

## Cholinesterase as a biomarker to identify cases of pesticide poisoning Colinesterasa como biomarcador para identificar casos de intoxicación por plaguicidas

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### Abstract:

The clinical care of the intoxicated, as well as the epidemiological surveillance activities of the population exposed to pesticides, require reference values to be able to make decisions based on science. Cholinesterase enzymes are biomarkers discovered in the early twentieth century that currently work as auxiliaries in the diagnosis of acute and chronic poisoning. **Objective:** To carry out a systematic review to identify the main variables that affect the measurement of cholinesterases in pesticide poisoning studies. **Methodology:** A systematic review was performed in the PubMed, Crossreff and Google Scholar search engines, using the keywords cholinesterase, acetylcholinesterase, pesticides and intoxication, only original articles were taken, which measured cholinesterases by colorimetric and pH methods, with the objective of relating them to acute or chronic poisoning by pesticides. **Results:** 71.42% of the articles reported a decrease in cholinesterase in the population under study and 28.57% report not having detected a decrease in cholinesterase in the study population. **Conclusions:** It is recommended that researchers always consider the delicacy of these enzymes and remember the most important factors that can affect cholinesterase values in addition to pesticides.

### Keywords:

cholinesterase, acetylcholinesterase, pesticides, poisoning

### Resumen:

La atención clínica de los intoxicados, así como las actividades de vigilancia epidemiológica de la población expuesta a plaguicidas demandan disponer de valores de referencia para poder tomar decisiones con base científica. Las colinesterasas son biomarcadores descubiertos a inicios del siglo XX que en la actualidad funcionan como auxiliares en el diagnóstico de intoxicaciones agudas y crónicas. **Objetivo:** Realizar una revisión sistemática que permita identificar las principales variables que afectan la medición de colinesterasas en los estudios de intoxicación por plaguicidas. **Metodología:** Se realizó una revisión sistemática en los buscadores PubMed, Crossreff y Google Scholar, mediante las palabras clave colinesterasa, acetilcolinesterasa, plaguicidas e intoxicación, se tomaron en cuenta solo artículos originales, que midieran las colinesterasas mediante métodos colorimétricos y de pH, con el objetivo de relacionarlas con intoxicaciones agudas o crónicas por plaguicidas. **Resultados:** El 71.42% de los artículos reportaron una disminución de las colinesterasas en la población bajo estudio y el 28.57% refiere no haber detectado disminución de las colinesterasas en la población en estudio. **Conclusiones:** Recomendamos a los investigadores tener en cuenta en todo momento la delicadeza de estas enzimas y recordar los factores más importantes que pueden afectar los valores de las colinesterasas además de los plaguicidas.

### Palabras Clave:

colinesterasa, acetilcolinesterasa, plaguicidas, intoxicación

### INTRODUCTION

Cholinesterases are a family of carboxy esterases, which are present from single-celled organisms to multicellular organisms such as plants, invertebrates and vertebrates in which they appear in embryonic development before synaptogenesis.<sup>1</sup> In 1914, the neurotransmitter acetylcholine (ACh) was discovered which is essential for the central nervous system, the peripheral nodes and

the neuromuscular plate. Acetylcholine binds to a specific receptor on the postsynaptic membrane; resulting in the change of membrane potential and may be the synapse.<sup>1</sup> Subsequently, the acetylcholine will be hydrolyzed by acetylcholinesterase (AChE) becoming acetic acid and choline, through the postsynaptic membrane return to the state of rest.<sup>2</sup> This process prevents overstimulation of muscle fibers due to accumulation of acetylcholine at the neuromuscular junction, which causes a

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wide range of neurotoxic effects (muscarinic, nicotinic, and neurological).<sup>2,3</sup> See Table 1. Cholinergic syndrome. The accumulation effects of acetylcholine can be evidenced biochemically by suppressed levels of serum pseudochoolinesterase (BChE) and erythrocyte cholinesterase (AChE).<sup>4</sup> These two types of cholinesterase are divided according to their substrate specificity, susceptibility to inhibitors, and energy tissues: Acetylcholinesterase (AChE), or true cholinesterase or E-type specific cholinesterase, is predominant in muscle, erythrocytes, and the nervous system, where BChE levels are lower.<sup>2</sup>

