread on slides until dry, to be subsequently fixed with a 3:1 glacial acetic acid methanol solution. The collected samples were worked according to the Feulgen reaction described by Stich and Rosin, with the modifications suggested by Gómez-Arroyo [8, 9].

For hydrolysis, the slides were immersed for 10 minutes in a 1N HCl solution at room temperature, consecutively in a 1N HCl solution at a temperature of 60 °C for 10 minutes to finally rinse them with distilled water and place them in Coplin boxes with the Schiff's reagent for a period of 90 minutes in refrigeration. To obtain the frequency of micronuclei and nuclear abnormalities, 1000 cells were counted for each participant in the microscope with objectives of 40x, using the criteria described by Tolbert et al., (1992) and Holland et al., (2008) for their identification [10,11].

2.3. Statistical analysis

With the data obtained through the survey, measures of centrality and dispersion were determined. Results are reported as mean \pm standard error for each determined nuclear abnormality. For the cytogenetic test, the Kolmogorov-Smirnov test was used to corroborate the normal distribution of the variables and the Mann-Whitney U test was used to determine the differences between the exposed group and the control group ($P < 0.05$). Likewise, the Spearman test

was applied to correlate the presence of micronuclei and other abnormalities with the study variables, statistical analysis was performed with the SPSS Statistics version 25 package.

3. Results

3.1. Survey data

The two study groups were made up mostly of men, for the control group an age range of 20 to 49 years was observed (mean age 25 ± 1.85 years), while in the exposed group it was 31 to 84 years (mean age 56.72 ± 2.02 years), finding significant differences between both groups ($P < 0.05$). As for the smoking habit in both groups, its consumption is minimal; otherwise it occurs in alcohol consumption, since it is more frequent (2 to 4 times a week) in the control group. Regarding the pathological history, diabetes and arterial hypertension were the most prevalent pathologies in the exposed group (10% and 8%), while for the control group it was arterial hypertension (12.5%).

Exposure to complex mixtures is varied among local agricultural workers, with herbicides being the most used, followed by insecticides and fungicides, the latter less frequently.

According to the exposure time, it was observed that 82% of the workers have an exposure greater than 6 years, of which 26% have worked with pesticides for more than 25 years. In the activities of preparation and application of pesticides, it was detected that 31.84% and 27.67% of them do not use any kind of personal protective equipment and in the case of workers who use it, it is not always adequate. Using mainly jackets or sweatshirts to cover the back and arms, as well as bandanas to protect the respiratory tract.

The most frequent symptoms were burning eyes (28.9%), irritated eyesight (17.8%), skin irritation (15.6%) and headache (8.9%). The most frequently cited application equipment is the backpack type manual sprayer (80%), as good hygiene practices, it was found that 42% of the workers say they do not consume any kind of food when they carry out some type of activity that involves the likewise handling, 91% wash their hands before carrying out any other type of activity, while 95.6% reported bathing when they got home.

3.2. Micronucleus assay

Regarding the micronucleus assay in epithelial cells of the buccal mucosa, an average of 4.54 ± 0.618 micronucleated cells/1000 cells analyzed was determined for the exposed group compared to the control group, an average of 1.75 ± 0.722 cells micronucleated/1000 cells. In both groups, cells with condensed chromatin (CC), karyolysis (KL), pyknosis (PN), karyorrhexis (KR), lobed nucleus (LN), binucleated cells (BC) were observed. When comparing the means of both groups evaluated, significant differences ($P < 0.05$) were found in CC, PN and MN (Table1). When relating the variables age, alcohol consumption, smoking, and exposure time with the presence of micronuclei in the exposed group through the Spearman correlation test, no correlation was observed with these variables.

Table 1 Frequency of micronuclei and nuclear abnormalities in oral mucosa cells of agricultural workers exposed to pesticides and control group.

Variable	Producers	Control group	Significance
CC	527.66 ± 30.004	766.69 ± 43.257	0.0001*
KL	218.10 ± 20.604	234.44 ± 37.782	0.6534
PN	42.16 ± 4.394	10.38 ± 5.093	0.0000*
KR	21.60 ± 3.302	7.81 ± 1.279	0.0309
LN	0.98 ± 0.314	0.06 ± 0.063	0.0368*
BC	6.58 ± 0.540	5.94 ± 1.24	0.2728
MN	4.54 ± 0.618	1.75 ± 0.722	0.0022*

Condensed chromatin (CC), karyolysis (KL), pyknosis (PN), karyorrhexis (KR), lobed nucleus (LN), binucleated cells (BC), micronuclei (MN). The data are expressed in $M \pm SE$ in percent for micronuclei and nuclear abnormalities in 50 exposed workers and 16 controls. * U de Mann-Whitney Test.

