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Evaluation of clinical parameters and acetylcholinesterase activity in agricultural workers exposed to organophosphate pesticides from Guasave, Sinaloa, Mexico

Evaluación de parámetros clínicos y actividad acetilcolinesterasa en trabajadores agrícolas expuestos a plaguicidas organofosforados de Guasave, Sinaloa, México

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ABSTRACT

Sinaloa is one of Mexico's most productive agricultural states. However, this activity involves intensive pesticide use, which can have detrimental effects on the health of those exposed. The present descriptive, cross-sectional, and analytical study evaluated the effects of organophosphate pesticides on the clinical and enzymatic parameters of agricultural workers in Guasave, Sinaloa. Clinical parameters and acetylcholinesterase activity in blood samples were monitored using a data-collection instrument. The findings indicated that workers were not utilizing adequate protective equipment, suggesting exposure through various routes. The most frequently reported symptoms were urination (45.8%), salivation (41.7%), and sweating (29.7%). While most clinical parameters were within the reference range for both exposed and unexposed individuals, a substantial decline in acetylcholinesterase activity was evident in the exposed cohort. It can be concluded that exposure to organophosphate pesticides influences acetylcholinesterase activity in agricultural workers. Nevertheless, incorporating a greater number of biomarkers from various biological samples is recommended to obtain a more comprehensive picture of the health effects resulting from this exposure.

KEY WORDS: Agrochemicals, Biomarkers, Agriculture, Occupational Exposure.

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RESUMEN

Sinaloa es uno de los estados agrícolas más productivos de México, pero esta actividad implica el uso intensivo de plaguicidas, que pueden afectar la salud de los trabajadores expuestos. Se realizó un estudio descriptivo, transversal y analítico para evaluar los efectos de los plaguicidas organofosforados sobre parámetros clínicos y enzimáticos de trabajadores agrícolas en Guasave, Sinaloa. Se aplicó un instrumento de recolección de datos y se monitorearon parámetros clínicos y la actividad de la acetilcolinesterasa en muestras de sangre. Los resultados mostraron que los trabajadores no utilizan el equipo de protección adecuado, lo que indica una exposición a través de diversas vías. Entre los síntomas reportados con mayor frecuencia se encuentran la micción (45.8%), salivación (41.7 %) y sudoración (29.7 %). Aunque la mayoría de los parámetros clínicos estuvieron dentro de los rangos de referencia tanto en las personas expuestas como en las no expuestas, se observó una disminución considerable de la actividad de acetilcolinesterasa en las personas expuestas. Se concluye que la exposición a plaguicidas organofosforados afecta dicha actividad en trabajadores agrícolas. No obstante, se recomienda incorporar más biomarcadores en distintas muestras biológicas para obtener un panorama más completo de las afectaciones a la salud derivadas de esta exposición.

PALABRAS CLAVE: Agroquímicos, Biomarcadores, Agricultura, Exposición ocupacional.

Introduction

Sinaloa is one of the wealthiest states in terms of agricultural development, making it essential to national agricultural production. However, this productivity has also led to excessive and inappropriate pesticide use, affecting a large proportion of the population (Arciniega-Galaviz, 2021). One of the most important agricultural areas in Sinaloa, in terms of both surface area and production, is the IR063 Irrigation District, located in Hydrological Region III of the North Pacific Basin, in the far north of the state, spanning the municipalities of Guasave and Sinaloa de Leyva. The district has 118,218 hectares (ha) of irrigated land, and the crops grown there (mainly corn, beans, chickpeas, potatoes, wheat, and sorghum) require the application of a high volume of pesticides (Hernández-Antonio & Hansen, 2011; González-Farias *et al*., 2014; CONAGUA, 2019; López-Gaxiola *et al*., 2021). The main chemical classes of pesticides used in Guasave, Sinaloa, in order of importance, are reported to be organophosphates, pyrethroids, organochlorines, carbamates, benzimidazoles, benzoic acid salts, and chlorophenoxy (Hernández-Antonio & Hansen, 2011; García-Gutiérrez & Rodríguez-Meza, 2012; González-Farias *et al*., 2014). The most widely used chemical class in DR063, organophosphates, induces toxicity by inhibiting the enzyme acetylcholinesterase (AChE) in nerve terminals in the central and peripheral nervous systems (Mitra *et al*., 2019). This leads to an inability to hydrolyze the neurotransmitter acetylcholine (ACh), resulting in elevated ACh levels at the synapse and overstimulation of both muscarinic and nicotinic receptors. This can cause toxic effects, both neuronal and non-neuronal, in exposed organisms (Bernal-González *et al*., 2023). In this regard, the dysregulation of

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the cholinergic system resulting from exposure to these compounds has been associated with sensory, motor, immunological, endocrine, and neurological alterations, leading to various pathologies such as cancer, hypersensitivity, neurodegenerative diseases, infections, and diabetes (Del Puerto-Rodríguez *et al*., 2014; García-Hernández *et al*., 2018; Martínez-Valenzuela *et al*., 2019; Saborío-Cervantes *et al*., 2019; Estremadoyro, 2022; Bernal-González *et al*., 2023). Recent research emphasizes that agricultural workers, indigenous communities, pregnant women, and children are among the groups most vulnerable to exposure to pesticides, including organophosphates. Therefore, special protection is required for these groups, particularly those who are occupationally exposed (Martínez-Valenzuela *et al*., 2019; Cestonaro *et al*., 2022; Chen *et al*., 2024; Vaezafshar *et al*., 2024).

As a biomarker of exposure and effect of organophosphate pesticides in occupationally exposed populations, the inhibition of cholinesterase (acetylcholinesterase and butyrylcholinesterase) in blood has been widely reported (Díaz *et al*., 2017; Bernal-Hernández *et al*., 2018; Dhananjayan *et al*., 2019; Caro-Gamboa *et al*., 2020; Herrera-Moreno *et al*., 2021; Kaur *et al*., 2023; Finhler *et al*., 2023; Kohoutova *et al*., 2024). In this regard, several studies have highlighted the impact on the health of agricultural workers due to exposure to organophosphate pesticides in the state of Sinaloa. Most of these studies are based on the administration of questionnaires to ascertain the relationship between exposure and specific symptoms, and/or the decrease in cholinesterase enzyme activity, specifically acetylcholinesterase, is also measured (Palacios-Nava, 2003; Palacios-Nava & Moreno-Tetlacuilo, 2004; Palacios-Nava *et al*., 2009; Palacios-Nava & Paz, 2011; Palacios-Nava, 2012; Arciniega-Galaviz, 2021; Ibarra-Ceceña & López de Haro, 2021; Arciniega-Galaviz & Fontalvo-Buelvas, 2024).

In this regard, among the studies conducted in Sinaloa, Palacios-Nava & Paz (2011) demonstrate the association between exposure to organophosphate pesticides and the presence of persistent symptoms (headache, stomach pain, myalgia, cramps, vertigo, weakness, and tearing) in agricultural workers. Demonstrating a probable adaptation of acetylcholinesterase levels in response to daily exposure to moderate but constant doses of pesticides.