Butyrylcholinesterase (BChE) or pseudochoolinesterase, plasma cholinesterase or cholinesterase type S is present in tissues such as intestine, kidney, heart, lungs and mainly the liver. After being synthesized, it is secreted into plasma.<sup>1,2</sup> The physiological function of BChE is still unknown, it is speculated that it plays an important role in the metabolism of lipids and lipoproteins, regulating the concentration of choline in plasma or preventing the accumulation of butyrylcholine, during the metabolism of fatty acids and lipogenesis. In addition, they have detected functions in the metabolism of drugs such as procaine, succinylcholine and acetylsalicylic acid, as well as the elimination of phosphates and carbamates.<sup>5</sup>

Cholinesterase enzymes are frequently used to biomonitor exposure to organophosphates and carbamates, which are determined to be neurotoxic and cause 80% of acute or chronic pesticide poisonings worldwide.<sup>6</sup> Pesticide absorption pathways are by inhalation, ingestion and through intact skin. Once in the body they suffer metabolic reactions of activation, detoxification and conjugation. Its mechanism of toxic action is the binding and inhibition of steric enzymes in a stable manner, and they are eliminated relatively quickly by the renal route.<sup>3</sup> The inhibition of AChE, caused by carbamates, is of less persistence since it is decarbamylated in a reversible and spontaneous way, with neurotoxic effects similar to organophosphates, but to a lesser extent and with usually rapid recovery.<sup>6</sup> Approximately 3 million people suffer from poisoning and 200,000 die from pesticide poisoning annually in the world.<sup>2,7</sup> Millions are exposed to hazardous work practices and insecure storage of pesticides; however, it is deliberate self-poisoning that causes most deaths, particularly in Asia.<sup>8</sup>

Table 1. Cholinergic syndrome.

Receptors	Signs and symptoms
Muscarinic	Sialorrhea, tearing, urinary incontinence, diarrhea, tenesmus, bronchospasm, bronchitis, wheezing, decreased blood pressure, bradycardia, nausea, vomiting, cramping, sweating, myosis and blurred vision.
Nicotinic	Muscle fatigue, muscle spasm, fasciculations, paralysis, decreased respiratory effort, tachycardia, diaphoresis, hypertension, hypoglycemia, paleness, cyanosis and mydriasis.

Central nervous system	Anxiety, nervousness, confusion, headache, seizures, ataxia, bradycardia, emotional lability, Cheyne-Stokes breathing, respiratory center. depression, central respiratory paralysis, dyslexia, coma and insomnia.
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Source: Adapted from Virú Loza MA. Current management of acute cholinesterase inhibitor poisoning: misconceptions and the need for updated Peruvian guidelines. *An. Fac. med.* 2015;76(4):431-7.

Chronic intoxications or exposures are associated with immunological effects, carcinogenic effects, reproductive disorders, developmental disorders, neurotoxicity, even exposure to pesticides is associated with the development of Parkinson's, Alzheimer's, anxiety disorders, deficit disorders. attention with hyperactivity, and depression, including areas with an intensive pesticide have a high suicide rate.<sup>9-11</sup>

### Cholinesterase levels as a diagnosis

Every good diagnosis should start with a complete medical history, through the suspicion or certainty of exposure to the pesticide, the route of absorption and a compatible clinical picture. Diagnostic confirmation should ideally be done by measuring cholinesterase activity.<sup>4</sup> However, the results should be interpreted with caution because a wide variability has been observed in the values considered normal because of the characteristics of cholinesterase, individual differences mainly the aggregate clinical conditions, substances that can alter cholinesterase levels, influence of the environment and the possibility of laboratory errors.<sup>7,11,12</sup>

## MATERIALS AND METHODS

A systematic review was performed in the PubMed, Crossref and Google Scholar search engines, using the keywords cholinesterase, acetylcholinesterase, pesticides and poisoning.

