

DOSAGE OF ACETYL- CHOLINESTERASE AS A PARAMETER OF PES- TICIDE POISONING IN FARMERS IN THE CITY OF MARIALVA/PR

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Abstract: Over the years, the use of pesticides in agricultural production has increased in Brazil, with the aim of ensuring excellent production of grains and cereals. The Southeast, South and Central-West regions are respectively those that make the most use of these agrochemicals. There are numerous classes of these substances, the most used of which are organophosphates (OF), a class responsible for combating the vast majority of agricultural pests and causing poisoning if they are handled incorrectly and without the use of personal protective equipment. Pesticides in general inhibit the function of the enzyme acetylcholinesterase, which has the physiological function of degrading the neurotransmitter acetylcholine. When the enzyme is inhibited, acetylcholine accumulates in the synaptic cleft, causing some signs and symptoms, as is the case with pesticide poisoning. Since farmers are responsible for producing food that supplies countries, they need to guarantee excellent products, where pesticides are applied in order to produce quality food. Thus, a survey was carried out with farmers, together with a questionnaire that was applied to them, where it was possible to achieve the objectives of this study, analyzing 81 farmers in their daily activities and measuring Cholinesterase in each of them, evaluating possible poisoning by pesticides. Based on the literature, it was possible to relate this study to existing studies.

Keywords: Agrochemicals; Enzyme; Personal protective equipment.

INTRODUCTION

Brazil is one of the main agricultural producers in the world, and as a result it becomes the largest consumer of pesticides, with Paraná standing out among the Brazilian states that use pesticides the most. These chemicals have a major impact on the environment and human health, and are

related to respiratory diseases, accidents, neurological diseases and poisoning (Lopes; Albuquerque, 2018).

The main classes of pesticides that cause poisoning are organophosphates and carbamates, which have the same mechanism of action, as they have high lipid solubility. These substances inhibit the enzyme acetylcholinesterase, accumulating acetylcholine (neurotransmitter) in the synaptic cleft, causing cholinergic syndrome and some clinical manifestations of cardiovascular, gastrointestinal, muscular, respiratory and urinary problems. The entry points for intoxication are the mucous membranes, respiratory tract, skin and gastrointestinal tract (Marques; Caixeta, 2016).

Acetylcholinesterase is an enzyme that has the function of hydrolyzing acetylcholine (ACh) into acetic acid and choline during synapses, with ACh transmitting signals from one neuron to another and allowing the cholinergic neuron to return to the resting state. Acetylcholine is an enzyme composed of three branches, directly linked by disulfide bonds, which is fixed to the cell membrane through collagen. Each of these three branches has four protein subunits that hydrolyze acetylcholine. Therefore, there are a total of twelve active sites for each acetylcholinesterase enzyme (Araújo; Santos; Gonçalves, 2016).

Cholinestrace activity is obtained through two enzymes, erythrocyte cholinesterase (AChE), which is present in the erythrocyte membrane, and plasma or serum cholinesterase (BChE). Erythrocyte cholinesterase is produced by erythrocytes during hematopoiesis, while serum cholinesterase is produced by the liver. The half-lives of the two enzymes are different, and this makes it possible to distinguish acute intoxication from chronic intoxication, since the half-life for AChE is three months

(parameter to evaluate poisoning suffered some time ago), while BChE is a parameter used for more recent poisonings (Ribeiro; Mella, 2007).

Pesticides can cause two levels of poisoning in humans: acute and chronic. Acute poisoning occurs due to prolonged and exacerbated exposure to agrochemicals, causing immediate signs and symptoms, such as nausea, vomiting, blurred vision, headache, tachycardia and fatigue. Chronic poisoning, on the other hand, occurs when signs appear late, that is, they take a long time to appear (months or years) and is caused by small or moderate exposure to toxic agents, causing irreversible damage (Santos; Santos, 2023).

Among the negative impacts generated on human health, we highlight the increased risk of cancer and other serious diseases in farmers who are exposed daily to pesticides, as well as the increased number of cases of autism in children, depression, infertility, Alzheimer's and heart disease (Basso; Siqueira; Richards, 2021).

