



Herbicide biomonitoring in agricultural workers in Valle del Mayo, Sonora Mexico

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Abstract

Valle del Mayo is an important agricultural area at the northwest of Mexico where up to 20,000 L of a mix composed of glyphosate and tordon is used in drains and canals. This study was carried out in order to evaluate the cellular damage caused by glyphosate, aminomethylphosphonic acid (AMPA), and picloram in agricultural workers. Biomonitoring was performed through the quantification of herbicides in urine using HPLC (high-performance liquid chromatography) to then evaluate the cellular damage in exposed people by means of an evaluation of micronuclei and cellular proliferation in lymphocyte cultures. The urine samples ($n = 30$) have shown a concentration of up to 10.25 $\mu\text{g/L}$ of picloram and 2.23 $\mu\text{g/L}$ of AMPA; no positive samples for glyphosate were reported. The calculation of the external dose reveals that agricultural workers ingest up to 146 mg/kg/day; however, this concentration does not surpass the limits that are allowed internationally. As for the results for the micronuclei test, 53% of the workers showed cellular damage, and the nuclear division index test reported that there was a significant difference ($P < 0.05$) between the exposed and the control population, which indicated that the exposure time to pesticides in the people of Valle del Mayo can induce alterations which can cause chronic damage.

Keywords Occupational health · Herbicide pollution · Picloram · AMPA · Glyphosate · Cellular damage · Urine

Introduction

The extensive use of herbicides has become in one of the biggest problems for environmental health (Bianco et al. 2017). The herbicides are chemicals which are widely used in agriculture, commerce, and home for the control of aquatic and terrestrial wide leaf grass; the herbicides can be applied

directly on the ground or grass. The most used herbicides are glyphosate (*N*-(phosphonomethyl) glycine), 2,4-D (2,4-dichlorophenoxyacetic acid), and paraquat (*N,N'*-dimethyl-4,4'-bipyridinium dichloride) (Imfeld and Vuilleumier 2012; Paulino 2013). It has been shown that the glyphosate and his metabolite aminomethylphosphonic acid (AMPA) produce health damage (IARC 2015); also, it has been proved that the herbicide 2,4-D is toxic to the environment and biodiversity (de Castro Marcato et al. 2017). These herbicides are commercialized mainly under the label Roundup® for the glyphosate (Nicolas et al. 2016) and in the case of the 2,4-D, Tordon®, which comes mixed with the herbicide picloram (4-Amino-3,5,6-trichloro-2-pyridinecarboxylic acid) (Bueno-Franco-Salla et al. 2019); in Mexico, glyphosate has been on sale for more than 40 years (Székács and Darvas 2012).

Agricultural production needs herbicides to eliminate the undergrowth (de Casthilos Guisi, 2012; Riley et al. 2014). The herbicides and their metabolized compounds by in the air, water, or ground can be bioaccumulated in living organisms (Nawaz et al. 2014; Parrón et al. 2014; Angelini et al., 2015); the most sensitive exposure routes are inhalation, dermal, and oral for consuming contaminated food or water (Acquavella

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et al. 2004; Curwin et al. 2007). Herbicides related to health problems are a cause of global concern. The most controversial one being glyphosate, which has been linked to cytotoxic, endocrine disrupting, immunotoxic, genotoxic, and carcinogenic effects (Gigante et al. 2018) that can derive into neurodevelopmental and congenital problems, Alzheimer's disease, Parkinson's disease (Rueda-Ruzafa et al. 2019), metabolic syndrome, thyroid disease, obesity, diabetes mellitus, hypertension, nephropathy (Samsel and Seneff 2015; Seneff and Orlando, 2018), pneumopathies, and various types of cancer (Gigante et al. 2018).