- Inclusion criteria: Only original articles in English or Spanish were taken into account, the search did not include a publication date limit, in their methodology they had to include the measurement of plasma cholinesterase, erythrocyte, or both through colorimetric chemical reactions or changes in pH, who would have carried out their measurements in vivo, included experimental and non-experimental investigations.
- Exclusion criteria: Those with a sample of less than 15 people and original articles that did not have the objective of relating cholinesterase measurements with acute or chronic pesticide poisoning.

## PROCESS

The review, synthesis and classification of the selected works was carried out by preparing a matrix that includes the name of the first author, date of publication, country of origin, methodology and type of cholinesterase measured, sample size, presence of signs of pesticide poisoning, number of people with inhibited or altered cholinesterase, if there is a statistical

association with cholinesterase, exposure time and exclusion criteria. The table is sorted by dates from oldest to most current. See Table 2. Studies with measurements of cholinesterases and pesticide poisoning.

## RESULTS

Twenty eight original articles that describe and analyze situations organophosphate or carbamate poisoning and its association with cholinesterase were used to generate this article. Twenty eight point five percent belong to PubMed, 35.7% to Crossreff and the remaining 35.7% is owned by Google Scholar.

Selected articles are conducted primarily in the Americas, predominantly Colombia with 8 items, with the exception of 5 items from which, 3 were conducted in India, 1 in Thailand and 1 in Serbia.

The analytical methods used were the Ellman method, the method of Ellman with DTNA (6-6'-ditiidinicotínico acid), Magnotti, Michel, Lovibond, Wiener, Merckotest, Aldridge and Randox which measure through chemical reactions or changes colored pH. The method most commonly found in the articles as the method of Ellman.

Normal values vary widely considered in each study, according to the methodology, the laboratory, the sample type and population in which was measured for both plasma cholinesterase to erythrocyte cholinesterase. 13 (46.42%) of the items evaluated plasma cholinesterase, 11 (39.28%) evaluated. and 4 erythrocyte cholinesterase (14.28%) of the items evaluated both. Only 10 (35.71%) of the studies collected describe the methodology with which the sample was taken. See Figure 1. Sampling and exclusion criteria for cholinesterase.

The study with the lowest sample had 15 participants and the largest sample had 46.290 participants. 10 (35.71%) of the articles included the exposed group and the control group in 4 (11.2%) of the studies reported taking between 3 and 5 serial samples per person and one article made measurements cholinesterase saliva.

The 28 (100%) articles described their population, as affected by pesticides, with risk factors and little or no protective equipment of these 20 articles (71.4%) reported signs and symptoms of poisoning, of these 20 articles that reported signs and symptoms, 6 (30%) focus on symptoms of chronic poisoning and 14 (70%) articles described signs and symptoms of acute poisoning.

Twenty (71.42%) of the articles reported a decrease in cholinesterase in the study population and 8 articles (28.57%) reported not having detected a decrease in cholinesterase in the study population.

Of the 20 articles that reported a decrease in cholinesterase:

- 9 (45%) articles indicated that less than 15% of their total population studied presented a decrease in measured cholinesterase enzymes.
- 5 (25%) articles reported that between 16% to 50% of their total population had a decrease in measured cholinesterase enzymes.
- 6 (30%) of the articles reported that between 51% to 100% of their study population selected a decrease in measured cholinesterase enzymes.

In 4 (14.28%) articles, they found no statistical association between pesticide exposure and cholinesterase levels. In 9 (32.14%) articles, the exposure time of their population to pesticides was not reported and in the remaining 19 (67.85%) articles, the exposure time was reported between 6 hours to 50 years. Only 14 (50%) of the studies used for this article mentioned or described their exclusion criteria when choosing their sample, ruling out physiological, pathological conditions or the presence of substances that can alter cholinesterase levels. For example, medications used in the treatment of Alzheimer's, such as donepezil or galantamine, have an inhibitory action against BuChE and AchE.<sup>13</sup>

