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Association of clinical and laboratory data of rural area residents to chronic exposure to glyphosate

Associação de dados clínicos e laboratoriais de residentes da área rural com exposição crônica ao glifosato

Asociación de datos clínicos y de laboratorio de residentes rurales con exposición crónica al glifosato

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Abstract
Commonly used by farmers, glyphosate has become the subject of many studies in recent years. However, these studies investigate and correlate glyphosate exposure to the most diverse physiological, motor, sensory, respiratory and hepatic functions, and possibly be related to causes of carcinogenicity and genotoxicity. Thus, the objective of the present study was to evaluate whether the population living in rural area of Paraguaçu, Brazil, presents evidence of chronic intoxication by glyphosate-based formulations through the evaluation of clinical signs and symptoms and laboratory data. A total of 162 samples were collected and analyzed using 53 samples from individuals who reported no contact with glyphosate for the control sample. Biochemical analyzes were performed on automated equipment, while cholinesterase was performed by Ellman's kinetic method. Data were submitted to statistical analysis, where changes in the nervous, respiratory, auditory, cutaneous and urinary systems were observed. Through the analysis of the data, associated with the literature information on the symptoms and damage generated by glyphosate, it can be concluded that the population living in the rural area, practicing family farming, has strong indications of having chronic poisoning by formulations based on glyphosate.
Keywords: Glyphosate; Chronic exposure; Environmental exposure; Clinical evaluation.

Resumo
Comumente usado por agricultores, o glifosato se tornou o assunto de muitos estudos nos últimos anos. No entanto, esses estudos investigam e correlacionam a exposição ao glifosato às mais diversas funções fisiológicas, motoras, sensoriais, respiratórias e hepáticas, e possivelmente estão relacionados a causas de carcinogenicidade e genotoxicidade. Assim, o objetivo do presente estudo foi avaliar se a população residente na zona rural de Paraguaçu,

Brasil, apresenta evidências de intoxicação crônica por formulações à base de glifosato por meio da avaliação de sinais e sintomas clínicos e dados laboratoriais. Um total de 162 amostras foi coletada e analisada e, foram usadas 53 amostras de indivíduos que relataram nenhum contato com o glifosato para a amostra controle. As análises bioquímicas foram realizadas em equipamento automatizado, enquanto a colinesterase foi realizada pelo método cinético de Ellman. Os dados foram submetidos à análise estatística, onde foram observadas alterações nos sistemas nervoso, respiratório, auditivo, cutâneo e urinário. Por meio da análise dos dados, associados às informações da literatura sobre os sintomas e danos gerados pelo glifosato, pode-se concluir que a população residente na zona rural, praticante da agricultura familiar, tem fortes indícios de intoxicação crônica por formulações à base de glifosato.
Palavras-chave: Glifosato; Exposição crônica; Exposição ambiental; Avaliação clínica.

Resumen
Utilizado comúnmente por los agricultores, el glifosato se ha convertido en objeto de muchos estudios en los últimos años. Sin embargo, estos estudios investigan y correlacionan la exposición al glifosato con las funciones fisiológicas, motoras, sensoriales, respiratorias y hepáticas más diversas, y posiblemente estén relacionadas con las causas de carcinogenicidad y genotoxicidad. Así, el objetivo del presente estudio fue evaluar si la población que vive en el área rural de Paraguaçu, Brasil, presenta evidencia de intoxicación crónica por formulaciones a base de glifosato a través de la evaluación de signos y síntomas clínicos y datos de laboratorio. Se recolectaron y analizaron un total de 162 muestras y se utilizaron 53 muestras de individuos que informaron no haber tenido contacto con glifosato para la muestra de control. Los análisis bioquímicos se realizaron mediante equipo automatizado, mientras que la colinesterasa se realizó mediante el método cinético de Ellman. Los datos fueron sometidos a análisis estadístico, donde se observaron cambios en los sistemas nervioso, respiratorio, auditivo, cutáneo y urinario. A través del análisis de datos, asociado a información en la literatura sobre los síntomas y daños causados por el glifosato, se puede concluir que la población residente en el área rural, practicando agricultura familiar, presenta fuertes signos de intoxicación crónica por formulaciones a base de glifosato.
Palabras clave: Glifosato; Exposición crónica; Exposición ambiental; Evaluación clinica.