Genotoxicity biomarkers are considered an essential tool for the study of potentially exposed individuals to contaminants that also complements the measurement of environmental and epidemiological risk (Lock and Bonventre, 2008; Katsikantami et al. 2017). Previous scientific contributions regarding the evaluation of herbicide exposure normally base their research on the determination of blood and urine herbicide concentrations in workers (Katsikantami et al. 2017). In order to assess cellular damage in a set population, the most widely used approach for occupational exposure studies is the usage of micronuclei assays with lymphocytes (Wultsch et al. 2019). Micronuclei and cell proliferation tests are based on the concept that micronuclei can be formed both from whole and fragmented chromosomes lagging behind the cell division; these tests can provide the following measures of genotoxicity and cytotoxicity: chromosome breakage, chromosome loss, chromosome rearrangement (based on the presence of nucleoplasmic bridges), cell division inhibition, necrosis, and apoptosis (Fenech 2000; Fenech 2007; Thomas and Fenech 2011).

In Mexico, people who live closer to agricultural areas are the most at risk to be exposed to herbicides since in these places, their application is constant (up to 45 times a year) (Johansen 2019), which implies continuous and direct contact with these chemical substances (Bianco et al. 2017). Valle del Mayo is one of the biggest agricultural producers in the north-west of Mexico with more than 93,000 ha of crops (INEGI, 2019). The most used herbicide is the glyphosate to 1%, predominantly in irrigation channels, while a mixture of glyphosate and tordon is used in drains, resulting in more than 20,000 L of glyphosate used yearly just in this region, mainly to remove undergrowth from the drains. The workers responsible for the application of the herbicide do not make use of the appropriate protection equipment at all times, and in previous studies, it has both been shown that in the communities of Valle del Mayo, concentrations of 5 µg/L of glyphosate and de 36.8 µg/L of AMPA can be found in private wells and that workers who consume this water tend to fall ill more frequently than the ones who drink tap water (Leyva-Soto et al. 2018), increasing the need for an evaluation of the dose to which workers are exposed, with the goal of establishing the appropriate guidelines which determine the permissible limits

applicable in the environment and evaluating the internal and external doses to which tissues and organs are exposed to (Katani et al. 2011). Therefore, the present investigation's aim is to quantify glyphosate, AMPA, and picloram concentrations in urine samples to later evaluate the nuclear division index and cellular damage of agricultural workers of the Valle del Mayo.

Materials and methods

Study population and ethical regulations considered

In order to carry out this study, agricultural workers were selected according to the following inclusion criteria: they must live in communities adjacent to agricultural drains and they must regularly apply herbicides. There are four small communities where herbicide applicators live, located next to irrigation channels in the Valle del Mayo (Leyva-Soto et al. 2018). The description of the participating population was made utilizing surveys which included general information such as occupation, consumption habits, and symptomatology (if present). A urine sample was taken for the study by providing the agricultural workers that met the inclusion criteria a 2-l wide-beaker sterile container in order to carry out the collection of urine 24 h after the application of the herbicides (glyphosate and tordon). The samples were collected at their homes and transported in 2-degree coolers. The request for the samples was performed as indicated by the regulations shared by the Research Ethics Committee of the University of Sonora (UNISON 2015).

Determination of glyphosate, AMPA, and picloram in urine by HPLC

The method was adapted and modified for urine samples (Stalikas and Konidari 2001; Peruzzo et al. 2008, Olivo et al. 2015).

Quality control and validation of the method To determine the concentration in the urine samples, a high-pressure liquid chromatography equipment was used. For this, analytical standards (PESTANAL, analytical standard, Sigma-Aldrich) were needed to obtain the calibration curve for each of the chemical compounds (glyphosate, AMPA, and picloram). The calibration curves were made using blanks and aqueous patterns of glyphosate and AMPA of different concentrations. The concentrations that were tested for glyphosate were from 5 to 25 µg/L; for AMPA, the concentrations were from 15 to 90 µg/L; and for picloram, they were from 15 to 90 µg/L (Leyva-Soto et al. 2018).

Preparation of the sample The samples were purified by filtration through a 0.45 µm cellulose acetate membrane (MF-Millipore™) (Peruzzo et al. 2008).