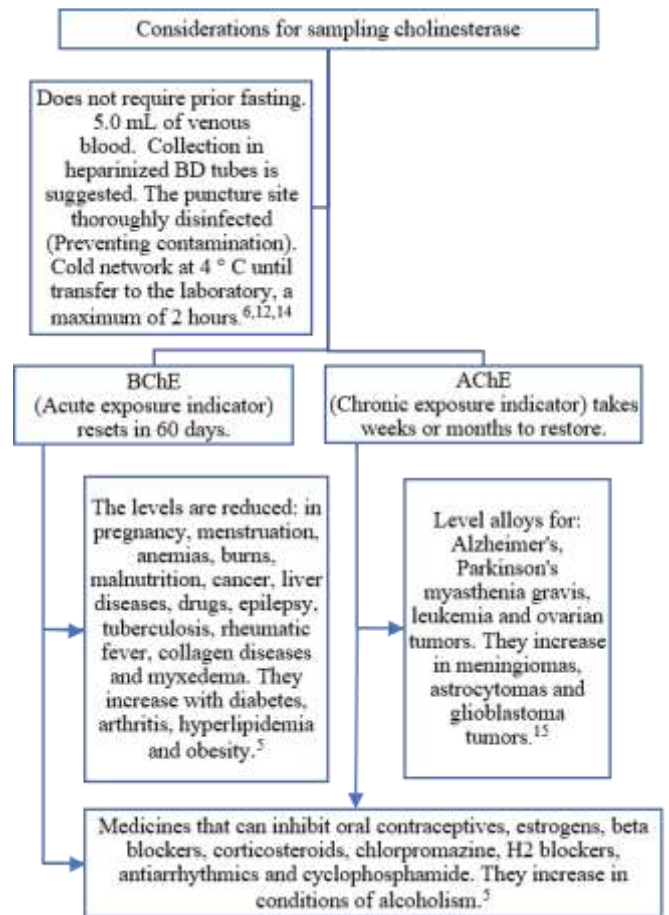


Figure 1 Sampling and exclusion criteria for cholinesterase.

Table 2. Studies with cholinesterase measurement and pesticide poisoning.

Studies	Enzyme and method	Sample size	Signs and symptoms	Cholinesterase inhibited	Statistical association with cholinesterase	Exhibition time	Exclusion criteria
Varona M. 1998 Colombia. <sup>16</sup>	AChE 4 methods: compare 190 samples by Merckotest, Michel in plasma, Michel and Aldrige in whole blood and Lovibond.	Between 1993 and 1995, a total population of 46,290 participated.	NA	Of a total of 46,290, 5,370 tests were below the normal range, equivalent to 11.6%.	The economic activities that presented higher prevalence of abnormal values for AChE, corresponded to the urban application, formulator and aerial application	NA	NA
Luna-Avilés I 2007 Nicaragua. <sup>17</sup>	AChE Lovibond Method BRV: 0.35-0.45 ml	60 samples 30 EX and 30 NE	Genetic damage in micronuclei, premature aging	6 people of the exposed (20%) and 1 of the non-exposed (3.3%)	AChE levels indicate chronic neurotoxic effect in pesticide traders. Significant difference between Control and Exposed groups ( $z = 2.5$ , CI: 99%)	Exposure range from 2 to 11 years	NA
Lamadrid-Boada I. 2011 Cuba. <sup>18</sup>	AChE Method regulated by the MINSAP. ARV: $0,3950 \pm 0,0275$ (0,340-0,450)	8 EX and 15 NE.	NA	0	BChE activity values, during the 2 years of the study, remained within the reference value for the Cuban population average.	Average working years: 7.6	Complete ***
Gutiérrez-Coronado ML. 2012 Mexico. <sup>19</sup>	BChE Colorimetric method (Randox diagnostic kit) BRV: $7,68 \pm 2,81$ KU/L	212 farmers	98.6% have some type of sign or symptom due to poisoning, symptoms more often headache and fatigue.	1.8% presented values below the reference values, 1% women with low values	No significant association was found.	35.3% less than 5 years	NA
Uzcátegui-Nava J. 2013 Venezuela. <sup>3</sup>	AChE human saliva ** Ellman Method	60 saliva samples (sputum) from farmers.	NA	80% enzyme inhibition at concentrations less than 100 ppm pesticide.	It was observed that organophosphorus pesticides and carbamates significantly affect the activity values of the enzyme acetylcholinesterase in saliva.	NA	NA
Prasad D. 2013 India. <sup>8</sup>	BChE ** 5 sampling BRV 5100 - 11700U/L	76 patients poisoned with phosphorous organ	Congested conjunctiva 87%, pointed pupil 83%, non-reactive pupil 75%, difficulty, respiratory 60% and abdominal pain 37%.	62 patients (90%) had BChE values of less than 5000 iu	Respiratory distress and fasciculation were related to > 75% reduction in BChE. constricted pupil and congested conjunctiva a 50-75% reduction	NA	Complete ***
Chaudhary SC 2013 India. <sup>4</sup>	BChE ** Spectrophotometry method BRV: 7,000-19,000 ui / l	70 patients 2 groups	60% required ventilatory support from group B and 48.57% from group A	BChE levels were reduced in all cases. 100%	About half of the patients have mild poisoning according to the classification of Kumar et al.	6 hours after exposure.	Complete ***
Ramírez-Santana M. 2015 Chile. <sup>20</sup>	BChE and AChE ** the acylpeptide hydrolase (ACPH) 3 samples exposed to 3 groups	100 exposed 100 internal controls and 1000 external controls	Memory, praxis constructive, attention, motor function and executive.	NA	These enzymes are inhibited when people are exposed to high doses of organophosphates, with clear signs and symptoms of acute poisoning; therefore, they do not serve to identify the risk of chronic exposure.	Minimum span 5 years	Complete
Lozano-Socarrás SL. 2015 Colombia. <sup>21</sup>	BChE Wiener Method Colorimetric BRV 3200-9000 U/L	80 farmers	NA	10 farmers (12.5%)	At higher exposure time, individuals presented high levels of the enzyme ( $Rho = 0.408$ ; $p < 0.05$ ).	$70,8 \pm 91,6$ months	NA