1. Introduction

Glyphosate, N-(phosphonomethyl)-glycine, is the active ingredient in various formulations of non-selective herbicides. It has a broad spectrum and is highly effective, which justifies its use in large volumes in the last forty years, under the assumption that the side effects are minimal (Van Bruggen et al., 2018). It is used intensively in transgenic crops of maize and soybean, modified genetically to be resistant to this substance (Perry et al., 2019). However, the use in other crops is common, mainly by the application in the pre-planting phase to desiccate the soil, replacing the mechanical cutting of the vegetation unwanted by the chemical treatment (Fernandes et al., 2019; Zhang et al., 2019). For this reason, it is common for small producers, who practice family farming, to use this herbicide indiscriminately, without considering precautions regarding the protection of human and environmental health.

When it comes to exposure to pesticides, it is common for critical eyes to turn to occupational toxicology, where an individual is exposed directly to the product, many of whom do not use the necessary protection. However, contamination can occur in an environmental way, since glyphosate remains in the soil for a long period of time and may also be mixed with groundwater and peri-domiciliary water, causing subclinical intoxication due to the low concentrations that are harmful to long term health (Bento et al., 2017).

Glyphosate acts as a mechanism of action to inhibit the enzyme 5-enoylpyruvyl-chiquimate-3-phosphate synthase (Lopes et al., 2018). Although its mechanism of action affects biochemical routes unique to plants, this herbicide has become the target of several studies due to the risks it promotes to human health when used incorrectly. The presence of glyphosate residues and their main metabolite, aminomethylphosphonic acid (AMPA), have already been described in all environmental compartments, such as soil and dust (Aparicio et al., 2013; Bento et al., 2017; Silva et al., 2018), in surface waters, in rivers and in the sea (Battaglin et al., 2014; Carles et al., 2019; Fernandes et al., 2019; Mercurio et al., 2014) and in the air (Ravier et al., 2019). In plants, glyphosate can be absorbed and transported to the parts used as food, where residues persist for a long time and are not degraded with cooking (Zhang et al., 2019). In addition to the fruits, grains and vegetables (Botero-Coy et al., 2013; Chen et al., 2013; Harris & Gaston, 2004), glyphosate and AMPA residues were found in infant formulas based on soybean (Rodrigues & de Souza, 2018), indicating an exposure to this herbicide ubiquitously.

The high solubility in water and the ionic nature of glyphosate retard penetration through the hydrophobic cuticular waxes of plants. For this reason, glyphosate is commonly formulated with surfactants, which decrease the surface tension of the solution and increase the penetration into the tissues of plants (Bolognesi et al., 2009; Giesy et al., 2000). However, the action of surfactants is similar on the skin, so they are used in medicines and cosmetics to increase the penetration of active ingredients (Lémery et al., 2015). Studies have suggested that the toxicity of commercial formulations based on glyphosate is due to synergistic effects between the herbicide and other products of the formulation, such as tallowamine polyethoxylate-like surfactants, belonging to the class of polyethoxylated amines (POEA), which facilitate the penetration of glyphosate through plasma membranes, potentializing its toxicity, besides possessing intrinsic toxicity in some cases (Cattani et al., 2014; Robin Mesnage et al., 2019).

Although some studies discuss the low uptake of glyphosate in the human body, recently published data show that this pesticide is absorbed at voluntary exposures in cases of suicide attempts (CHO et al., 2019; GARLICH et al., 2014; ZOUAOUI et al., 2013) and in occupational exposures (Connolly et al., 2017, 2019), besides correlations between cases of respiratory and dermatological diseases and environmental exposure to glyphosate after aerial spraying of this pesticide (Camacho & Mejía, 2017). Several symptoms related to acute intoxication have already been described, such as gastrointestinal symptoms, altered consciousness, hypotension, respiratory distress and renal failure (H.-L. Lee et al., 2000; Zouaoui et al., 2013).