Finally, Palacios-Nava (2012) conducted a study to determine the prevalence of symptoms, their relationship with working conditions, and the level of acetylcholinesterase activity. The study found that 63 of 100 workers experienced persistent symptoms and that the average enzyme activity was within normal ranges (3.69 ± 0.65 U/mL). Anemia was present in 65% of day laborers, most likely due to exposure to pesticides. However, the results showed no association between persistent symptoms and acetylcholinesterase activity levels.

Given the importance of agricultural activities in the state of Sinaloa and their association with the application of large quantities of organophosphate pesticides, this study aimed to evaluate the impact of exposure to these pesticides on clinical parameters and acetylcholinesterase enzyme activity in agricultural day laborers in the municipality of Guasave, Sinaloa. Despite the available information, there are currently no studies in Sinaloa and few in other Mexican states that address the potential adverse health effects of exposure to organophosphate pesticides.

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Material and Methods

Study population

A descriptive, cross-sectional, analytical study was conducted in two groups of agricultural workers in the municipality of Guasave, Sinaloa, Mexico. The first group comprised individuals with no occupational contact with organophosphate pesticides (unexposed, n = 11), while the second group consisted of workers with varying degrees of exposure (application or exposure in the field) to organophosphate pesticides (exposed, n = 25).

Questionnaire design and validation

A semi-structured questionnaire was designed to assess the potential health effects of pesticide exposure on agricultural workers in the municipality of Guasave. The questionnaire was structured in three sections. The first section included questions on sociodemographic factors (name, age, weight, sex, among others) and general health conditions considered dangerous for an agricultural worker exposed to organophosphate pesticides, as well as to confirm that the worker applies this type of pesticide. The second section of the questionnaire focused on the occupational characteristics of the participants, their job position, exposure risk factors, and the use of protective equipment during handling and application. The third questionnaire inquired about the frequency of symptoms associated with potential organophosphate pesticide poisoning. The questionnaires were administered in print, while those experiencing difficulties with reading and writing received assistance in completing the instrument.

The questionnaire used as an instrument was reviewed by a panel of six experts in the field of pesticide exposure. Following a thorough review of the questions, a consensus was reached regarding the relevance of each to occupational exposure to pesticides. Those deemed irrelevant were subsequently modified to align with the sociocultural context of the study population. This process evaluated the instrument’s validity. Subsequently, to assess its feasibility, a pilot test was conducted on 13 exposed workers, the results of which enabled a reliability analysis using Cronbach’s alpha (Rodríguez-Rodríguez & Reguant-Álvarez, 2020; Cancino *et al*., 2023; Torres-Sánchez *et al*., 2024).

Selection criteria

The study population consisted of male and female agricultural workers aged 18 and above who had voluntarily consented to participate by signing an informed consent form. Individuals who did not wish to participate, those with a doctor-diagnosed chronic illness, pregnant women, and/or individuals without a duly signed consent form were excluded from the study. In the non-exposed group, individuals who met the same criteria as the exposed group were invited to participate, except that they did not work in activities related to the use/handling of pesticides.

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Sampling

A non-probabilistic, intentional sampling method was employed, in which exposed workers were selected according to the criteria established by Arciniega-Galaviz & Fontalvo-Buelvas (2024). The inclusion criteria for the study were as follows: 1) the worker was employed in the application of pesticides or exposure in the field; 2) the subject was committed to providing precise and reliable information; 3) the subject was willing to reside and work in locations that were located within Irrigation District 063; and 4) the subject was willing to answer the questionnaire and provide a biological blood sample for the measurement of biochemical parameters and acetylcholinesterase enzymatic activity.

Ethical considerations

The ethical considerations outlined in the Declaration of Helsinki and the Nuremberg Code were taken into account in the implementation of this research. The present study was approved by the Bioethics Committee of the Autonomous University of the West (UAdeO) (CM-UAdeO 29.08/2021) (see supplementary material). Each participant signed an informed consent form, and the confidentiality of their data was guaranteed (the form is available in the supplementary material).

Analytical determinations

Collection and processing of biological samples: Blood samples (10 mL) were obtained by venipuncture during the fall-winter period (which corresponds to the high exposure season) using tubes with ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. All samples were analyzed on the day of collection within 8 hours (Herrera-Moreno *et al*., 2021).

Blood biometry and blood chemistry: A series of routine laboratory tests was conducted to evaluate the health status of the day laborers. A complete blood count encompassing various parameters (erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelets, platelet volume, leukocytes, neutrophils, lymphocytes, monocytes, and eosinophils) and blood chemistry (glucose, urea, creatinine, uric acid, total cholesterol, triglycerides, and urea nitrogen) in blood samples were performed in a certified clinical analysis laboratory, where impedance and colorimetric methods were employed to achieve the desired analytical outcomes, respectively.

Cholinesterase activity in whole blood: Determination of cholinesterase activity was performed according to the method described by Tor *et al*. (1994), with minor modifications. The samples were diluted 1:100 with Triton X-100. Subsequently, 500 µL of each sample was diluted to 1 mL with 0.1 M phosphate buffer (pH 8). To initiate the reaction, 250 µL of the diluted sample was added to each well of a 96-well microplate. The wells were then assigned to samples, replicates, and controls. Thereafter, 25 µL of DTNB (5,5’-dithio-bis(2-nitrobenzoic acid)) was added, and the

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wells were maintained at 25 °C. Subsequently, 25 µL of acetylcholine iodide was added to the sample wells. Twenty-five microliters of phosphate buffer were added to the designated "substrate blank" wells. The reaction was then allowed to reach equilibrium for 1 minute. The absorbance was measured at 405nm at 8-second intervals for 5 minutes in a plate reader (Multiskan FC, Thermo Scientific) with automated mixing. The analyses were performed in triplicate. Enzyme activity (EA) was corrected for hemoglobin according to Herrera-Moreno *et al*. (2021):

$$AE\ U/g\ de\ Hb = \frac{AE\ en\ U/mL}{\left(\frac{Hb\ en\ g/dL}{100}\right)}$$ Equation (1)

Statistical analysis

A descriptive statistical analysis was performed, yielding means, standard deviations, minimums, and maximums for continuous variables and frequencies and proportions for categorical variables. A subsequent investigation was conducted to perform a correlation analysis on the variables obtained from the clinical parameters and acetylcholinesterase activity. Furthermore, a correlation analysis was performed among all the variables evaluated in this study to explore the strength of the relationships. In this regard, the normality assumption for the variables was evaluated using the Shapiro-Wilk test to determine the optimal test for performing the correlation analysis. Subsequently, a linear regression analysis was performed using the variables with the highest positive or negative correlation values to evaluate the dependence among variables. Similarly, all models were evaluated for compliance with the assumptions of normality (Shapiro-Wilk test) and homoscedasticity of variance (Breusch-Pagan test). The latter test rejected the model's homoscedasticity at the 0.05 level.

Analyses were conducted to evaluate differences in the clinical and acetylcholinesterase activity parameters measured in blood between exposed and unexposed workers. Initially, Student's t-tests were conducted as a preliminary approach. To this end, the assumptions of normality were evaluated; if they were not met, the nonparametric Kruskal-Wallis test was performed. In instances where substantial disparities were identified, a comparison of means was conducted utilizing the Tukey test.