Studies	Enzyme and method	Sample size	Signs and symptoms	Cholinesterase inhibited	Statistical association with cholinesterase	Exhibition time	Exclusion criteria
Blanco AR 2016 Honduras. <sup>22</sup>	AChE Michel Method 2 groups EX y NE VRA: 154 unids de pH/hour	95 farmers, 60 EX and 35 NE	42.5% have headache, nausea, dizziness, muscle weakness, eye and skin irritation	3 (5%) EX y 5 (14.28%) NE	Slight inhibition 133 units of pH / hour and values less than 120 units of pH / hour as moderate inhibition.	50% of the farmers have worked more than 10 years.	NA
Farrukh J. 2016 India. <sup>23</sup>	BuChe Maw the Accucare kit method of Knedel et al. (1967)	374 workers 187 EX and 187 NE	Anxiety, insomnia, severe depression, disorders that affect psychomotor speed, selective attention, divided attention, verbal memory, eczema, fatigue, headache, sweating, abdominal pain, nausea, muscle weakness	NA	The rates of anxiety and insomnia (p = 0.030) and severe depression (p <0.001) were significantly higher in organophosphorus pesticide sprayers than in controls.	Years 9.88 ± 7.72	Complete ***
Restrepo-Cortés B. 2017 Colombia. <sup>5</sup>	AChE y BChE ** Ellman with DTNA BRV 12120 -2176 u/l ARV 10567-2227 u/l	819 farmers (480 men and 339 women).	NA	36% had inhibition of AChE activity and 51% of BChE*	AChE was significantly lower among fumigants (P = 0.022).	NA	Complete ***
Toro-Osorio MB. 2017 Colombia. <sup>6</sup>	BChE ** Ellman. Method BRV 4659-14 443 u/l	1 098 farmers.	15 % headaches, 12 % dizziness and 8 % blurred vision and muscularaches.	3.8% of farmers	Less time after the last application of the insecticide, there is a greater inhibition of the enzyme.	Range of one to ten days since the last application	NA
Cuaspu J. 2017 Ecuador. <sup>24</sup>	AChE Ellman Method with DTNA ARV EX 4199-2320,5 u/l ARV NE 4745-3081 u/l AV NE: 3625,41 u/l	145 samples 95 EX and 50 NE.	18 people- neurological symptoms, 35 people-muscarinic symptoms and 43 people-nicotinic symptoms.	42% of farmers	AChE (3154,99 U/L) Below the average level of the unexposed group.	NA	Complete ***
Matheus T. 2017 Venezuela. <sup>25</sup>	BChE BRV 3528-1162,45u/l and the presence of micronuclei	82 samples 41 EX and 41 NE.	Prevalence of eye redness 48.8% and headache 36.6%	NA	BChE activity in EX with statistically significant differences, a negative correlation of exposure time was observed.	Working age of at least 6 months. the range of 6-15 years	NA (exclusion criteria only for micronuclei)
Butinof M. 2017 Argentina. <sup>14</sup>	BChE BRV: 3200 a 9000 u/l	47 farmers	Breast cancer and total cancer	NA	The cumulative exposure to pesticides and BChE activity were negatively correlated	NA	Complete ***
Díaz SM. 2017 Colombia. <sup>26</sup>	BChE y AChE** Michel method.	125 farmers	Eye burning with 72.0% (90), headache with 68.0% (85) and dizziness with 56.0%.	8.0% (n = 10) of individuals have AChE inhibition	AChE inhibition was minimal, the use of organophosphorus pesticides, carba mates, among others, was reported. There is no difference between age and gender	Range 9 months to 50 years. Minimum previous exposure 5 days.	NA
Pedrozo ME, 2017 Paraguay. <sup>27</sup>	BChE	15 EX	Nausea and vomiting, followed by abdominal pain, headache, fever, pruritus, conjunctival hyperemia and sweating	0	Samples were taken in order to establish the renal and hepatic profile, as well as serum cholinesterase, with results within the reference values.	48 hours prior exposure	Complete ***