The interest in chronic toxic effects from exposure to glyphosate-based formulations is constantly increasing, given the accumulation of these substances in the environment. There is strong evidence that exposure to this compound may cause genotoxic damage, based on studies in humans and experimental animals (IARC monographs on the evaluation of carcinogenic risks to humans, 2017). In addition, according to (Van Bruggen et al., 2018), correlations were found between the increased use of glyphosate and a wide range of diseases including various forms of cancer, kidney damage and mental illnesses such as Attention Deficit Hyperactivity Disorder (ADHD), autism spectrum disorder, Alzheimer's and Parkinson's (Fluegge & Fluegge, 2016; Fortes et al., 2016; Jayasumana et al., 2015; R. Mesnage et al., 2015; Swanson et al., 2014). In addition, glyphosate is also related to decreased acetylcholinesterase activity in rats (Cattani et al., 2017; Van Bruggen et al., 2018).

Therefore, in the absence of population information on chronic glyphosate poisoning, the objective of the present study was to evaluate whether the population living in rural areas, a family farming practitioner, has evidence of chronic poisoning by glyphosate-based formulations, by evaluating of clinical signs and symptoms and laboratory data.

2. Methodology

It is an evaluative research of mixed epidemiological nature (quali-quantitative), using a questionnaire with closed questions, previously validated, associated with biochemical data of the study volunteers (Lima-Costa & Barreto, 2003; Pereira et al., 2018). The technique employed for this scientific research work is that appropriate to population survey studies. The study was conducted in Brazil, in the southern region of Minas Gerais state, in the rural area of Paraguaçú, a region that has a predominance of coffee and maize, where there is potential exposure to glyphosate-based formulations as previously mentioned by Silveiro et al (Silvério et al., 2017). In this region the manual extermination of weeds has been abolished and the desiccation of the land is carried out employing chemical agents, with Roundup® being the main commercial product used.

Data were collected in August and September 2018. As a criterion, all those who were environmentally exposed to glyphosate-based formulations (n = 162), chosen according to the answer given, were included in the group of exposed volunteers. For the control group, volunteers living in urban areas who were not exposed to any type of pesticide were chosen (n = 53). No dietary exposure was assessed in any group of volunteers. Data collection and biological samples were performed with the approval of the Research Ethics Committee of the University of José do Rosário Vellano (UNIFENAS), under opinion number 149718. Informed consent was obtained from each volunteer.

2.1 Clinical evaluation

A standardized questionnaire, previously validated, was used to collect demographic data (age, sex, etc.), lifestyle (smoking, coffee and alcohol consumption, diet, etc.), occupation (number of hours worked by day, number of days worked per year and personal protective equipment), as well as epidemiological and clinical data of rural residents, obtained at the State University of Campinas (Unicamp) and modified by researchers who used this in other previously published studies (Silvério et al., 2017). The application of the instrument and clinical evaluation were performed by a team of trained applicators for this purpose.

In addition to the questionnaire, heparinized blood samples were collected for biochemical measurements and determination of cholinesterase activity. Biochemical measurements (AST, ALT, GGT and CRE) were performed on a Wiener lab autoanalyzer CM 250 (Wiener lab Group, Argentina), using commercial kits kindly donated by Gold Analisa (Gold Analisa Diagnóstica Ltda, Minas Gerais, Brazil). The evaluation of cholinesterase activity was determined according to the Ellman kinetic method, modified by Harlin and Ross (Ellman et al., 1961; Harlin & Ross, 1990), on a Shimadzu UV 180 spectrophotometer using the wavelength of 430 nm. It is based on the colorimetric measurement of the rate of acetylthiocholine hydrolysis by blood cholinesterases. For the interpretation of acetylcholine activity results, the regional reference value was used since it was not possible to determine individual reference values for workers due to the prolonged exposure time without leave greater than 30 days. Regional reference values were estimated from the measurement of cholinesterase activity of 100 individuals of both sexes and without occupational exposure to pesticides residing in the Alfenas urban zone. The enzymatic activity range obtained for TChE was from 12.7 to 30.5%, for PChE from 1 to 6.4%, and for AChE from 31.1 to 59.4%. All samples and data were subjected to blind analysis.