METHODOLOGY

SAMPLES AND COLLECTIONS

The scientific project involves field research with farmers in the city of Marialva/PR, who use pesticides on their crops. Blood collections from workers were carried out at home in the period October-November/2023, and following ethical and biosafety standards, both for those who carried out the collection and for the research volunteers. Disposable and sterile materials were used and subsequently disposed of (sharp and contaminated materials). Data was also collected through a questionnaire made available to each research participant.

The collected samples were stored correctly until they reached the laboratory, where they were centrifuged in order to separate serum from the cellular part. To carry out this

analysis, the sample serum was used, in which the cholinesterase in the serum is stable for up to 15 days, if stored between 2°C and 8°C.

CHOLINESTERASE DOSAGE

Acetylcholinesterase enzyme measurements were carried out using a blood sample from each farmer who participated in the research, which were later analyzed in the Clinical Analysis laboratory at Unicesumar-Maringá/PR.

Cholinesterase is an enzyme with the function of catalyzing the hydrolysis of the neurotransmitter Acetylcholine, with two types of Cholinesterase, Acetylcholinesterase, which is found in red blood cells and Pseudocholinesterase, which can be found in plasma, liver, smooth muscle and adipocytes. The measurement of Pseudocholinesterase is of great importance for evaluating and diagnosing patients with pesticide poisoning (GoldAnalisa,2013). For this, the GoldAnalisa Cat.415 Cholinesterase Kit was used, which is a Kinetic-Colorimetric method, and the Labor control 1 Control Serum, with the aim of ensuring precision in the results issued by the Bioplus equipment. The Control Serum was used before the start of sample dosing and at the end of the samples dosed on the day, with the aim of releasing reliable and accurate results to patients.

RESULTS ANALYSIS

The results of the assessment were related to reference values for men and women according to the manufacturer's instructions. After analyzing the results of each laboratory test, feedback was sent to each participant informing the result of their test. The discussion was carried out using the results obtained through this research and consultation in SciELO (Scientific Eletronic Library On Line) databases, PubMed and Google Scholar.

This research was submitted to the

Ethics and Research Committee (CAAE: 72908623.5.0000.5539) and approved by it. All research participants signed the Free and Informed Consent Form and were aware of the completion of the research.

DEVELOPMENT

RESULTS

The project was carried out with 81 farmers, of both sexes, from the city of Marialva/PR and its districts. Among the research participants, the male public prevailed, with 63 men, aged 18 to 72. The female audience was smaller, totaling 18 participants, ranging in age from 29 to 66 years old. Seven workers from an agricultural products company were also evaluated.

The age range of research participants was subdivided into four categories, with 15-30 years corresponding to 13 participants (16.04%), 31-45 years corresponding to 28 participants (34.56%), 46-60 years corresponding to 25 people (30.86%) and the last category are participants aged 61-75, comprising 15 people (18.51%).

Among the 81 research participants, 27 (33.33%) of them use some type of PPE such as gloves, masks, aprons, boots, visors or overalls. Still within the 81 participants, 4 (4.93%) of them have some symptom when they enter pesticides such as headache, dizziness, malaise, tiredness, pain in the lower limbs, chills, nausea or tremor.

Of the total number of participants, only 5 (6.17%) of them reported that they had already been poisoned by pesticides at some point in their lives. They reported symptoms such as vomiting, tremors, chills, headache, diarrhea, weakness, malaise, or nausea.

The research was also carried out with 7 workers (8.64%) from an agricultural input company who have indirect contact with pesticides, with the purpose of analyzing the toxicity of agrochemicals causing any harm

to individuals who are in daily contact, but without make use of them in farming. It was reported that none of the workers use PPE in the workplace, have never been poisoned by pesticides and do not have any symptoms when handling them.