Studies	Enzyme and method	Sample size	Signs and symptoms	Cholinesterase inhibited	Statistical association with cholinesterase	Exhibition time	Exclusion criteria
Lutovac M. 2017 Serbia. <sup>28</sup>	AChE 6 groups, 4 EX and 2 NE. A kinetic method with propionaldehyde as substrate and DTNB 2618-6971 U / L 1 shows per year	175 workers	Nervous disorders, headache, dizziness, paralysis, anxiety, stomach pain, vomiting, diarrhoea, asphyxia or increased sweating, salivation, tears.	72%, there is a slight drop of AChE activity each year.	In workers who are constantly exposed to pesticides, a cetylcholinesterase is within the reference value. It observed in 72% a slight drop of AChE activity each year.	Range 1-13 years.	Complete
Guytingcoa. 2017 Thailand. <sup>29</sup>	AChE ** colorimetric method below 75 units / ml	3,431 workers gave blood samples	The most common self-reported symptoms were cough (5.7%), fatigue (5.4%), dizziness (5.1%), dry skin and irritation (4.8%).	The prevalence of abnormal results of AChE was 12.5%.	Male agricultural workers had a significantly higher risk than women farmers after adjusting for sex and level of risk (OR = 1.56, P <0.05).	NA	Complete
Lindao-Córdova VA. 2018 Ecuador. <sup>11</sup>	BChE The kinetic spectrophotometric method (405 nm at 37 ° C). (Kruskal Wallis)	170 farmers	NA	NA	The relative humidity is directly proportional to the cholinesterase level, however with the temperature it has an inverse relationship	Range of 1 to 10 years	NA
Marrero S. 2018 Venezuela. <sup>30</sup>	BChE 4,970-13,977 U/L	20 EX and 10 NE	30% allergies and 30% itching, statistically significant compared to those not exposed (Z = 2.21; p = 0.01)	3 (15%) workers had BChE inhibition.	Three exposed workers are below the range normal, this being statistically significant (p = 0.005).	NA	Complete
Caro-Gamboia LJ. 2019 Colombia. <sup>31</sup>	BChE Ellman. Method BRV: 8063,78 ± 1934.01 u/l	76 farmers	NA	1.32% (1/76) had values below 3930 u / l.	A decrease was observed that did not show significant differences during the three annual measurement periods (p = 0.25).	At least six months prior to the study	NA
Luna-Rondón JM. 2019 Colombia. <sup>32</sup>	AChE** 4 measurements	256 fumigators, a control group was also included.	37.2% with symptoms for chronic intoxication. Affective responses such as depression, reluctance that may be due to other endogenous or exogenous causes.	The average of the 4 measurements ranges between 33.8 and 27.6 U / g of oxyhemoglobin, with progressive decreases in the initial value (67%) in the 3rd and 4th measurements.	The AChE figures in non-fumigators remained in the normal range and the values of the fumigators showed fluctuations outside the normal range, being interpreted as the presence of a affectation or inhibition.	8 to 28 years	Complete***
Polanco-López-de-Mesa. 2019 Colombia. <sup>33</sup>	AChE	40 farmers 21 NE and 19 EX	No symptoms associated with pesticide poisoning were found.	NA	According to the levels of erythrocyte cholinesterase in individuals who use or not pesticides, the results showed a significant value in those who do not use them (p = 0.042)	NA	NA