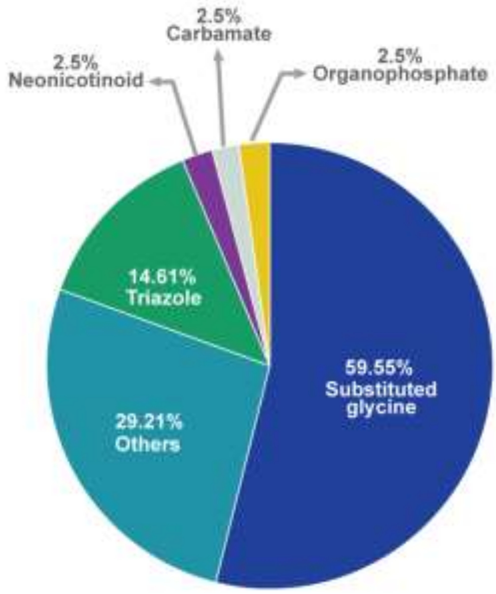
2.2 Statistical analyzes

Data were analyzed using the program BioStat®, version 5.0 (AnalystSoft Inc.), for the frequencies of categorical variables and measures of position and dispersion of quantitative variables (descriptive statistics). The normality of the data was evaluated using the Shapiro-Wilk test. One-way variance analysis (ANOVA) was used to compare parametric data, while non-parametric data were evaluated using the Mann-Whitney tests. The relationships between different qualitative variables were determined by the Chi-Square test. The level of significance was 5%.

3. Results

Data from 162 rural residents were evaluated, with an average age of 47.70 ± 14.96 years, of with 63.58% were male. The control group consisted of 53 volunteers living in the urban area, who reported no exposure to any type of pesticide, with 43.40% male, with a mean age of 34.57 ± 12.67 years. All rural volunteers reported exposure to glyphosate base formulations, with 55.42% of volunteers reporting that this was the last pesticide to which they were exposed occupationally. In addition, 59.55% of volunteers reported that glyphosate-based formulations are the most frequently used among the most commonly used pesticides, as shown in Figure 1.

Figure 1 - Pesticides used frequently by volunteers.



Source: Authors (2021).

In order to evaluate possible interferences, smoking and alcohol consumption among the study volunteers were evaluated. Both groups have about 43% of smoking volunteers. There was no statistical difference between the data when they were evaluated separating groups of smokers and non-smokers ($p > 0.05$). The same occurred when data were evaluated separating groups of consumers from alcoholic and non-consumers ($p > 0.05$). In this case, the exposed group had a frequency of 42.59% of alcohol consumers, while the control group had a frequency of 7.55%. The average schooling of urban volunteers was 15.34 ± 8.70 years, while for rural volunteers it was 5.79 ± 3.36 years. Other data that characterize the rural resident population are presented in Table 1.

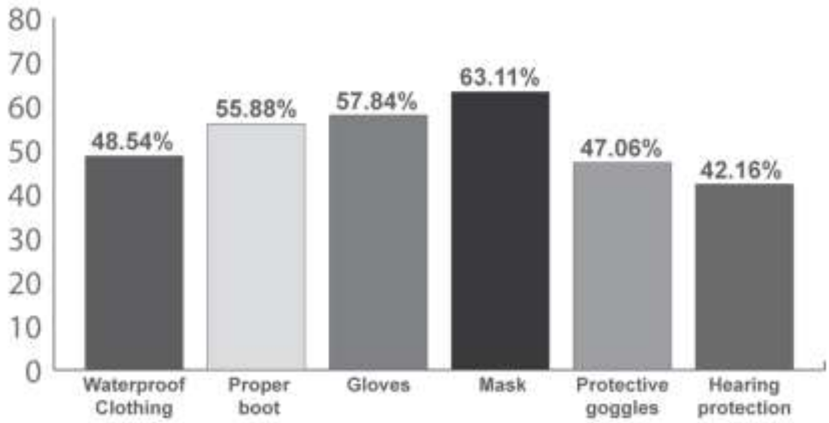
Table 1 - Characterization of volunteers living in rural areas.