All statistical analyses were performed using SPSS version 22 for variables obtained from the semi-structured questionnaire and R version 3.6.2 for variables obtained from biological samples. Statistically significant values were defined as $p < 0.05$.

Results and Discussion

Questionnaire validation

The validation results indicated an overall Cronbach's alpha of 0.742 across all items, while individual items ranged from 0.70 to 0.75. The minimum acceptable value for Cronbach's alpha is 0.70; below this, the scale's internal consistency is deemed low. It can thus be concluded that, in

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general, the instrument demonstrates adequate consistency for the proposed purpose. This test has been extensively documented across various scientific disciplines as a reliable method for evaluating the precision of data-collection instruments (González-Alonso & Pazmiño-Santa Cruz, 2015; Toledo-Morales & Sánchez-García, 2015; Tuapanta-Dacto *et al*., 2017).

Muñoz-Quezada *et al*. (2019) evaluated the reliability and validity of a questionnaire designed to assess exposure to organophosphate pesticides in agricultural workers in Maule, Chile. The results demonstrated that the instrument generated had good internal consistency (Cronbach's alpha > 0.7), indicating high reliability for predicting probable pesticide exposure. The factors that were evaluated were as follows: The following factors were considered in the study: 1) working conditions during the application of pesticides; 2) the use of personal protective equipment; 3) workplace conditions related to exposure to pesticides; and 4) home conditions related to exposure to these compounds. The researchers concluded that the questionnaire developed had sufficient metric properties to characterize probable pesticide exposure in agricultural workers in the region under study.

Characteristics of the study population and occupational exposure to pesticides

Of the total population under study, 76 % were male and 24 % were female. The mean age of the subjects was 32.9 years (±12.7 years), with a range of 19 to 54 years. All participants who had been exposed to organophosphate pesticides reported participating in activities involving contact with these chemicals. The data indicate that 29 % of respondents reported being agricultural day laborers, 29 % reported being fumigators/applicators, 21 % reported performing pesticide preparation activities, 15 % reported being harvesters, and 2 % reported performing various activities.

In a study of agricultural day laborers, Palacios-Nava & Paz (2011) reported that the most common job for which subjects were employed was cutter (43.4 %), followed by pesticide applicator (26.4 %), general assistant (11.3 %), supervisor or steward (11.3 %), and application assistant (7.5 %). Ibarra-Ceceña & López de Haro (2021) reported that, in an indigenous community in El Fuerte, Sinaloa, approximately 25% of respondents identified as agricultural day laborers, with the remaining respondents comprising housewives (45 %), teachers (20 %), students (5 %), and ejido members (5 %).

The findings of this study indicated that 50 % of the participants had between one and 10 years of experience working with organophosphate pesticides, while the remaining participants exhibited a similar response percentage (16.7 %), categorized into the following groups: less than one year, between 11 and 20 years, and more than 20 years. The results of this study differ from those reported in the Culiacan Valley, where 81 % of agricultural workers are between 16 and 35 years old (Haro-García *et al*., 2002).

Palacios-Nava (2003) reported in a study conducted in an agricultural field in Sinaloa that the total population studied consisted of 75% individuals aged 15-26, which differs from the present

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study. However, Palacios-Nava states that among the agricultural day laborers considered in that study, 83% were migrants from other Mexican states. Palacios-Nava (2012) conducted a study in which she found that 87% of the agricultural day laborers surveyed were male, aged 15-31, of whom 31% were illiterate and 71% were migrants.

The findings of this study are consistent with those reported by Arciniega-Galaviz (2021) in Ahome, Sinaloa, where agricultural day laborers reported initiating employment in activities related to pesticide handling at ages 20 to 25 (45 %) and 15 to 20 (28 %). In a similar vein, Arciniega-Galaviz *et al*. (2021) reported that in Ahome, Sinaloa, 3% of the indigenous agricultural workers surveyed reported commencing work in agricultural activities involving pesticides while minors. In this regard, the association between occupational exposure to pesticides and the health of agricultural workers has been examined in a reductionist manner. This is because the latter typically belong to the lowest socioeconomic level in the agricultural regions of Sinaloa (Haro-García *et al*., 2002).

Regarding pesticide exposure, in the present study, 83.3% of respondents reported currently using organophosphate pesticides for pest control in crops established in the municipality of Guasave. Of these, 58.3 % reported using these pesticides permanently, while the remaining 41.7 % reported using them seasonally. Furthermore, 87.5% of respondents reported initiating activities related to the use of these substances at an early age (i.e., under 15 and between 16 and 25). This is a salient factor, as it has been documented that infants and the elderly are most vulnerable to the deleterious effects of pesticides, due to their immune systems being in development or in decline, respectively (Muñoz-Quezada *et al*., 2021; Prahl *et al*., 2021). However, it is essential to note that in Mexico, regulations prohibit individuals under 18 from engaging in agricultural activities involving direct contact with pesticides (STPS, 1999).

Regarding occupational exposure time from pesticide use, a study of agricultural day laborers in the Culiacan Valley found that 65% of subjects reported having been hired for more than 5 years on a piecework basis (Haro-García *et al*., 2002). This finding was consistent with that of workers hired for 4 to 8 hours/day, suggesting no statistically significant differences between the two groups. One of the primary factors influencing pesticide exposure is the duration of exposure during the working day. In this regard, the subjects in the present study were asked about the frequency of their most recent pesticide use. The predominant response indicated that the last application of pesticides occurred within a timeframe spanning more than a week to less than a month prior (54.2 %), followed by a period between more than a month and less than a year (37.5 %), and finally, a timeframe of more than a year and less than two years ago, as well as more than two years ago, exhibiting equivalent levels of usage (4.2 %) (Figure 1).

The questionnaire items regarding protection used during pesticide application, as reported by respondents, are summarized in Figure 2. The proportion of respondents who reported using personal protective equipment (PPE) was 75%, although the frequency of use varied. The items reported as being utilized consistently were boots or shoes (75 %), long-sleeved shirts and long-sleeved trousers (88 % for both), and raincoats or coveralls (71 %), while the remaining items of clothing exhibited usage frequencies of less than 30 %. Face masks or gas masks, gloves,

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goggles, and hats or helmets were the items most frequently reported as never having been used, with percentages of 58%, 62%, 46%, and 58%, respectively.

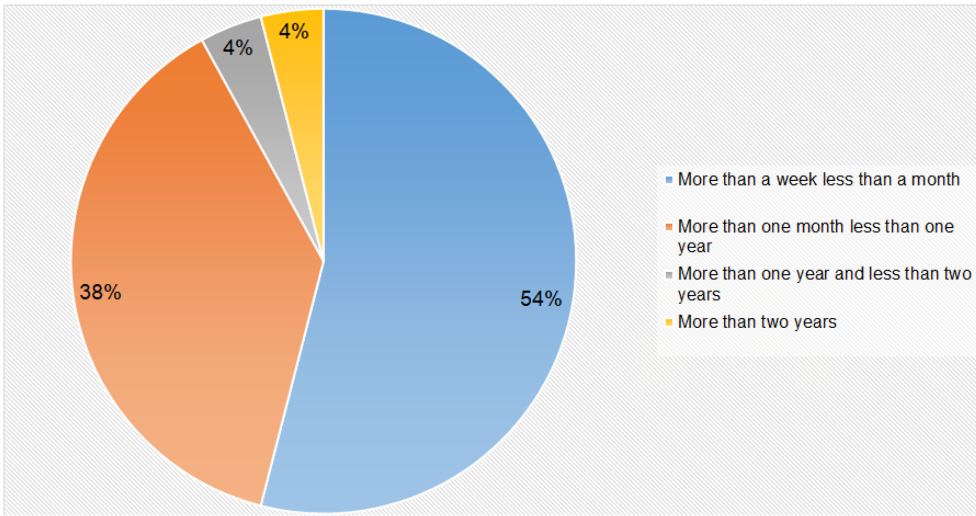

Figure 1. Frequency of application with respect to the last use of organophosphate pesticides reported by the people interviewed in the municipality of Guasave.