Studies	Enzyme and method	Sample size	Signs and symptoms	Cholinesterase inhibited	Statistical association with cholinesterase	Exhibition time	Exclusion criteria
Serrano-Medina A. 2019 México. <sup>10</sup>	AChE** Ellman modified colorimetric (worek method). Samples processed immediately.	140 farmers 2 groups EX and NE	31.4% have a diagnosis of major depression, numbness (51.4%), dizziness (51.4%), abdominal discomfort (40.7%), dyspnea (31.4%), nocturia (34.3%) and insomnia (25.7%).	44 people with suicidal risk and AChE inhibition. High risk 27 (63.36%) Moderate Risk 9 (20.45%) Low risk 8 (18.18%).	The mean value of AChE enzyme activity for the exposed population was significantly lower (178.88 mE / min $\pm$ 37.48) than the enzyme activity of the unexposed group but the difference was not statistically significant (t (33) = 0.677, p = 0.503 ).	2.96 years (between 0.5 and 20 years).	NA
Magalhães AFA. 2019 México. <sup>34</sup>	BChE Maw DiaSys commercial kit (Diagnostic Systems GmbH) VRB women: 3930 to 10800 U / L) and for men (4620 to 11500 U / L).	382 workers	5 to 30% have headache, blurred vision, pruritus, nausea, dyspnea, dry cough, watery eyes and ocular hyperemia.	68% show decrease in BuChE activity.	The majority (68%) presented decreased BuChE activity, mainly farmers (85.9%); 57.3% of workers were considered intoxicated, 61.6% by agrochemicals and 37.9% by industrial chemicals.	55.2% had up to 9 years of activity	NA
Dutta S. 2019 India. <sup>35</sup>	AChE and BChE Ellman method 4 groups EE, NE and consumers of alcohol and snuff	225 Individuals	Like headache, abdominal pain, nausea, vomiting and watery eyes	Exposed (N = 95 is 42.2%) SD $\pm$ 1.85 6.43 AChE BuChE and SD 3.50 $\pm$ 1.89	AChE activity was half in individuals exposed to pesticides than controls (p $\leq$ 0.001). BuChE activity also decreased significantly in individuals exposed to pesticides than in controls (p $\leq$ 0.001	Working range 1 to 30 years	Complete ***

NA: Not applicable, no data are mentioned in the study.

DTNA: 6-6'-dithiodinicotinic acid

MINSAP: The Ministry of Public Health of the Republic of Cuba

BRV: BChE reference values, ARV: AChE reference values y AV: average value

EX: Exposed to organophosphorus and carbamates. NE: Not exposed

\* Taking as reference the 90th percentile, a 25% decrease in the values of cholinesterase activity.

\*\* It refers to methodological characteristics of the sampling.

\*\*\* Excludes participants for physiological, pathological conditions or presence of substances that can skew the results.