Variables		**Frequency (%)**
Work relationship		
	Owner	48,70
	Salaried	23,38
	Sharecropper/ tenant	7,14
	Others	20,78
Worker role		
	Administrative	10,07
	Agricultural Technician/Agronomist	2,88
	Pesticide Applicator/Preparer	19,42
	Family farming	67,63
Time of contact with pesticides		
	Up to 5 years	13,27
	6 to 10 years	16,81
	11 to 20 years	34,51
	> 20 years	35,40
Mode of apllication		
	Costal pump	60,36
	Hose	3,60
	Tractor without protective cabin	27,93
	Tractor with protective cabin	4,50
	Others	3,60
IPE usea		
	Complete	37,61
	Incomplete	33,33
	None	29,06
Poisoning		19,51

a Individual protection equipment. Source: Authors (2021).

The Table 1 show work relationship, worker role, time of contact with pesticides, mode of application, use individual protection equipment and poisoning. The evaluated volunteers, when working with pesticides, mostly did not follow safety standards such as the use of personal protective equipment (PPE) and proper cleaning techniques of these equipment, correct instructions for the application of pesticides and adequate time for harvesting. Regarding the use of PPE, only 37.61% of volunteers said they use it correctly. However, when asked which PPE were used, there was no item cited by 100% of the volunteers (Figure 2), which confirms the lack of information on the correct use of these equipment.

Figure 2 - Items belonging to the most used personal protective equipment.



Source: Authors (2021).

The percentages of change in each system of the organism in the volunteers studied are presented in Table 2, which shows the percentage of changes in the two groups under study, exposed to glyphosate and not exposed (control group) and the p with the significance between them. The statistical analysis showed significant alterations in symptoms related to the central nervous system, respiratory, urinary, auditory and skin and mucosal functions when comparing the data from the two groups studied.

Table 2 - Systems featuring changes in the three different groups.

System featuring changes		Exposed to glyphosate (%)	Not exposed to glyphosate (%)	p[a]
Cardiovascular		32,69	26,42	0,3937
Central nervous				
	Muscle weakness	16,56	3,77	0,0179
	Blurred vision	14,65	1,89	0,0116
	Irritability and restlessness	22,29	7,55	0,0170
	Tingling limbs	19,75	7,55	0,0394
Digestive		37,50	39,62	0,7826
Respiratory				
	Respiratory difficulties	11,61	1,89	0,0339
	Cough	20,65	5,66	0,0118
Hearing				
	Tinnitus	15,13	3,77	0,0296
Skin and mucous membranes				
	Sensitizing contact dermatitis	8,72	0	0,0262
	Eye irritation	8,72	0	0,0262
Urinary				
	Dark / bloody urine	9,87	0	0,0175
Spontaneous abortions		12,77	18,87	0,2812
Children with congenital malformations		2,76	0	0,2219

[a] Chi-square test, with 5% significance. Source: Authors (2021).

Laboratory tests were performed to confirm information from clinical data analysis, which were self-reported by study volunteers. Decreases in acetylcholinesterase activity were found in 13.19% of volunteers and in butyrylcholinesterase in 2.72%. There was no evidence of dependence between the time-of-exposure data (relative to the time elapsed from the last contact with pesticides) and the decrease in acetylcholinesterase activity using the chi-square test ($p > 0.05$). Data from volunteers showing inhibition of butyrylcholinesterase were excluded from the analyzes in order to avoid statistical bias. The frequency of volunteers who presented changes in markers of hepatic and renal function are presented in Table 3, which explains the differences in the percentages of changes between the group of those exposed to glyphosate and those not exposed, and also the significance between the data.

Table 3 - List of hepatic and renal marker results.