Source: own elaboration.

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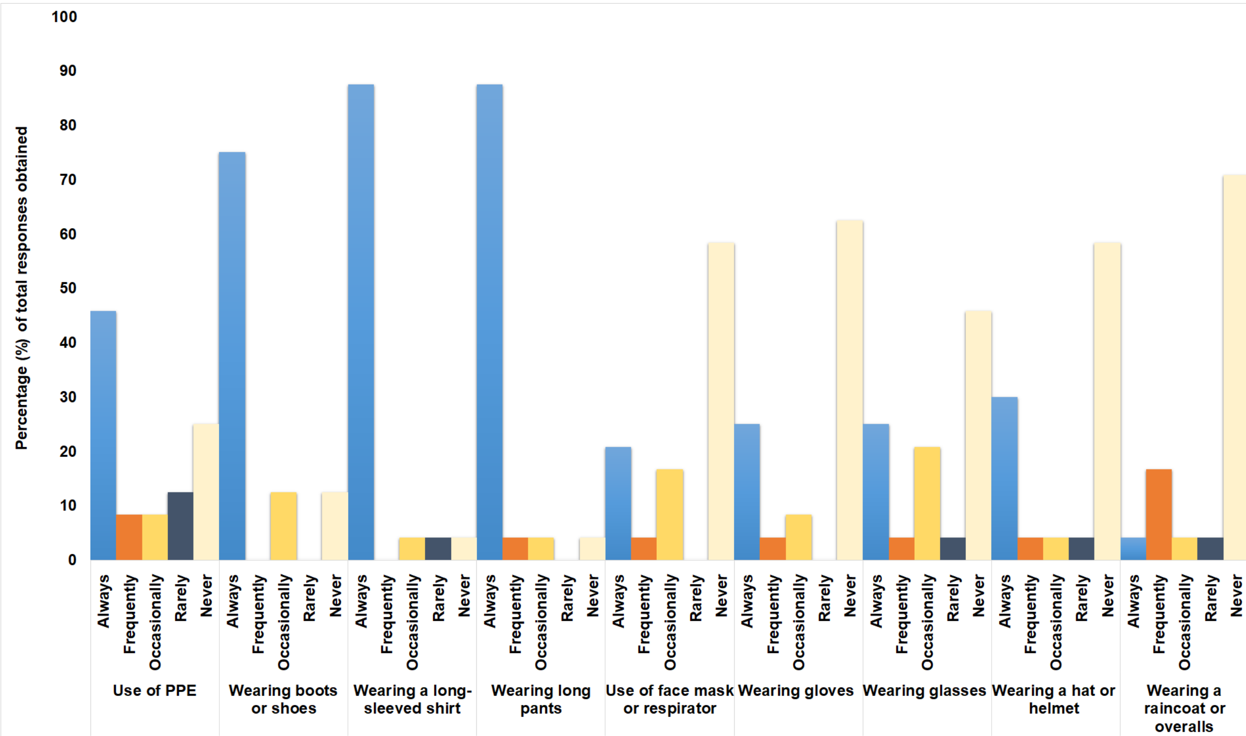

Figure 2. Percentage of frequency of use of personal protective equipment (PPE) by agricultural workers during handling with organophosphate pesticides in Guasave, Sinaloa, Mexico.

Source: own elaboration.

Palacios-Nava (2003) asserts that most agricultural workers in Sinaloa utilize rudimentary PPE, including hats, bandanas, and sandals. Palacios-Nava (2011) highlights that in an agricultural field in Sinaloa, 23.5 % of the workers interviewed stated that they did not use PPE; while 46.8 % used hats, bandanas, and sandals, 16.2 % used hats, masks, raincoats, gloves, and shoes (referred to by them as complete equipment). Finally, 13.5 % reported using hats, bandanas, raincoats, and gloves together.

Arciniega-Galaviz (2021) and Arciniega-Galaviz *et al*. (2021) reported results similar to those observed in the present study, in which the PPE most commonly used by agricultural workers during pesticide handling included long pants, long-sleeved shirts, bandanas, masks, and sneakers. These authors indicate that the PPE is rudimentary, in contrast to the recommended PPE for pesticide application, which poses a greater risk of exposure (Arciniega-Galaviz & Fontalvo-Buelvas, 2024).

Regarding the above, it is important to note that agricultural workers in Sinaloa do not use the required PPE. Consequently, manufacturers' recommendations for use are not being followed, potentially exposing workers to pesticides through various routes, including dermal contact, inhalation, and ingestion. A potential contributing factor to this issue is employers' apparent failure to provide adequate PPE to their employees. This is in direct contravention of the stipulations outlined in NOM-003-STPS-1999, which explicitly obliges employers to ensure the provision of PPE to their employees. Furthermore, the meteorological conditions in the study area are frequently extreme, primarily characterized by elevated temperatures, leading workers to refrain from wearing specific articles of clothing. Furthermore, the absence of training on safety measures for pesticide application has been highlighted (Arciniega-Galaviz, 2021; Arciniega-Galaviz *et al*., 2021; Arciniega-Galaviz & Fontalvo-Buelvas, 2024).

Presence of symptoms related to exposure to organophosphate pesticides

Regarding symptoms, the prevalence of symptoms reported by respondents upon exposure to organophosphate pesticides varied widely. The most frequently reported symptoms were urination (45.8 %), salivation (41.7 %), and sweating (29.7 %), while the remaining participants reported never having experienced symptoms or a frequency of less than 29 % (**Figure 3**). Palacios-Nava (2003) stated that among the symptoms reported by agricultural workers in a field in Sinaloa, the most frequent were fatigue or weakness (35 %), headache (27 %), muscle pain (25 %), blurred vision (23 %), irritated eyes (21 %), and dizziness and/or vertigo (19 %). Concurrently, Palacios-Nava & Paz (2011) discovered an association between days of exposure to pesticides and persistent symptoms, as well as between the level of exposure and the presence of probable symptoms (e.g. vertigo, difficulty breathing, chest pain, weakness, nausea) and specific symptoms (e.g. blurred vision, nervousness, cramps, tingling, salivation, body tremors) in an agricultural field in Sinaloa.

A more detailed study conducted by Palacios-Nava (2012) also in an agricultural field in Sinaloa reported that the most common symptoms were headache (26.7%), followed by weakness or fatigue (20.9%) and watery eyes (20.3%). The presence and number of symptoms were associated with various factors, including age, frequency of application, time of re-entry to the field after pesticide application, hours worked per week, and personal protective equipment used, among others.