## DISCUSSION

Cholinesterase enzymes are widely used as biological markers to detect pesticide poisoning, but the variability of the range considered "normal" prevents comparison between the results in different studies. As already mentioned, the range of normality can vary from individual to individual, from population to population, by pathologies, substances, the environment for example high temperature and humidity less than 40% favor skin absorption by decreasing cholinesterase levels and even by the laboratory and the method used for its analysis. It is true that there is no standardized and universally accepted laboratory technique or method for these types of studies, however, since 1961 international organizations have recommended the Ellman method, which is reflected in its predominance in the analyzed articles.<sup>12,36,37,38</sup> It is desired to highlight some of the main errors that were detected, for which the results of the measurements with acetylcholinesterase are not significant, the lack of standardized analytical and preanalytical parameters can have a great influence on the variability of results since there are modifications to the conventional method there are laboratories that prepare their own reagents, while others use commercial kits.<sup>34</sup> It is important to select whether to investigate acute or chronic intoxications to identify the characteristic signs and symptoms, while choosing the most appropriate type of cholinesterase with our study. Butyrylcholinesterase is recommended for acute intoxications and acetylcholinesterase for chronic intoxications.<sup>6</sup> At the time of selecting the sample, in most of the studies there are no mentioned exclusion criteria aimed at ruling out factors of the individual that could alter the values considered as "normal", this reason may explain why in some studies they reported cholinesterase inhibition within the control group. The methodology in the sampling is referred to in less than 50% of the studies, but it is of vital importance, considering that it can be contaminated from the venous puncture (by pesticides present in the skin) and by requiring special conditions for its transport (network from cold to approximately 4 degrees Celsius for a maximum of 2 hours) that prevent the inactivation of enzymes during transport. It has been reported that the activity of the AchE in the laboratory at -20 degrees Celsius is stable during the first 7 days, subsequently its activity slowly decreases, it is 91.8% at 34 days, while the activity of BChE in plasma remained unchanged for 34 days. Only one of the studies reported that after the arrival of the laboratory it took 10 hours to perform the analysis and another study reported the analysis immediately.<sup>6,10,12,14</sup> It is believed that better results are obtained in studies where the baseline cholinesterase levels of everyone can be taken prior to exposure to pesticides, but it is known that this is not always possible, it is recommended the use of control groups that function as regional baseline samples. Good results are also seen in studies where they take serial samples and compare them to measure enzyme inhibition.

An investigation was added to this study where the measurement of AChE and BChE in saliva is carried out, which is an interesting proposal for the silver problem in some studies that showed the loss of population, who refused to take venous blood because it was an invasive technique; however, it is considered that more research is required for the validation and standardization of this method.<sup>3</sup> For the analysis of cholinesterase levels, it was found interesting the proposals where the level of intoxication was associated, with cholinesterase levels on a percentage scale. Example. "According to serum PCE levels, the severity of the poisoning was defined according to the scale of Kumary col."<sup>4</sup>

- Latent: BChE level > 50% of normal or > 3,500 IU / L.
- Mild poisoning: BChE level 20-50% of normal or > 1,401-3,500 IU / L
- Moderate poisoning: BChE level 10-20% of normal or 701-1,400 IU / L
- Severe poisoning: the BChE level is <10% of normal or <700 IU / L.<sup>4</sup>

And the Proudfoot classification, where mild organophosphorus toxicity is defined as a BChE reduction of less than 10% (fatigue, headache, nausea and vomiting), moderate toxicity as a 10-50% reduction (myosis, weakness general, dysarthria, fasciculation) and severe toxicity such as > 50% of the reduction (general fasciculation, flaccid paralysis, respiratory distress, loss of consciousness).<sup>8</sup>

## CONCLUSION

According to what it was found, it is recommended that researchers who carry out studies with cholinesterase enzymes do not lose sight of the delicacy of these enzymes in order to have the best possible results, not necessarily seek that the results are below a range established by the laboratory and take into account notes that the appearance of symptoms of intoxication depends more on the rate of cholinesterase inhibition than on the absolute level of activity found.<sup>32</sup>

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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