Marker		**Exposed to glyphosate**	**Not exposed to glyphosate**	p^a
AST (%)		6,41	3,77	0,4760
	Average (± SD) (U/L)	24,79 (± 22,57)	25,75 (±10,80)	
	Interval (U/L)	10 – 92	10 - 86	
	Median (U/L)	21	24	
	p^b	0,003		
ALT (%)		10,26	1,89	0,0540
	Average (± SD) (U/L)	25,15 (22,14)	18,60 (12,39)	
	Interval (U/L)	10 - 181	10 - 92	
	Median (U/L)	19,50	16	
	p^b	0,007		
GGT(%)		2,04	3,77	0,6050
	Average (± SD) (U/L)	21,39 (24,58)	23,78 (17,19)	
	Interval (U/L)	8 - 182	8 - 95	
	Median (U/L)	16	18	
	pb	0,121		
CRE		5,23	0	0,0900
	Average (± SD) (U/L)	0,92	0,76	
	Interval (U/L)	0,34 – 1,54	0,43 – 1,13	
	Median (U/L)	0,89	0,71	
	p^b	< 0,01		

Notes: a Chi-square test; b Mann-Whitney test; AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; GGT = Gamma glutamyl transferase; CRE = Creatinine; SD = standard deviation. Source: Authors (2021).

In addition to the data presented in Table 3, the levels of total cholesterol, high density lipoprotein (HDL), triglycerides, urea and glycemia of the exposed volunteers were evaluated. Of these, 49.67% of the volunteers living in the rural area presented total cholesterol levels above the desirable level (range of 113 to 311 mg dL-1), 2.04% had below-desirable HDL levels (range of 35 to 96 mg dL-1), 10.46% had higher than desirable levels of triglycerides (range 29 to 437 mg dL-1), 22.37% had high levels of urea (range of 16 to 90 mg dL-1) and 5 , 92% presented alterations in glycemia levels (range of 50 to 150 mg dL-1).

The glomerular filtration rates of the exposed volunteers were estimated using the Cockcroft-Gault equation (Cockcroft & Gault, 1976), based on the observation of the significant difference in mean plasma creatinine levels between the groups. Changes in glomerular filtration rate were observed in 54% of the evaluated volunteers, and among these 88% of volunteers presented glomerular filtration rate between 60 and 89 mL min-1 / 1.73 m2, 6% between 45 and 59 mL min -1 / 1.73 m2 and 6% between 30 and 44mL min -1 / 1.73 m2.

4. Discussão

Currently, glyphosate is the most widely used herbicide in the world, being presented in several different commercial formulations (Perry et al., 2019). In Brazil, glyphosate is the most commercialized pesticide (Fernandes et al., 2019) and the indiscriminate use has been causing great concern related to the health of people exposed in a direct and indirect way, in this case, residents of the rural area, who are exposed occupational, environmental and food form of this pesticide. In addition to ease of purchase, lack of care in handling the product can create dangerous exposure, leading the worker, and consequently people living near the farm, to present serious health problems.

In the region where the study was carried out, which is composed of large areas of crops belonging to small farmers, there is a predominance of family farming. This information was fundamental for the evaluation of the health conditions of the population. Within family farming, all family members, regardless of age or gender, participate in all stages of production, from land preparation and planting to financial management (Cattelan et al., 2018). Volunteers from this study who did not self-refer as participants in family farms were mainly involved in the preparation and application of pesticides, which resulted in more than 87% of the volunteers directly exposed (Table 1).

Although the period chosen for collecting the data is a harvest season, when little or no pesticide use is expected, more than 50% of the volunteers reported use of glyphosate between 7 and 60 days. In addition, 59.55% of the volunteers cited glyphosate as the most widely used pesticide. The main form of pesticide application reported by the study volunteers was the costal pump, which is cited by several authors as an additional factor of exposure, followed by application using tractors without a booth, which is mistakenly treated as safe by volunteers using majority, only ear protectors during application using this vehicle. These results are consistent with other studies carried out in other regions of Brazil, showing a great use of this pesticide and the absence of care at the time of use (Cattelan et al., 2018; Kahl et al., 2018; Santos et al., 2019).