The Cholinesterase test is widely used to evaluate organophosphate and carbamate poisoning in individuals who handle them in some way, whether on crops or in agrochemical companies. According to the leaflet for the Cholinesterase Kit, GoldAnalisa, the reference values for this test vary according to gender, being 3,930-10,800 U/L for women and 4,620-11,500 U/L for men. The results of this test below the reference values provided by the manufacturer have great clinical significance, being found in poisoning by organophosphate insecticides, hepatocellular diseases, malnutrition, acute infections, pulmonary embolism, muscular dystrophy and myocardial infarction. Results above reference values may be related to obesity, diabetes and nephrotic diseases.

The control serum was measured between the patient samples, as a way to guarantee a safe and accurate result, making it possible to offer reliable results. The Labor control 1 control serum was used, in which the values assigned for cholinesterase in automatic analyzers have an acceptable range of 5,544 U/L - 8,316 U/L. The average results obtained were $6,073.58 \text{ U/L} \pm 359.14$.

Among the 81 research participants, 63 of them (77.77%) were men, and the average results obtained in this category was 8,668 U/L, with the lowest result being 4,658 U/L and the highest result being 13,563 U/L. The rest of the participants who correspond to the female audience (18 people/ 22.22%) obtained an average result of 7,426 U/L, with the lowest result being 4,658 U/L and the highest result being 11,200 U/L (Table 1)

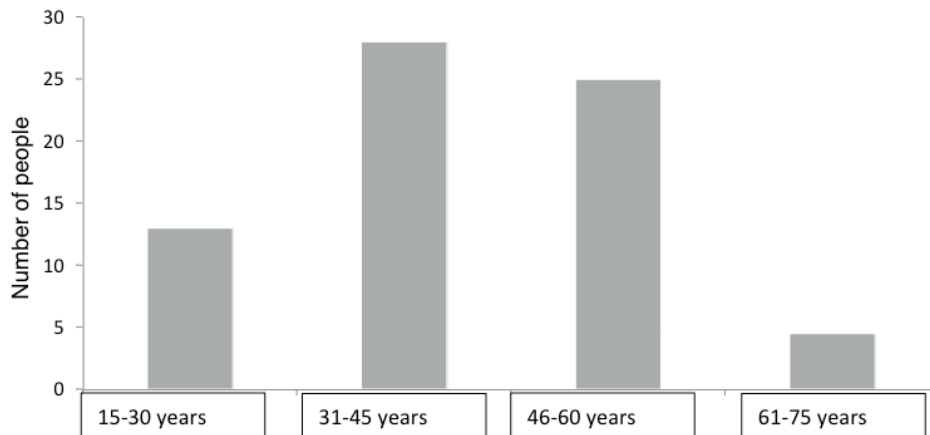


Figure 1: Age range of participants.