Regarding the frequency of micronuclei, we can observe that its presence increases according to the age of the exposed workers (Figure 1), and since age is considered as the main factor associated with degenerative damage, it could be suggested as the response to this evaluation, however, it was also possible to note a lower frequency (2 MN/1000 cells) in those workers older than 76 years. When carrying out the correlation test, no relationship was found between the frequency of micronuclei and the age of the workers ($P>0.05$).

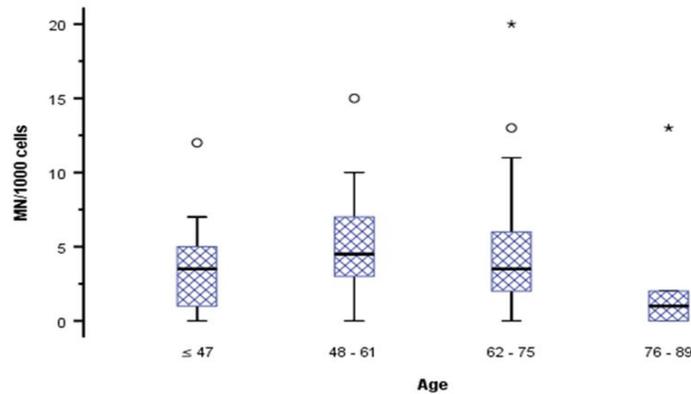


Figure 1 Micronuclei frequency observed in the epithelium of the buccal mucosa of agricultural workers with respect to age.

Another factor considered in the face of genotoxic damage from exposure to pesticides is the exposure time (Figure 2), since it has been positively correlated with the presence of cytogenetic alterations in agricultural workers with minimal exposures and with more than one exposure period. For this study, a higher frequency of micronuclei was detected in those workers with an exposure of more than 6 years, although no significant difference was found among these ($P>0.05$). It should be noted that those workers who currently do not use pesticides showed a higher mean than that observed in the group of workers exposed to a range of 1 to 5 years (4 ± 2.67 and 2.50 ± 2.50 respectively).

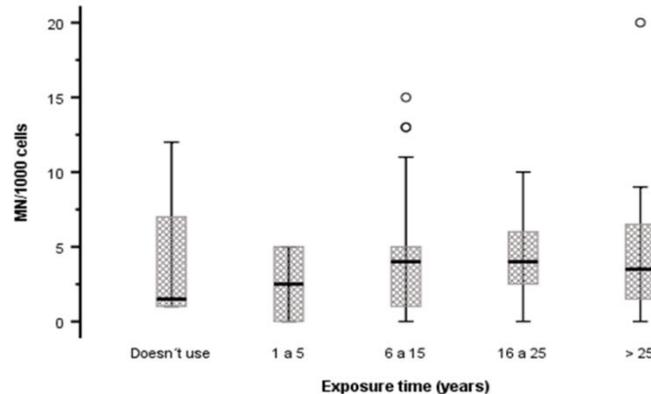


Figure 2 Micronuclei frequency in the epithelium of the buccal mucosa in agricultural workers with respect to exposure time.

4. Discussion

The development of activities outside the field aimed mainly at other productive sectors such as industry, transportation and construction, has favored the use of herbicides among agricultural workers in the region [12]. Due to the diversity of crops grown in the community, the exposure presented by workers can be considered cyclical, since the mentioned pesticides are used constantly in each agricultural and chronic cycle, having an exposure greater than one year.

In this study, as in others with agricultural workers exposed to pesticides, a significant increase was obtained compared to the control group in the analysis of exfoliated buccal cells [13,14], although no correlation was found with the exposure time and frequency of micronuclei in none of those studies, nor in the present.

2,4-D is a selective systemic herbicide used more frequently by agricultural workers in this study, its exposure has been significantly related to the probability of presenting NHL and oxidative DNA damage [3], it has also been identified that the exposure to mixtures containing this herbicide, represent a high risk in the probability of breaking the DNA chain [15]. It is currently classified as a possible carcinogen (Group II B) by the IARC [16].