Published articles focus on the contribution of the low level of schooling to the inappropriate use of pesticides, since there are difficulties in reading and understanding the labels and absence in the association of health problems with the use of these substances. This can be translated by the difficulty that the workers exposed to these substances possess in using personal protective equipment (PPE) correctly (Feola et al., 2012; Viero et al., 2016). In this study it can be observed that besides the percentage of individuals who said to use the PPE correctly is relatively low, there was no PPE cited by all volunteers, which clearly demonstrates the lack of understanding of the population about the correct use of these equipment, despite the problems, they are still the best form of protection against exposure to these substances. Similar data was found by Cattelan et al. 2018, Rezaei, Damalas and Abdollahzadeh 2018, which show the importance of the implementation of public policies aimed at adequately informing, within the limitations of this population, about the hazardousness and forms of protection during the use of pesticides.

The indiscriminate consumption of pesticides, which can lead to contamination of the work environment and peri-home, among others, combined with inadequate use of PPE when occupationally exposed, may justify the data obtained through the clinical evaluation of the volunteers of this study. Symptoms related to cholinergic syndrome such as muscle weakness, blurred vision, agitation and irritability, tingling of limbs and breathing difficulty, and tinnitus were mentioned by the rural area residents, with a statistically significant difference when compared to data reported by the population living in the urban area ($p < 0.05$). Although these symptoms are directly related to acute and chronic organophosphate poisoning (Huen et al., 2012; Silvério et al., 2017), only 4.5% of the volunteers in this study reported the use of these classes of cholinesterase inhibitor pesticides (Figure 1) and only 2.72% showed a decrease in butyrylcholinesterase activity. Published papers on acute poisoning by glyphosate formulations have clinical symptoms like organophosphate poisoning. Some authors cite severe clinical signs such as respiratory distress and cardiac arrhythmias following suicide attempts by ingesting glyphosate-based formulations (H.-L. Lee et al., 2000; Zouaoui et al., 2013). Glyphosate, together with AMPA, has also been reported to increase blood-brain barrier permeability, and can lead to various neurological effects such as unconsciousness and tremors (Martinez & Al-Ahmad, 2019).

In this study, 13.19% of the volunteers presented a decreased acetylcholinesterase activity. According to Silvério et al. (2017), this alteration may be related to incomplete recovery after exposure to organophosphates, even after a non-exposure period, as some volunteers reported exposure to these pesticides. However, there are published studies claiming that glyphosate-induced acetylcholinesterase inhibition may lead to an accumulation of acetylcholine in the brain synaptic clefts, which may trigger events that contribute to the depressive behaviour observed in rats exposed to this pesticide, although no changes are observed on acetylcholinesterase activity evaluated in peripheral blood (Cattani et al., 2017). In another study, a significant inhibitory effect on the activity of this enzyme was observed in a work performed on fish (Menéndez-Helman et al., 2012). In addition, both glyphosate and AMPA, in concentrations associated with acute intoxication, decreased acetylcholinesterase activity in erythrocyte cultures (Kwiatkowska, Nowacka-Krukowska, et al., 2014). A case report of a patient with parkinsonism after chronic occupational exposure to glyphosate has also been published (Wang et al., 2011). Therefore, new studies should be conducted to clarify how the effect of glyphosate on acetylcholine metabolism may interfere with human health, considering that to date there are no data in the literature that assess the interaction between organophosphate pesticides and glyphosate.

Data regarding to problems related to the respiratory, auditory, skin and mucous membranes also presented statistical differences when compared to those of urban residents. Studies evaluating acute glyphosate exposure cite respiratory distress as one of the main complications caused by this pesticide (Roberts et al. 2010; Zouaoui et al. 2013; Lee et al. 2000). In addition, a study that evaluated the incidence of respiratory problems after aerial spraying of glyphosate shows an increased probability of exposed respiratory and dermatological problems (Camacho & Mejía, 2017). However, no published work has been found to explain how these problems occur. In the case of respiratory problems in acute intoxications, studies suggest that respiratory distress is derived from laryngeal lesions that lead to infiltrations, due to the irritant properties of the formulations (H.-L. Lee et al., 2000; Zouaoui et al., 2013).