The derivatization was carried out, mixing 3 mL of the purified sample and 2 mL of FMOC-Cl (0.005 M) (Sigma-Aldrich, 23,186) prepared with chloroform (Sigma-Aldrich, 650,498). The derivatization is maintained for 45 min avoiding radiation; after the reaction concluded, 3 mL of methyl chloride (Sigma-Aldrich, 270,997) was added to remove the excess FMOC-Cl; subsequently, the supernatant is filtered using polymeric SPE columns (Strata C-18 Phenomex) (Olivo et al. 2015). The collected filtrate is then taken to the HPLC equipment (Agilent 1200 series).

Chromatographic conditions The modifications of the chromatographic conditions were established as follows: Column Agilent C18 250 × 4.6 mm, injection volume 20 µL, mobile phase water-acetonitrile (65:35), flow 1 mL/min, UV 240 nm, FL excitation 266 and emission 315, and retention time 30 min.

Calculation of the internal dose

The internal dose is available systemically for what was calculated by the following equation:

$$ID = \frac{C \times V}{BW}$$

where ID is the internal dose (µg/kg bw), *C* is the urinary concentration of herbicide (µg/L), *V* is the volume of urine per person per day, and BW is the body weight (Niemann et al. 2015).

Calculation of external dose

With the result of the internal dose, the external dose (µg/kg bw) was calculated as follows:

$$ED = \frac{ID \times 100}{\%A}$$

where %A is 0.2, the percentage of absorption (Niemann et al. 2015).

Collection of blood samples of agricultural workers exposed to herbicides

Participants who tested positive for herbicides in urine were asked for a blood sample. About 3 to 5 mL of peripheral blood were extracted using the venipuncture technique, which was performed using Vacutainer heparin-coated tubes (Becky Dickinson) in order to avoid blood coagulation. The samples were then identified with the respective name and number of

the patient following the indications of the Clinical and Laboratory Standards Institute (CLSI, 2017) and the Official Mexican Standard NOM-253-SSA1-2012.

Transportation of the samples Once the blood samples were taken from the previously identified Vacutainer tubes, they were placed in a rack which was placed inside a cooler with cooling gels (approximately 5 coolants) to keep the blood fresh; all this was carried out using the CLSI criteria regarding the transportation of terrestrial samples. All samples were processed no later than 24 h after sampling. Control samples for this study were obtained from volunteers who declared the following: (1) not to have contact with herbicides and (2) not to live in agricultural communities.

Cell damage evaluation A RPMI 1640 medium (Gibco-Invitrogen) was used, which was already supplemented with non-essential amino acids, and 1% L-glutamine (Gibco-Invitrogen) was added per 100 mL of the total volume. Two cultures were prepared per sample. The mixture was prepared with 0.5 mL of peripheral blood and 0.2 mL of phytohemagglutinin M (Gibco-Invitrogen). The medium was first added to the 15-mL Falcon tubes with a micropipette, and then 0.5 mL of blood was added by slowly dripping it over the walls of the tubes, resuspending in order to avoid the formation of bubbles. Subsequently, the cultures were incubated at 37 °C for 44 h.

Blocking cytokinesis Cytochalasin B 5 mg (Sigma-Aldrich) or Cyt-B was prepared with DMSO (Sigma-Aldrich) according to the Thomas and Fenech (2011) protocol. After 44 h, the Cyt-B (4.5 µg/mL) was added to the lymphocyte cultures, and the tubes were gently resuspended with the seeding, after which the whole volume was withdrawn using a syringe to pass it to a sterile 15-mL falcon tube. The cultures were then incubated once more at 37 °C for 24 more hours.

Lymphocyte harvest The lymphocyte harvest was performed by Thermo Fisher centrifugation at 1200 rpm for 15 min; after 72 h, the supernatant was removed leaving 0.3 mL, which then was vortexed and fixed up to 10 with a Carnoy solution prepared with methanol-acetic acid 3:1 (Baker); this was done while vortexing until the samples were mixed well. The samples were centrifuged 5 more times until the white cell button was observed (without impurities). When the last wash was made and after removing the supernatant, a Falcon tube was filled with 7 mL in order to be stored under refrigeration for a maximum of 2 days until staining.