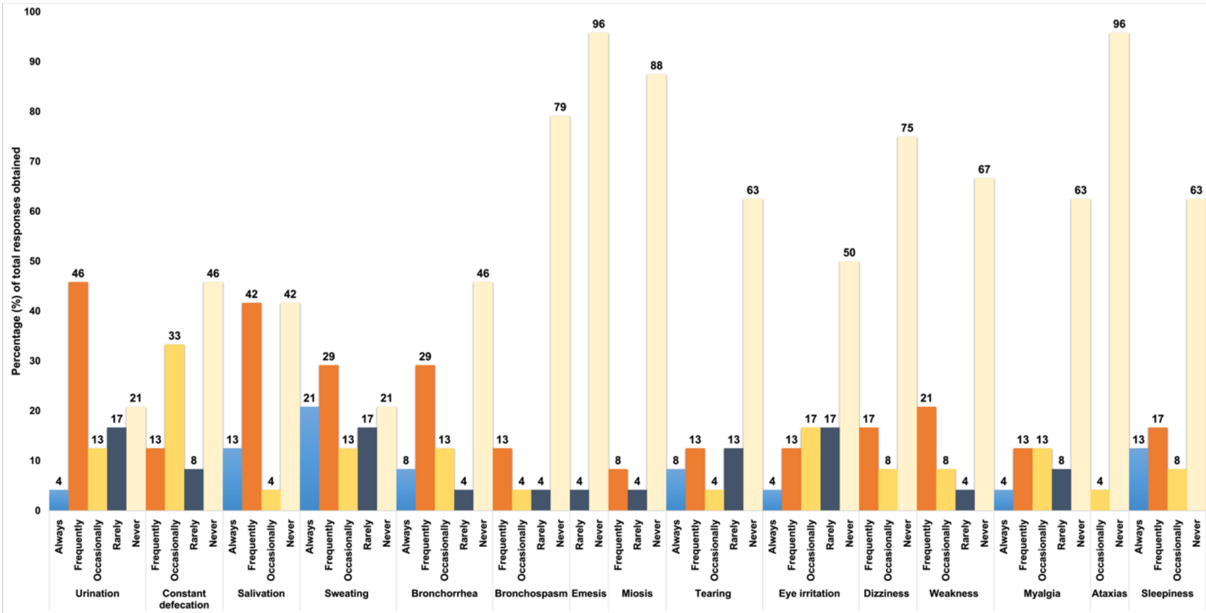

Figure 3. Percentage of frequency of symptoms reported by agricultural workers related to poisoning from handling organophosphate pesticides in Guasave, Sinaloa, Mexico.

The categories in symptom frequency are: Always, Frequently, Occasionally, Rarely and Never. In the figure, responses with 0% were omitted to improve its visualization.

Source: Own elaboration.

Arciniega-Galaviz (2021) reported that the principal symptoms experienced by agricultural workers in Ahome, Sinaloa, when performing activities involving direct contact with pesticides are: skin irritation (24 %), headache (19 %), and dizziness (16 %). However, the workers themselves have reported that the effects of the substance are minimal and only temporary, or that they tend to diminish or disappear by the following day. Consequently, 75 % of workers do not consider it necessary to seek medical attention. This may be attributable to the fact that daily wages are low, and seeking medical attention necessitates an absence from work, thereby reducing earnings.

On the other hand, Arciniega-Galaviz *et al*. (2021) reported that indigenous agricultural day laborers in San Miguel Zapotitlán, Ahome, Sinaloa reported experiencing symptoms related to pesticide exposure, including headaches (65 %), followed by dizziness (39 %), nausea (26 %), skin irritation (16 %), and blurred vision (3 %); while 26 % reported no symptoms. This observation highlights the importance of meticulous interpretation of reported symptoms, as they may occasionally be misinterpreted as manifestations of climatic influences, such as heat or the intensity of solar radiation characteristic of the region.

Perception of risk from pesticide exposure among the exposed population

About the perception of potential health risks associated with exposure to organophosphate pesticides, 95.8 % stated that they totally agreed, and 4.2 % agreed that continuous and unprotected exposure to pesticides can directly compromise health. Furthermore, 83.3% and 16.7% of respondents expressed “strongly agree” and “agree” with the assertion that pesticides not only compromise human health but also have a substantial detrimental impact on the environment.

Arellano *et al*. (2009) assessed perceptions of health risks associated with exposure to a mixture (r = 0.02) in the Mexicali and San Quintín valleys of Baja California, Mexico. The findings of this study suggest that participants can only recognize acute conditions but cannot recognize chronic effects. Furthermore, the perception of risk is closely related to an individual’s level of education and income; lower levels of education and income are associated with a lower perception of risk.

In a study conducted in Chiapas by Hernández-Valdés *et al*. (2017), the perception of health risks from exposure to pesticides among corn, flower, and vegetable producers was evaluated. Irrespective of the crop they reported cultivating and their declared educational attainment, farmers lack the requisite convictions to mitigate the risks posed by exposure to these substances, thereby engendering behavioral patterns that favor scenarios that compromise their health.

Rangel-Ortiz *et al*. (2023) conducted a study that identified pesticide use practices and perceptions of risk from exposure during handling in Guanajuato, Mexico. Furthermore, 69 % of the farmers surveyed acknowledged the environmental impact of pesticides, while among the suppliers surveyed, this statement was observed in 63 % of the participants. Perceptions of health risks from pesticide exposure vary across groups. 74% of farmers recognize health effects associated with the use of these substances, while only 54% of suppliers report this problem.

Analysis of biochemical parameters and acetylcholinesterase activity

A total of 36 blood samples were obtained from workers who were either occupationally exposed or unexposed to pesticides. The samples were then used to analyze blood biometric parameters, including erythrocytes, hemoglobin, hematocrit, MCV, MCH, MCHC, RDW, platelets, platelet volume, leukocytes, neutrophils, lymphocytes, and monocytes. Furthermore, blood chemistry results were obtained from the same workers for the following parameters: glucose, urea, creatinine, uric acid, total cholesterol, urea nitrogen, and triglycerides (Tables 1 and 2). A thorough investigation of the blood biometry parameters revealed that all values were within the established reference ranges (Table 1).

For the parameters considered in blood chemistry, both exposed and unexposed individuals recorded values within the reference ranges established by the method used for determination (Table 2). Statistical analyses between the exposed and unexposed groups revealed statistically significant differences in erythrocytes (p = 0.006), hemoglobin (p = 0.004), hematocrit (p = 0.01), MCHC (p = 0.03), eosinophils (p = 0.02), and total cholesterol (p = 0.0008). The parameters of erythrocytes, hemoglobin, hematocrit, and MCHC showed higher concentrations in exposed workers, whereas eosinophils and total cholesterol showed higher concentrations in unexposed workers (Tables 1 and 2).

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Table 1. Parameters evaluated in blood counts in workers exposed and not exposed to organophosphate pesticides in Guasave, Sinaloa, Mexico.