Skin is one of the main routes of exposure to pesticides in the absence of proper use of PPE. Contact dermatitis, together with eye lesions, are the most common effects of exposure to pesticides, either through irritative or allergic mechanisms, often reported in clinics (Sanborn et al., 2007). Some authors report that irritant skin and mucosal properties of glyphosate-based formulations may be related to the surfactant used in the formulation (Robin Mesnage et al., 2019). There are no reports in the literature of effects related to the auditory system caused by formulations based on glyphosate. However, it is an effect that has been discussed in the exposure to other pesticides (Silvério et al., 2017).

Although hepatic damage was reported in a study that evaluated acute exposure to glyphosate-based formulations in 131 volunteers (H.-L. Lee et al., 2000) and in studies evaluating chronic exposure to glyphosate in animals (Çağlar & Kolankaya, 2008; Robin Mesnage et al., 2015), the results of this study did not show significant changes in hepatic markers when compared to the frequency of volunteers with alterations between rural and urban residents. Likewise, there was no statistical difference when comparing the frequencies of changes related to renal damage between exposed and non-exposed volunteers. However, when the glomerular filtration rates of the exposed volunteers were calculated, possible cases of mild renal insufficiency were observed, when plasma urea and creatinine levels were still normal, or moderate where already high levels of plasma urea and creatinine were observed (Romão Junior, 2004). These data justify the statistical difference found in the clinical evaluation, in relation to changes in the urinary system, in addition to the percentage of exposed individuals who presented elevated levels of urea in the blood.

Several studies provide evidence for the nephrotoxic properties of glyphosate (Garlich et al., 2014; Jayasumana et al., 2014, 2015; D. H. Lee & Choi, 2017; Robin Mesnage et al., 2015; Roberts et al., 2010). In addition, glyphosate is capable of inducing oxidative stress in animals and cell cultures at low concentrations (Jasper et al., 2012; Kwiatkowska, Huras, et al., 2014). Since this pesticide is predominantly eliminated by the renal pathways (Martinez & Al-Ahmad, 2019) and this organ is particularly susceptible to oxidative stress and is one of the main pathological mechanisms contributing to tubulo-interstitial nephritis (Nanayakkara et al., 2012), attention should be given to possible kidney problems caused by glyphosate in rural residents who are frequently exposed to this pesticide. These data are reinforced by studies conducted by Jayasumana, Gunatilake and Siribaddana (2015), which correlate chronic exposure and renal damage, in addition to the results of studies conducted by Zouaoui et al. (2013) and Lee and Choi (2017), who cite severe kidney damage after acute exposure to glyphosate.

In this study, almost 50% of exposed volunteers presented higher than desired levels of cholesterol, as well as lower frequencies of triglyceride, HDL and glycemia levels. According to El-Shenawy (2009), oxidative stress induced by glyphosate may also be responsible for the increase in serum levels of cholesterol and triglycerides. Studies performed on rats show a significant increase in the levels of serum lipoproteins (LDL and HDL), cholesterol and triglycerides in groups exposed to formulations based on glyphosate (Çağlar & Kolankaya, 2008; El-Shenawy, 2009). However, no data on humans were found.

5. Conclusion

The indiscriminate use of glyphosate can harm the health of the exposed population, not only occupationally, but also environmentally, especially in rural residents, where crops are located around homes. Although there are several recent studies, little is known about the potential harmful effects of this pesticide on the health of the population. Based on the clinical and laboratory data found, in association with the existing information in the literature on the symptoms and damage generated by glyphosate, it can be concluded that the population living in rural Paraguaçú, Brazil, a family farming practitioner, has strong indications of being carriers of chronic poisoning by glyphosate-based formulations. Thus, further studies should be conducted to verify biochemical, hematological and systemic effects in workers exposed to glyphosate.

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