Preparation, staining, and mounting The cooled samples were removed and centrifuged at 1200 rpm for 5 min. The supernatant was removed, and approximately 1 mL was left of the solution to be used later for the resuspension of the cell button. Before this, the slides were placed in the

freezer in a beaker with methanol for about 1 h so that they were moist and could spread more easily when used. When removing the slides, the excess methanol was cleaned and with the help of a Pasteur pipette, 5 drops of the lymphocyte suspension were added and allowed to dry for 2 days (it is recommended to leave the slides in the hood to dry well to then perform the staining). After the drying time (2 days) was over, staining was carried out using 5% Giemsa (Sigma-Aldrich). This staining colors and reveal erythrocytes, basophils, eosinophils, polymorphonuclear cells, lymphocytes, platelets, and the chromatin of the nuclei; the nuclei of lymphocytes are observed violet and the cytoplasm blue. The staining was made in a Coplin glass box (model CRM-20339) which has a stainless-steel basket, with a capacity for 24 slides, which was filled with 80% deionized water and 5% Giemsa. After the slides were introduced and left to act for 1 h, they were removed and rinsed with deionized water to then leave them to dry. Later, they were seen under a microscope (Zeiss) to corroborate good staining. Blue-purple cells should be observed with a well-defined cytoplasm, and the nucleus should have a more violet texture. Once the slides were stained, they were kept in refrigeration, from approximately 2 to 3 days so that the staining could dry properly; it is important to examine the slide without it having any fresh drops or bubbles. We then proceeded with the assembly of the slides, which was carried out by placing 2 drops of Entellan (Merck) followed by the placing of the coverslips, avoiding the formation of bubbles, to then let them dry for 2 more days. After this time, the slides can be stored in a lamellae holder without risk of scratching until further analysis can be performed under a microscope.

Evaluation of slides For a proper examination, the microscope was focused with a 10× lens, and later with a 100× lens. The slides were placed under the microscope with the emery on the left side, counting in a zigzag direction (Fenech 2000).

Nuclear division index calculation

The nuclear division index is calculated for each cell culture, counting 500 binucleated, trinucleated, or polynucleated mononuclear cells to calculate the proliferation index. Optimal conditions in an MN assay require a percentage of binucleated cells between 35 and 60% over 500 viable cells; the nuclear division index is calculated as it follows

$$\text{NDI} = \frac{X_1 + 2(X_2) + 3(X_3) + 4(X_4)}{500}$$

where NDI is cellular proliferation, the “X” quantity of molecules: X_1 mononuclear, X_2 binuclear, X_3 Trinuclear, and X_4 polynuclear, divided by 500.

The characteristics of the cells evaluated were reviewed using the protocol described by Torres-Bugarin and Ramos-Ibarra (2013) and Šiviková et al. (2018).

The observation of micronuclei (MN), nucleoplasmic bridges (PNP), cariorrexsis (CR), condensed chromatin (CC), lobulated nucleus or nuclear gelation (GM), and if there is presence of cariolysis (CL), apoptosis (AP), and necrosis (NEC) indicates the presence of cytotoxic damage (Fenech 2007).

Statistical analysis

The calibration, concentration, and internal dose curves were processed using Microsoft Excel 2016 spreadsheets. The survey data of the exposed participants and controls were entered into the Epi Info program (version 7.2 CDC 2018). Differences in symptomatology, age, alcohol consumption, and tobacco were also analyzed. Variables were grouped into the following categories: exposure (no exposure/exposure), gender (male/female), age, consumption of substances, and symptomatology. Any significant differences that were reported by the program were evaluated using Bartlett’s methods for the evaluation of differences between variables, and Kruskal-Wallis’s method for evaluating the differences between groups.