Parameters	Reference values		Exposed		Not exposed	
	Men	Women	Mean ± S.D.	Range	Mean ± S.D.	Range
Erythrocytes (10^6/μL)	4.3 - 5.8	4.0 - 4.9	**5.2 ± 0.4** [a]	4.5 - 5.7	**4.9 ± 0.3** [b]	4.5 - 5.4
Hemoglobin (g/dL)	13.0 - 17.0	12 - 14.6	**14.6 ± 1.2** [a]	11.4 - 16.7	**13.5 ± 0.8** [b]	12.5 - 14.7
Hematocrit (%)	39.0 - 52.0	36.0 - 44.0	**44.2 ± 3.1** [a]	36.5 - 49.4	**41.8 ± 2.3** [b]	39.1 - 45.3
MCV (fL)	83.0 - 100	83 - 100	84.8 ± 3.7 [a]	74.2 - 92.1	85.3 ± 4.0 [a]	79.6 - 93.6
MCH (pg)	28.0 - 32.0	27.0 - 32.0	28.1 ± 1.6 [a]	23.2 - 30.4	27.6 ± 1.5 [a]	25.2 - 30.0
MCHC (g/dL)	32.0 - 34.5	31.5 - 34.5	**33.1 ± 0.8** [a]	31.2 - 34.4	**32.4 ± 0.8** [b]	31.7 - 34.5
RDW (%)	11.6 - 13.7	11.6 - 13.7	14.0 ± 0.9 [a]	13.0 - 17.7	14.2 ± 1.2 [a]	13.2 - 17.1
Platelets (10^3/μL)	150 - 400	150 - 400	247.8 ± 67.2 [a]	155.0 - 385.0	270.1 ± 48.8 [a]	199.0 - 352.0
Platelets volumen (fL)	8.0 - 12.0	8.0 - 12.0	8.4 ± 0.9 [a]	6.7 - 10.0	8.3 ± 0.9 [a]	7.1 - 10.0
Leukocytes (cells/μL)	5000 - 10000	5000 - 10000	7268.0 ± 2404.8 [a]	3500 - 13600	7445.5 ± 2162.6 [a]	4700.0 - 11900.0
Neutrophils (cells/μL)	2500 - 7000	2500 - 7000	4523.6 ± 2123.3 [a]	1750.0 - 10880.0	4421.2 ± 1641.3 [a]	2726.0 - 8092.0
Lymphocytes (cells/μL)	1000 - 4000	1000 - 4000	2362.7 ± 1098.6 [a]	1128.0 - 6825.0	2567.3 ± 587.7 [a]	1551.0 - 3358.0
Monocytes (cells/μL)	100 - 1000	100 - 1000	242.4 ± 109.1 [a]	117.0 - 525.0	264.5 ± 89.9 [a]	171.0 - 476.0
Eosinophils (cells/μL)	0 - 400	0 - 400	139.2 ± 72.1 [a]	47.0 - 300.0	192.5 ± 77.8	76.0 - 357.0

S.D. = standard deviation; MVC = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width. Different letters indicate significant differences between groups ($p < 0.05$).

Source: own elaboration

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Table 2. Parameters evaluated in blood chemistry in workers exposed and not occupationally exposed to organophosphate pesticides in Guasave, Sinaloa, Mexico.

Parameters	Reference values		Exposed		Not exposed	
	Men	Women	Mean ± S.D.	Range	Mean ± S.D.	Range
Glucose (mg/dL)	65 - 110	70 - 110	89.6 ± 9.3 [a]	72.0 - 108.0	93.1 ± 6.3 [a]	84.0 - 103.0
Urea (mg/dL)	20 - 60	10 - 50	24.8 ± 4.9 [a]	16.0 - 34.0	26.4 ± 5.1 [a]	21.0 - 37.0
Creatinine (mg/dL)	0.7 - 1.4	0.6 - 1.1	0.8 ± 0.1 [a]	0.6 - 1.0	0.8 ± 0.1 [a]	0.7 - 1.1
Uric acid (mg/dL)	3.5 - 7.2	3.0 - 5.5	4.7 ± 0.9 [a]	3.1 - 6.5	4.5 ± 1.3 [a]	3.4 - 7.9
Total cholesterol (mg/dL)	Low risk <200 Moderate risk 200-239 High risk >240		**120.0 ± 31.2 [a]**	78.0 - 198.0	**161.2 ± 28.2 [b]**	116.0 - 211.0
Triglycerides (mg/dL)	Desirable <150 Limit values 150-199 High values 200-500 Very high values >500		113.3 ± 53.0 [a]	45.0 - 217.0	104.9 ± 45.6 [a]	60.0 - 231.0
Urea nitrogen	9.3 - 28.0	4.7 - 23.4	11.6 ± 2.3 [a]	7.5 - 15.9	12.3 ± 2.3 [a]	9.8 - 17.0

S.D. = standard deviation. Different letters indicate significant differences between groups ($p < 0.05$)
Source: Own Elaboration.

In this regard, it has been reported that hemoglobin concentration is directly associated with acetylcholinesterase levels (Palacios-Nava *et al*., 2009; Sosan *et al*., 2010; Gupta *et al*., 2018; Kori *et al*., 2019). Gupta *et al*. (2018) conducted a study examining the relationship between acetylcholinesterase and two other variables: hemoglobin and RDW. Their findings indicated a negative correlation between acetylcholinesterase and hemoglobin, and a positive correlation between acetylcholinesterase and RDW. Conversely, a decline in AChE activity has been associated with decreases in lymphocyte, erythrocyte, and platelet counts. A negative correlation has been observed between the subject and increases in blood urea nitrogen (BUN), as well as decreases in the absolute counts of red blood cells and lymphocytes (Pardío *et al*., 2007). This behavior has also been observed in people exposed directly or indirectly to pesticides, in which AChE activity values tended to increase with the number of platelets and red blood cells (dos Santos Barreto *et al*., 2025). In a similar vein, Kori *et al*. (2019) investigated the relationship between cholinesterase inhibition and various health markers in agricultural workers in the Sagar district of India. These markers included hematological, biochemical, and oxidative stress parameters. The study found that AChE activity exhibited a negative correlation with hemoglobin, hematocrit, total red blood cells, uric acid, and triglycerides.

Several studies have reported alterations in renal function among agricultural workers occupationally exposed to pesticides, observing increases in blood urea, creatinine, and uric acid concentrations (Singh *et al*., 2011; Mendoza *et al*., 2015; Contreras-Trejo, 2019). In contrast, the present study found no significant differences in these parameters between the evaluated groups, with values within the method's reference ranges in all cases.

On the other hand, enzyme activity was determined in exposed and unexposed workers using the acetylcholinesterase activity parameter. A statistical analysis of results obtained with this parameter showed a significant difference (p = 0.00002), with the exposed group exhibiting lower values than the unexposed group (Figure 4). Statistical analyses were also performed to evaluate the possible relationship between enzyme activity and the frequency of general PPE use by exposed workers. Pearson's correlation analysis revealed no association between the two variables (r = 0.02). Conversely, an analysis of variance (ANOVA) was employed to compare enzyme activity by PPE use frequency (always, frequently, occasionally, infrequently, and never). The findings revealed no statistically significant differences in any of the cases. Kapeleka *et al*. (2019) obtained similar results, showing that enzyme activity did not differ significantly between exposed workers who frequently used PPE and those who were not usually protected with equipment.