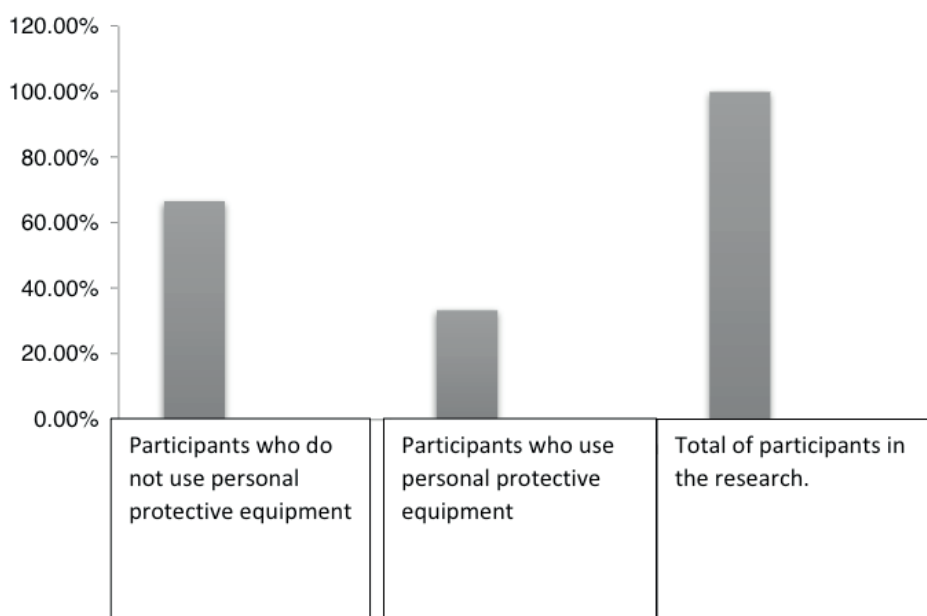


Figure 2: Use of personal protective equipment

Male public	9.453 U/L
4.658 U/L	9.658 U/L
5.686 U/L	9.727 U/L
5.754 U/L	10.001 U/L
6.165 U/L	10.070 U/L
6.165 U/L	10.070 U/L
6.302 U/L	10.549 U/L
6.507 U/L	10.549 U/L
6.576 U/L	10.549 U/L
6.713 U/L	10.580 U/L
6.781 U/L	10.600 U/L
6.781 U/L	10.805 U/L
6.800 U/L	10.823 U/L
6.850 U/L	11.165 U/L
7.096 U/L	11.345 U/L
7.192 U/L	13.239 U/L
7.192 U/L	13.289 U/L
7.192 U/L	13.358 U/L
7.261 U/L	13.563 U/L
7.398 U/L	
7.535 U/L	
7.878 U/L	Female public
7.946 U/L	4.658 U/L
7.946 U/L	5.452 U/L
8.014 U/L	5.822 U/L
8.015 U/L	6.096 U/L
8.151 U/L	6.644 U/L
8.288 U/L	6.713 U/L
8.288 U/L	7.124 U/L
8.288 U/L	7.192 U/L
8.357 U/L	7.287 U/L
8.357 U/L	7.535 U/L
8.562 U/L	7.689 U/L
8.631 U/L	7.877 U/L
8.699 U/L	7.894 U/L
8.768 U/L	8.151 U/L
8.768 U/L	8.253 U/L
8.768 U/L	8.562 U/L
8.836 U/L	9.521 U/L
8.836 U/L	11.200 U/L
8.836 U/L	
8.836 U/L	
8.905 U/L	
8.905 U/L	
9.179 U/L	

Table 1: Cholinesterase test results for each participant.

DISCUSSION

The Cholinesterase test plays a fundamental role in analyzing the health of farmers who use pesticides on their crops daily. This class of workers is the most affected by agrochemical poisonings, as they have direct contact with these products that can cause harm to human health, with Organophosphates and Carbamates being the most responsible for causing poisoning.

The farmer is not only exposed to a chemical product, but to many of them and other occupational risks in an attempt to guarantee excellent quality final products for the world's population, as they are responsible for producing the majority of food that reaches the table of consumers. Brazilians.

The data collected through the application of the questionnaire to the research participants showed that 78% of the research volunteers were male, and 22% were female. Faria et al (2009), mentions that in their research in the first stage with rural fruit workers in Bento Gonçalves/RS, 95.2% of the participants were male, and only 4.8% of the volunteers were female.

Workers aged 31-45 are those who carry out the most rural work. In relation to rural work, participants reported that they use pesticides on soybean, corn, wheat and/or grape plantations, in addition to a small proportion working in the agricultural input industry, and for these purposes, 27 participants (33.33%) use some type of PPE, which means low adherence to Personal Protective Equipment. Participants who do not use PPE correspond to 54 people (66.66%), demonstrating that the majority of the public investigated in the research does not use PPE, resulting in possible future poisoning, given that in the current research results there were no signs of poisoning. The present research differs from a study in the city of Botucatu/SP, where all research participants used PPE

and, in the same way, cholinesterase activity was below reference values (Brega et al 1998).

In relation to symptoms after exposure to pesticides, it was evidenced that only 4 individuals (4.93%) had any clinical manifestation after handling/application of the products on their crops and in the agricultural company, symptoms such as headache, nausea, vomiting, miosis, tachycardia and diarrhea, as presented by the authors Ogaet al (2008). It was also observed that 5 people (6.17%) had acute poisoning from agricultural products at some point in their lives, and at the time they did not use PPE and after experiencing the poisoning they started using some type of Personal Protective Equipment. Through a study carried out by Faria et al (2009) with 241 rural workers, it was possible to observe that 14.9% of the research volunteers had already suffered previous pesticide poisoning.