Results and discussion

The workers who participated in the urine study belong to the small communities (population of less than 100) of Saucobe, Colonia Soto, Sebampo, and La Esquina of the municipalities of Etchojoa and Huatabampo belonging to Valle del Mayo, Sonora.

Herbicide concentrations in urine

For this investigation, a total of 30 urine samples were obtained from agricultural workers in the Valle del Mayo. Samples were analyzed for glyphosate, AMPA, and picloram, of which 17 samples resulted positive for these herbicides.

Quality control in urine tests

In the AMPA calibration curve, a correlation coefficient (R^2) 0.994 was obtained, while for glyphosate, it was $R^2 = 0.994$ and for picloram of $R^2 = 0.9917$. The retention times obtained were the following: 26.5 min for AMPA, 8.3 min for glyphosate, and 3.9 min for picloram. The limit of detection of picloram in this study was 15 µg/L; for AMPA, it was 15 µg/L; and for glyphosate, it was 5 µg/L.

The concentrations of glyphosate, AMPA, and picloram were quantified. The analyzed samples ($n = 30$) reported that 62% were positive, of which 56% were for picloram and 6% for AMPA; no positive concentrations were obtained for

glyphosate (Table 1). Comparing our results with other studies, we found that Mesnage et al. (2012), Markard (2014), and Honeycutt and Rowlands (2014), as well as our research, did not detect glyphosate in 24-h urine samples; however, in a study conducted by Connolly et al. (2019), concentrations of up to 7.4 µg/L of glyphosate were quantified in Irish horticulturists; in addition, Rendón-von Ostén and Dzul-Caamal (2017) obtained concentrations of up to 16 µg/L of glyphosate in 24-h urine samples from farmers from Campeche, Mexico; Parvez et al. (2018) found glyphosate concentrations of 34 µg/L in pregnant women in rural agricultural areas in the USA; and Sierra-Diaz et al. (2019) reported 263 µg/L of glyphosate in 24-h urine samples of children and adolescents living in agricultural areas in Jalisco, Mexico.

In a study conducted by Mesnage et al. (2012), the urinary concentrations of glyphosate in farmers were measured, registering a concentration of up to a maximum of 9.5 µg/L 2 days after the application of glyphosate, but glyphosate was not detected in the urine samples taken the day after the application; however, in a similar study conducted by Acquavella et al. (2004), the urinary excretion of glyphosate was determined in 48 farmers in 24-h urine samples after 1 day of the application of the herbicide, obtaining concentrations with a geometric mean of 3.2 µg/L of glyphosate for the whole group of agricultural workers.

Table 1 Herbicide concentrations in urine samples of agricultural workers and the estimate of the internal and external doses

Positive samples ^a (n = 30)	Concentration in urine samples ^b (µg/L)	Internal doses (µg/kg)	External doses (µg/kg)	
1	Picloram	0.249	0.007	3.5
2		0.607	0.017	8.5
3		0.477	0.013	6.5
4		0.070	0.002	1
5		0.630	0.018	9
6		0.650	0.0185	9.25
7		0.485	0.013	6.5
8		1.94	0.055	27.5
9		2.81	0.080	40
10		0.033	0.000	0.00
11		0.355	0.010	5
12		4.260	0.030	15
13		10.25	0.292	146
14	8.29	0.236	118	
15	2.99	0.085	42.5	
16	AMPA	0.42	0.012	6
17		2.23	0.063	31.5

^a Displayed of the positive samples from a total of 30

^b Glyphosate was not detected in the urine samples

Glyphosate can also be metabolized by bacteria in the human organism, which then excrete AMPA (IARC 2015). In a study by Motojyuku et al. (2008), glyphosate poisoning with AMPA concentrations of up to 15.1 µg/mL was found in blood. Bernal et al. (2010), after rats who received a high single oral dose of glyphosate (100 mg/kg/weight) were analyzed, found small amounts of AMPA in the plasma. In the present study, similar concentrations of AMPA were obtained in relation to those reported by Hoppe (2013), who reported a concentration of 0.18 µg/L of AMPA in urine, and that of Markard et al. (2014), who obtained a concentration of 1.31 µg/L for AMPA.