Organophosphate compounds have been shown to inhibit the activity of cholinesterases (acetylcholinesterase and butyrylcholinesterase), which play a pivotal role in neurotransmission in the nervous system. Consequently, cholinesterases have been used as biomarkers of pesticide exposure to assess health risks to the exposed population (Dhananjayan *et al*., 2019; Finhler *et al*., 2023). As posited by Caro-Gamboa et al. (2020), the activity of acetylcholinesterase in blood may be influenced not only by exposure to organophosphate pesticides but also by several factors. These include the chemical composition of the pesticide itself and the solvents utilized in its preparation. Additionally, interindividual variability, encompassing ethnic and genetic traits, and intraindividual variability, such as age, sex, reproductive status, health status (including medication use), and the presence of certain diseases, have been identified as contributing factors. In this regard, acetylcholinesterase levels were compared by sex and then correlated with age. The findings revealed that cholinesterase activity levels in exposed individuals were lower than in unexposed individuals. However, when workers were categorized by sex as exposed or unexposed, no significant differences in enzyme activity were observed. The final correlation analysis revealed no relationship between enzyme activity and workers' age (Table 3).

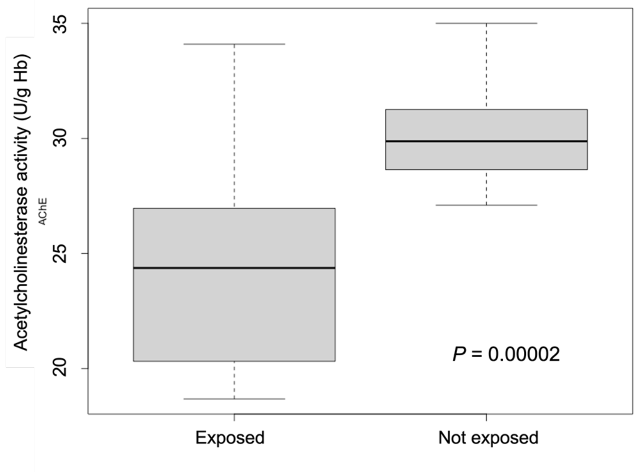

Figure 4. Comparison of total acetylcholinesterase activity in workers exposed and not occupationally exposed to organophosphate pesticides in Guasave, Sinaloa, Mexico.

Source: Own elaboration

Table 3. Statistical analysis of total acetylcholinesterase activity with exposure variables, sex, and age of agricultural workers in Guasave, Sinaloa, Mexico.

Cholinesterase VS	Group	Statistical test	Results	Description
Exposition	Total	t-student	0.00002	Lower total cholinesterase in exposed individuals
Gender	Total	t-student	0.072	No significant differences
	Exposed	t-student	0.109	No significant differences
	Not exposed	t-student	0.499	No significant differences
Age	Total	Correlation	-0.12	The correlation analysis showed no relationship between the two variables.

Source: own elaboration.

The absence of significant differences in acetylcholinesterase activity when classifying participants exposed to organophosphate pesticides by sex suggests that these compounds exert inhibition on the enzyme, regardless of gender. Cuaspud & Vargas (2010) conducted a study to evaluate the acetylcholinesterase values of a group of workers exposed to organophosphate and

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carbamate pesticides in Ecuador. These values were then compared with those of a non-exposed control group. The results demonstrated a significant decrease in enzyme activity in the exposed group; however, when analyzing the differences by sex within the exposed group, no significant variations were observed. Concurrently, studies such as that conducted in Colombia by Cortés-Iza *et al*. (2017) have demonstrated that exposure to organophosphate pesticides tends to inhibit enzyme activity similarly in both men and women.

Several studies have been conducted to ascertain a correlation between exposure to pesticides and acetylcholinesterase levels in agricultural workers within the state of Sinaloa (Palacios-Nava *et al*., 2009; Palacios-Nava, 2012; Galindo-Reyes & Alegría, 2018). Palacios-Nava *et al*. (2009) determined baseline acetylcholinesterase levels in agricultural workers in Sinaloa; the authors found that acetylcholinesterase activity and hemoglobin were higher in men than in women, with a high prevalence of anemia. In the present study, no instances of anemia were detected among the participating workers. In the study conducted by Palacios-Nava (2012), in which 172-day laborers from Sinaloa participated, acetylcholinesterase activity levels were reported to be within the normal range. It is important to note that both studies lack a comparison of acetylcholinesterase activity levels between the group of exposed day laborers and an unexposed group. Several studies have been conducted on populations with varying levels of exposure to organophosphate pesticides in different regions worldwide. These studies have reported lower levels of acetylcholinesterase activity in these groups when compared to unexposed groups. These findings are consistent with those reported in the present study (Díaz *et al*., 2017; Caro-Gamboa *et al*., 2020; Rodríguez-Gil *et al*., 2023).

To date, there has been a paucity of studies reporting on the analysis of biochemical parameters in populations occupationally exposed to pesticides that attempt to relate these measurements to enzymatic activities. In this regard, Molina-Pintor (2017) evaluated acetylcholinesterase enzymatic activity and its association with biochemical parameters among urban fumigators in Nayarit, Mexico. The study found that enzymatic activity correlated with biochemical parameters, depending on participants' body mass index (BMI). In the present study, participants were not classified by BMI, and only two groups were studied: exposed and unexposed, without distinction in exposure level within the exposed group. Furthermore, the correlation between acetylcholinesterase activity and the clinical parameters evaluated varied significantly depending on whether it was assessed in the exposed group, the unexposed group, or all participants. No positive correlations were observed between acetylcholinesterase activity and any blood biometry or blood chemistry variable. However, a negative correlation was detected with erythrocytes (-0.54) when the total number of participants was considered. Amongst exposed workers, negative correlations were observed with erythrocytes (-0.62), hemoglobin (-0.51), hematocrit (-0.52), and uric acid (-0.56). Among unexposed workers, a positive correlation with MCV was observed ($r = 0.59$). Regarding the remaining parameters, marginally positive and negative correlations were observed, with values below 0.5 in both groups and overall (Table 4 and Figure 5). These correlations have not been previously reported and could be attributed to other risk factors such as alcohol and tobacco consumption, nutritional status, and age, among others (Herrera-Moreno *et al*., 2018).

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Table 4. Correlations between total acetylcholinesterase activity and biochemical parameters assessed through blood counts and blood chemistry.