According to Ribeiro and Mella (2007), the lower the cholinesterase enzyme activity of an individual means that they have greater exposure to Carbamates and Organophosphates, and it is not necessarily that individuals who have signs and symptoms are having greater exposure than those who do not. clinical signs.

The fact that the current research does not present any result of cholinesterase activity lower than the reference values may suggest some hypotheses such as, for example, agricultural machines used in the application of pesticides contain a protective cabin, which prevents the operator from coming into contact with directly with agricultural pesticides. Another hypothesis would be a reduction in the concentration of the active ingredient in the products, in addition to a significant reduction in the doses of agricultural pesticides applied by farmers.

In addition to the hypotheses already mentioned, the market for biological inputs has been increasing every year, and farmers are

adept at substituting the use of synthetic inputs for biological inputs, which are less harmful to human health, as they are developed and extracted from plants, microorganisms and secondary metabolites (Embrapa, 2020).

Another parameter that can be analyzed by applying the questionnaire is that all 81 participants (100%) are aware of the harm pesticides can cause to human health, but only a third of farmers use PPE. According to research carried out by Marques et al. (2010), workers reported not using Protective Equipment for reasons such as heat, discomfort and, in addition to not considering it necessary, they allege carelessness and lack of time. In a study carried out by Cerqueira et al (2016), the reasons reported by farmers for not adhering to PPE were the high cost of the materials, in addition to the discomfort caused by them.

According to Veiga et al (2007), in Brazilian agriculture it is common to find farmers who do not use PPE during the preparation and application of pesticides, one of the main reasons being the fact that PPE causes thermal discomfort, which can lead to thermal stress of rural workers. To date, no independent laboratories have been found in Brazil that analyze the correct suitability of PPE, and existing laboratories are restricted to obtaining the certificate of approval and commercialization of PPE in Brazil.

CONCLUSION

Brazilian agriculture is of paramount importance for the country's economy, contributing to the growth of the national GDP and recognized as a generator of food that supplies the homes of the world's population, as more Brazilian agricultural products are exported every day, with China standing out as the largest importer of Brazilian soybeans. With the growth and intensification of agricultural production, more and more

jobs are becoming available in this area.

Brazil is one of the largest agricultural producers in the world, being the world champion in the use of pesticides due to the increase in the area of agricultural production, which is increasingly expanding. These chemical products have the function of defending crops against attacks from pests and insects, guaranteeing excellent grain and cereal production. Agricultural pesticides must meet the safety standards established by the Ministry of the Environment and ANVISA, which guarantee safety for rural producers and food consumers.

Rural workers who undergo the application of agrochemicals on their crops have direct and indirect contact with these products, and often without the use of PPE, putting their own health at risk in favor of food production. One way to monitor the health of these workers is to measure the enzyme Cholinesterase, which is responsible for the hydrolysis of acetylcholine present in synapses, which have the function of transmitting nerve impulses. Pesticides from the carbamate and organophosphate groups have the ability to inhibit cholinesterase, causing acetylcholine to accumulate in the synaptic cleft, causing a cholinergic syndrome, causing symptoms of poisoning (acute or chronic) to appear in rural workers.

According to data from this research, 81 rural workers were evaluated, who apply pesticides on their crops, or work in agrochemical companies, reaching the final result that no acetylcholinesterase results were found outside the reference values, which means that none of the workers evaluated has signs of poisoning caused by pesticides.

Given this, it was possible with this research to measure the cholinesterase enzyme in the Clinical Analysis Laboratory of the Unicesumar campus Maringá-PR, contributing to knowledge about the real

values dosed by farmers who deal with pesticides on a daily basis. This work will add knowledge to academics and university students in the health field, collaborating with reliable and real information from field research, ensuring trust and contributing to studies on the same topic.

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