Calculation of the internal and external dose

For the calculation of the internal dose, the geometric means of the concentrations detected in the study were used, 2 L daily volume of urine (Tortora and Derrickson 2013) and 70 kg as average weight. The estimation of internal doses is seen in Table 1. An internal dose of 0.292 µg/kg (sample 13) is expected to result from a fully ingested amount of 10.25 µg; if it is considered that dietary exposure is the most likely route of picloram or AMPA intake, an oral absorption of 20% should be taken into account as toxicokinetic data shows (EFSA 2014). Following the general principle of toxicological risk assessment, these exposure estimates were compared with the recently proposed reference doses for glyphosate, i.e., an ADI (acceptable intake) of 0.5 mg/kg body weight (EFSA 2014). Based on urinary excretion, it is possible to estimate the approximate physical burden glyphosate has on the study participants (Niemann et al. 2015). The estimation of the external dose is observed in Table 1.

In other picloram absorption studies, 24-h urine samples were analyzed by liquid gas chromatography of 80 forest workers who applied herbicides, where they were asked to sample 1 day before the application, the day of the application, and 12 days after the application, a higher concentration was reported after the application in comparison to the day before it; however, it was found that between days 2 and 3, there was no significant difference in the average concentration reported (0.0260 mg/kg) (Lavy et al. 1987); similar concentrations were obtained in our study (Table 1). Hall et al. (1989) analyzed a urine sample from a volunteer who donated a 24-h urine sample from which determination of picloram was done by RIA, obtaining a 0.25 µg/L concentration for picloram; higher concentrations were obtained in our study. The last study of picloram which can be found in the literature was done by Deschamps et al. (1990), who analyzed picloram concentrations from a sample of synthetic urine that was analyzed using an immunoassay; however, the tests were not satisfactory due to contamination of the sample.

Comparing our results of external doses with acceptable doses from other countries, we found that in Canada, an

ADI of 0.03 mg/kg (HC, 1995) is handled, while in the USA, it is 0.1 mg/kg (USEPA, 2015) and in Europe of 0.5 mg/kg (EFSA 2014). Considering the Canadian ADI, we have 4 samples (23.5%) that exceed the intake; on the other hand, according to American regulations, 2 of the samples (11.76%) are above the permissible limits; according to European guidelines, none of the samples in this study are above the acceptable daily intake (EFSA 2014).

Cellular damage

Of the 17 samples that tested positive for herbicides, 15 blood samples from agricultural workers were analyzed for cell damage analysis, since 2 participants died during the sampling period. In addition, 13 blood samples from control participants were evaluated; the characterization of the study population is seen in Table 2, where it can be seen that most agricultural workers are male; however, it is well established that there are no significant differences regarding the evaluation of cell damage between women and men (Martínez-Valenzuela et al. 2017).

Table 3 details the results of the evaluated cells and the nuclear division index, where it can be observed that there is a significant difference of 99% between the exposed and control participants regarding the trinucleated and polynucleated

Table 3 Cell counting and nucleation index

Cell type		Exposed (n = 15)	Control (n = 13)	P value
Mononucleated	Average	282.46	240	0.13
	SD	52.31	78.27	
	Min	196	113	
	Max	373	370	
Binucleated	Average	211	208.5	0.9
	SD	49.5	55.5	
	Min	123	129	
	Max	299	299	
Trinucleated	Average	4.2	28.73	0.00001
	SD	5.2	18	
	Min	0	6	
	Max	18	54	
Polynucleated	Average	2.4	25.46	0.00002
	SD	4.1	21.22	
	Min	0	2	
	Max	15	72	
NI	Average	1.4	1.7	0.01
	SD	0.1	0.3	
	Min	1.3	1.3	
	Max	1.6	2.1	