Exposure to a mixture of different types of pesticides is more toxic than exposure to one pesticide alone. (4) In this study, it was observed that agricultural workers make different mixtures with pesticides that, according to the WHO [17], are classified as moderately dangerous (2, 4-D, cypermethrin, lambda cyalothrin and tebuconazole) and little dangerous (atrazine). Agree Gómez-Arroyo et al., (2013) this type of action is common in 83% of the investigations carried out in Latin America, making it difficult to attribute the effects to any specific compound, making it difficult to compare the different investigations given the large number and variety of products applied [4].

Regarding the identified nuclear abnormalities, a significant difference was detected with respect to the control group ($P < 0.05$) in cells with CC, KL, PN, KR and LN, the latter considered as an indicator of chromosomal instability or DNA damage, while the previous ones are related to cell death processes [18]. Regarding the frequency of cells with CC, it is notable that the value obtained for both groups is high (527.66 ± 30.00 vs 766.69 ± 43.25) compared to the values recorded in other investigations [13, 14, 19]. However, Martínez-Valenzuela et al., (2017) observed high frequencies in the group exposed to complex mixtures and with little use of protective equipment [20].

Lack of dexterity and shortness of breath are some of the main drawbacks for which agricultural workers use their personal protective equipment incompletely, proving to be inadequate in most cases. This situation facilitates the entry of these substances into the body, through the respiratory tract and the absorption of the skin. Its correlation with the presence of damage to genetic material has not been significant among those who use it and not for this study, as well as in others [14, 19]. However, a significant correlation has been demonstrated between the lack of personal protective equipment and the presence of symptoms caused by exposure to pesticides, making its absence a risk factor [21, 22].

The most frequent symptoms in exposed workers are headache, irritation of the mucosa of the eyes and nose, allergies or skin reactions, as well as nausea and digestive problems [19,23], correlated with complex mixtures of organophosphate pesticides, carbamates and pyrethroids [14]. Particularly skin contact with pyrethroid insecticides results in skin irritation such as burning, itching and tingling [24] a symptom reported more frequently among the producers of this study.

The frequency of micronuclei can be altered by various factors that may influence its presence, age is a factor that has been significantly correlated with the presence of damaged cells in workers exposed to pesticides [19], on the other hand, Balderrama-Carmona et al., (2020) determined that age does not influence the increase in micronuclei by not finding cellular damage in samples of agricultural workers aged 30 years or less, as well as 45 years of age, a situation that was observed for this study [25].

Another factor that can modify the frequency of micronuclei is the state of health. In long-term follow-up studies, a higher frequency of micronuclei has been shown to be a predictive biomarker of cardiovascular mortality within a population of healthy subjects, as well as of the main adverse cardiovascular events in patients with coronary artery disease [26]. On the contrary, Matheus et al., (2017) they did not observe any correlation with the presence of cells with genetic damage in agricultural workers who presented a pathological history of arterial hypertension and chronic drug use [14]. Situation that is similar to that observed for this study, where diabetes and arterial hypertension are the most reported conditions, with a 16.7% consumption of drugs by agricultural workers.

Regarding the consumption of cigarettes and alcohol, they were not considered as confounding factors for this study, since these habits are minimal among workers (4% and 6% respectively). However, according to Fenech and Bonassi, the number of cigarettes smoked and the consumption of alcohol do not significantly increase the frequency of micronuclei; however, it is important for biomonitoring studies that involve occupational exposure to genotoxic agents such as pesticides [27].

5. Conclusion

The micronuclei trial has been considered a marker of early stages of chronic diseases such as cancer, therefore its implementation in populations exposed to pesticides should be considered as an integral part of medical surveillance. For this study, levels of damage were detected in the genetic material of agricultural workers, so it can be concluded that these are induced by exposure to pesticides. However, it is necessary to carry out more biomonitoring studies that include the use of other genotoxicity markers to reinforce the results obtained. Variables such as exposure time, the

absence of personal protective equipment, the type of pesticides used, as well as exposure to complex mixtures represent risk factors for workers, which is why they should be considered when performing a cytogenetic evaluation.

Compliance with ethical standards

Acknowledgments

All authors listed have made a substantial and intellectual contribution to the work, and approved it for publication.

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of informed consent

The informed consent included the following aspects: Name of the research project in which it will participate, objectives of the study, that is, what is intended to be obtained with the research and procedures and maneuvers that will be performed on the people in that study. And it was approved by the ethics committee of the Benemerita Universidad Autonoma de Puebla (MasAgro/Tesis/2018-2020).

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