Group	Complete blood count							
	Erythrocytes	Hemoglobin	Hematocrit	MCV	MCH	MCHC	RDW	Platelets
Total	**-0.54**	-0.42	-0.35	0.27	0.02	-0.41	0.03	0.07
Exposed	**-0.62**	**-0.51**	**-0.52**	0.07	-0.05	-0.27	0.15	-0.21
Not exposed	-0.03	0.29	0.47	**0.59**	0.39	-0.29	-0.20	0.27

Group	Blood chemistry			
	Glucose	Urea	Creatinine	Uric acid
Total	0.04	0.05	-0.06	-0.28
Exposed	-0.06	0.09	-0.09	**-0.56**
Not exposed	-0.21	-0.20	-0.33	-0.01

Group	Complete blood count					
	Platelets vol.	Leukocytes	Neutrophils	Lymphocytes	Monocytes	Eosinophils
Total	0.21	-0.02	-0.12	0.14	0.07	0.23
Exposed	0.17	-0.18	-0.24	0.02	0.04	0.12
Not exposed	0.48	0.05	-0.09	0.43	0.02	-0.07

Group	Blood chemistry			
	Uric acid	Total cholesterol	Urea nitrogen	Triglycerides
Total	-0.28	0.47	0.05	-0.04
Exposed	**-0.56**	0.12	0.09	-0.06
Not exposed	-0.01	0.45	-0.20	0.17

Source: own elaboration.

Study limitations and future perspectives

Our study has several limitations that should be considered when interpreting the results. First, the sample size is limited, which may affect the generalizability of the findings. In addition, the classification of symptoms was based on the existing literature on exposure to organophosphate pesticides without distinguishing between the symptoms they cause (cholinergic, muscarinic, and/or nicotinic). However, no specific biomarkers were used to confirm 100% exposure (metabolite measurements in blood and urine and studies of cellular-level toxicity mechanisms in blood cells). The lack of information on pesticide application in the study area also limits the interpretation of

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the results. To address these limitations, it is suggested to: 1) Collect information on pesticide application in the study area through the dissemination of results and outreach to key individuals in the agricultural sector; 2) Measure clinical parameters before and after pesticide exposure, ideally with a baseline value prior to exposure; 3) Using specific biomarkers to confirm exposure to organophosphate pesticides and rule out confounding variables; 4) Considering environmental exposure to pesticides in future studies; 5) Measuring acetylcholinesterase (AChE) activity before and after pesticide exposure in the exposed group. By addressing these limitations and considering future perspectives, we can improve our understanding of the effects of organophosphate pesticide exposure on the health of occupationally exposed individuals and develop more effective strategies to prevent and mitigate these effects.

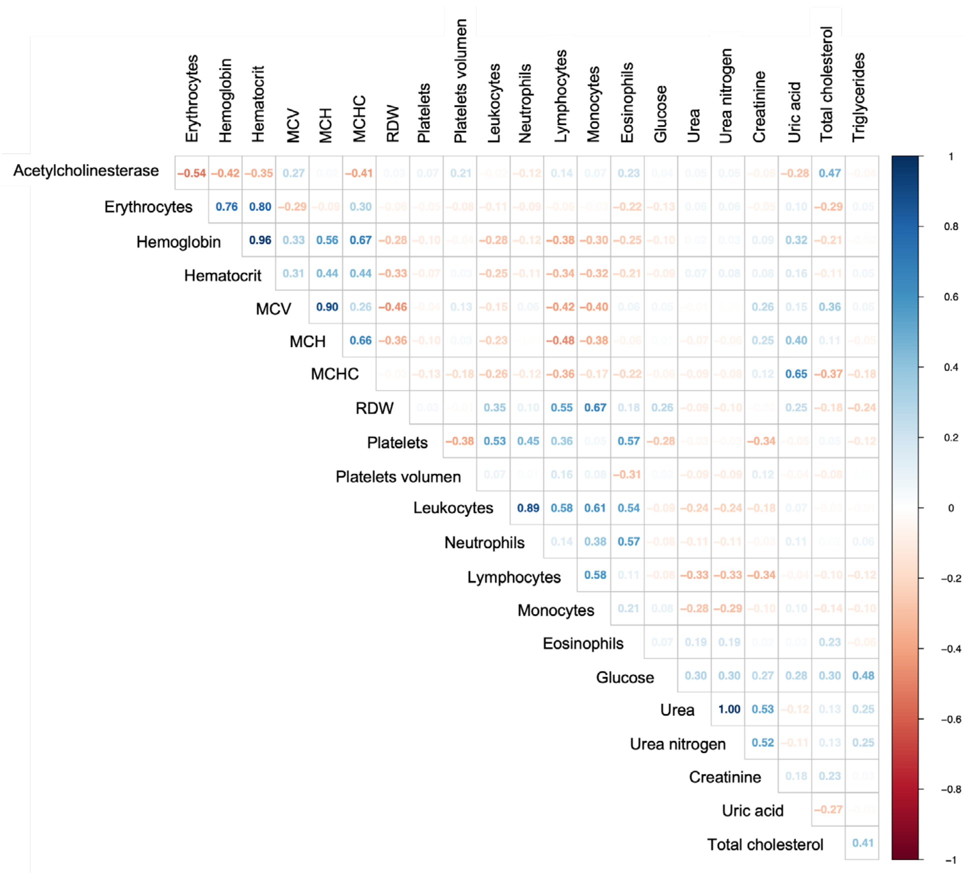

Figure 5. Correlation analysis between acetylcholinesterase and blood and chemistry variables with the total number of participants exposed and not occupationally exposed to organophosphate pesticides in Guasave, Sinaloa, Mexico.

The color scale shows the correlation coefficient (r) between the variables.

Source: Own elaboration.

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Conclusions

This is the first study conducted in the agricultural area of Guasave, Sinaloa, Mexico, that attempts to establish a correlation between the effects of clinical parameters and acetylcholinesterase activity in a population occupationally exposed to organophosphate pesticides. The research will serve as a baseline for establishing the possible effects of occupational exposure to pesticides in the municipality of Guasave, given the conditions found in terms of years worked and the personal protective equipment used during pesticide application, which is insufficient. It is evident from the findings that workers have limited awareness of the health risks associated with their occupational activities.

The biochemical parameters evaluated through blood biometrics and blood chemistry were generally within the reference ranges established in the methods used for their determination for both exposed and unexposed individuals. At the same time, acetylcholinesterase enzyme activity was lower in the exposed group. Despite the presence of both positive and negative correlations between cholinesterase activity and the biochemical parameters under scrutiny, these correlations were found to be very weak. Consequently, further studies are needed to determine whether such an association exists.

The findings obtained provide substantial support for the use of two or more clinical parameters, as evaluated in this study, in human biomonitoring of pesticide exposure. Furthermore, the use of a robust and validated questionnaire will facilitate the establishment of intervention programs aimed at reducing exposure and the risk of adverse health effects associated with pesticide exposure.

Authors' contribution

Conceptualization, JBLM, XPPD, BARA; Methodological development, JBLM, EMC, XPPD, JSA, IGMA; Software management, BARA, JBLM, EMC; Experimental validation, JBLM, BARA, XPPD, EMC; Analysis of results, JBLM, BARA, XPPD, JSA, IGMA, EMC; Data management, JBLM, BARA, XPPD, EMC; Manuscript writing and preparation, JBLM, BARA, XPPD; Writing, review, and editing, EMC, JSA, IGMA, BARA, XPPD, JBLM; Project administrators, JBLM, XPPD; Fund acquisition, JBLM, JSA, IGMA, XPPD.

All manuscript authors have read and accepted the published version.

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Ethical statements

This study was approved by the Bioethics Committee of the Autonomous University of the West (UAdeO) (CM-UAdeO 29.08/2021).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

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Conflict of interest

The authors declare that they have no conflict of interest.

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