NI nucleation index, SD standard deviation, Max maximum, Min minimum

Table 2 Characterization parameters of the study population

		Exposed (n = 15)	Control (n = 13)
Age	Average	40.4	27.5
	SD	10.6	11.8
	Max	58	50
	Min	21	19
Gender (%)	Feminine	13.3	69.23
	Masculine	86.67	30.77
Consumption (%)	Coffee	86.67	53.85
	Alcohol	86.67	30.77
	Tabaco	33.33	46.15
	Prescription drugs	40	38.43
Symptoms (%)	Illegal drugs	0	0
	Weakness	6.67	7.69
	Irritability/insomnia	6.67	15.38
	Sicknesses	0	7.69
	Dermic problems	13.33	23.08
	Headache	40	15.38
	Digestive problems	6.67	7.69
	Respiratory problems	0	7.69
Dizziness	26.67	15.38	

SD standard deviation, Max maximum, Min minimum

counts. On the other hand, a significance of 95% is observed in the results of the nuclear division index, which evaluated the cytostatic effects, and measures the mitogenic response, which serves as a biomarker for immune function. According to other pieces of research, a decrease of the proliferation index in the presence of pesticides has been found (Pastor et al. 2001; Šiviková et al. 2018).

Table 4 shows the results of the cell types that were assessed for cell damage. It was found that in those that were exposed to herbicides, greater damage was encountered by observing micronuclei, nuclear buds, nucleoplasmic bridges, apoptosis, and necrosis.

Meanwhile, in the control, population apoptosis was present but in very small amounts. Similar results were reported in a study by Carbajal-López et al. (2016), where a higher incidence of nuclear abnormalities was found in participants who were exposed to pesticides in contrast to the control population; Doğanlar et al. (2018) reported that residents of agricultural areas exhibit higher micronucleus counts than those who are not exposed, and Aiassa et al. (2019) reported an increase in the values of genetic damage in applicators with respect to people dedicated to other activities.

In the analysis of consumption habits (Table 2), a high percentage of control participants who consume coffee and tobacco are highlighted. In the investigation of Ballén et al. (2006), coffee and tobacco were related to the presence of

Table 4 Cellular damage estimation

Cell type		Exposed (n = 15)	Control (n = 13)	P value
Micronuclei	Average	0.2667	0	0.18
	SD	0.7988	0	
	Min	0	0	
	Max	3	0	
Nuclear buds	Average	0.5333	0	0.01
	SD	0.8338	0	
	Min	0	0	
	Max	3	0	
Nucleoplasmic bridges	Average	0.8	0	0.01
	SD	1.1464	0	
	Min	0	0	
	Max	3	0	
Apoptosis	Average	3.0667	0.4615	0.08
	SD	4.2167	1.1266	
	Min	0	0	
	Max	11	3	
Necrosis	Average	0.667	0	0.35
	SD	0.2582	0	
	Min	0	0	
	Max	1	0	
Cellular damage ^a		8	2	0.27

SD standard deviation, Max maximum, Min minimum

^a Frequency

cellular damage, since these two substances contain chemical compounds which can act as precursors for disease. The control population that consumed coffee and showed damage (Table 5) may be affected by a substance formed during the coffee roasting process known as acrylamide, which when introduced into the body can become glycidamide, which can damage proteins and DNA. According to these investigations, we can justify the genotoxic damage presented in some of the control population since the substances they reported to consume the most were coffee and tobacco.

Table 5 Relationship between the exposure time and the age of the participants that were exposed with detection of cellular damage

Years	With damage ^a	Without damage	P value
Exposure			
Low (0–3)	0	4	P = 0.001
Medium (4–12)	4	2	
High (> 12)	5	0	
Age			
15–30	0	3	P = 0.15
30–45	6	1	
> 45	3	2	

^a Number of agricultural workers with detected cellular damage

Age, smoking, and other factors such as diet, physical activity, infections, gender, and exposure to ultraviolet radiation can affect the analysis and lead to discrepancies during the study of cell damage (Intranouvo et al. 2018). However, in the present study, there are no significant differences between cellular damage and consumption of tobacco or coffee ($p \geq 0.05$); on the other hand, there are numerous studies (most of them carried out in mammals and cell cultures) that have investigated the nutritional and toxicological potential of coffee, where an increase in the frequency of nuclear abnormalities was found, as well as in the exchange of genetic material in sister chromatids of lymphocytic cells (Stadler et al. 1994).

Our results have shown that 53.33% of the exposed participants showed damage, while 46.67% did not (Table 4). Within the control samples, there was no damage in 84.62% and in 15.38%, damage was found. In the research of Gentile et al. (2012) and Jacobsen-Pereira et al. (2018), a positive correlation between the age and the finding of damaged cells is reported; however, in this study, it was found (Table 5) that agricultural workers that were 30 years old or younger, as well as 2 workers that were over 45 years of age, did not show cellular damage; this observation was confirmed with the statistical analysis which concludes that there is no significance between the age of the participants and the presence of cellular damage (p value 0.15); this result is backed by research such as that of Bolognesi (2003), Bhalli et al. (2006), Carbajal-Lopez et al. (2016), and others; however, it is important to take into consideration that the susceptibility of lymphocytes to oxidative stress due to the exposure to pesticides depends on the antioxidant defense capabilities of the exposed individual (Bianco et al. 2017).

As for the exposure, in Table 5, the results of the statistical analysis are shown which infer that there are significant differences between the time of exposure of the participants and the presence of cellular damage (p value 0.001); scientific evidence suggests that the chronic exposure to low doses of pesticides induces genetic damage (Alleva et al. 2018; Aiassa et al. 2019); however, previous studies do not correlate the years of exposure with the presence of abnormal cells (Gentile et al. 2012; Jacobsen-Pereira et al. 2018).

Taking into account that in this study the samples were taken the morning after the exposure to pesticides, it is inferred that the years of exposure are more relevant to the cellular damage than a previous exposure; this theory is confirmed by Omari's (2011) research in which it is shown that cellular damage in agricultural workers is less after avoiding exposure for 8 months, which indicates that occupational exposure to pesticides induces cumulative effects (Lebailly et al. 2015).

The results of pesticide exposure in this study were significant, making it impossible to specify the potential effect of herbicides in particular because these chemicals generally consist of mixtures of different substances. In the research of Sultana et al. (2016), the idea that herbicide mixtures increase

genotoxicity in lymphocytes was presented. The results support our hypothesis that individuals living in agricultural areas are more exposed to high levels of agrochemicals than those living in urban areas. This situation indicates that these people are exposed to agrochemicals which can enter orally, respiratory, or dermally and accumulate in different parts of the body. Individuals at high risk of exposure to pesticides are essentially agricultural workers, mainly applicators who do not wear the adequate personal protective equipment (Doğanlar et al. 2018). The most logical and accepted explanation of the genotoxic damage observed is the lack of preventive measures taken by the workers.

Conclusions

To our knowledge, this study represents the first monitoring of picloram and AMPA residues in urine samples from agricultural workers in Valle del Mayo. The biomonitoring of picloram, AMPA, and glyphosate in the present investigation was a useful tool to estimate exposure and potential health risk that derive from the exposure to herbicides.

It is recommended that people living near agricultural areas be examined regularly to prevent certain diseases associated with the accumulation of agrochemicals in general at chronic or acute levels. It would also be useful to give talks to the community where information is provided regarding the environmental and health problems related to the usage of herbicides in order to promote a culture of prevention in the area. It is very important that pesticide distribution companies provide training in the usage of the appropriate personal protection equipment for applicators and compliance with safety regulations. To ensure the safety of consumers and agricultural workers, education and continuous training of those involved in the production and distribution of products, the safety of their work, potential risks, and recent advances in crop protection are needed.

Genotoxic monitoring in humans is a useful tool to estimate the genetic risk caused by exposure to components and mixtures of chemical substances that can be used to establish an early warning system for genetic alterations, reproductive problems, and diseases such as cancer. It also allows for the proper development of control measures that can be implemented for the protection of human populations and the environment.

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