Evaluation of lifestyle, biochemical and toxicological alterations in family farmers in the far west region of Santa Catarina, Brazil

Avaliação do estilo de vida, alterações bioquímicas e toxicológicas de agricultores familiares da região do extremo oeste De Santa Catarina, Brasil

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ABSTRACT
Introduction: Farmers use agricultural pesticides for crop maintenance and pest control. The exposure to these compounds generates a multitude of harmful effects to human health, causing biochemical changes. Objective: The aim of this study was to evaluate possible changes in pesticide intoxication markers, liver, kidney and behavioral profiles resulting from exposure to agricultural pesticides in rural workers. Methods: A cross-sectional study was conducted with 34 farmers (8 women and 26 men). A quality of life questionnaire was applied and blood samples were collected for analysis of liver (ALT, AST, GGT, ALP), kidney (Urea, Creatinine) and intoxication (Cholinesterase) profile biomarkers. Data were analyzed using an analysis of variance (ANOVA). Results: The renal and intoxication profile values showed no significant differences between the groups by ANOVA statistical test, however, one liver profile value (ALT) showed significant difference between the groups. It was also observed that in all groups there were values of all profiles outside the reference limits. Conclusion: The results of the study show abnormalities in the liver profile of the "Good" group, by the change in ALT reference values, possibly due to the non-use of PPE, however, there were metabolic changes in all groups, including the marker of intoxication from pesticides, which leads
us to believe that regardless of lifestyle the rural population is exposed to this class of substances.

**Keywords:** 1. Farmers, 2. Pesticides, 3. Poisoning, 4. Life style.

**RESUMO**

Introdução: Os agricultores fazem uso de pesticidas agrícolas para manutenção da lavoura e controle de pragas. A exposição a esses compostos gera uma infinidade de efeitos nocivos à saúde humana, provocando alterações bioquímicas. Objetivo: O objetivo desse estudo foi avaliar as possíveis alterações de marcadores de intoxicação por agrotóxicos, perfil hepático, renal e comportamental provindas da exposição aos pesticidas agrícolas em trabalhadores rurais. Métodos: Foi realizado um estudo transversal com 34 agricultores (8 mulheres e 26 homens). Foi aplicado um questionário referente à qualidade de vida e colhidas amostras de sangue para análise dos biomarcadores do perfil hepático (ALT, AST, GGT, ALP), renal (Ureia, Creatinina) e de intoxicação (Colinesterase). Os dados foram analisados por meio de uma análise de variância (ANOVA). Resultados: Os valores de perfil renal e de intoxicação não demonstraram diferenças significativas entre os grupos. No entanto, um valor de perfil hepático (ALT) mostrou diferença significativa entre os grupos. Observou-se ainda que, em todos os grupos houve valores de todos os perfis fora dos limites de referência. Conclusão: Os resultados do estudo evidenciam anormalidades no perfil hepático do grupo “Bom”, pela alteração nos valores de referência de ALT, possivelmente pela não utilização de EPI, contudo, houve alterações metabólicas em todos os grupos, inclusive do marcador de intoxicação de agrotóxicos, o que nos leva a crer que independentemente do estilo de vida a população rural está exposta a essa classe de substâncias.

**Palavras-Chave:** 1. Agricultores, 2. Agrotóxicos, 3. Intoxicação, 4. Estilo de vida.

**1 INTRODUCTION**

The use of agricultural pesticides is essential for crop management and pest control, however, an inadequate exposure to these substances has a linear relationship of undesirable effects to human health, both in acute and chronic toxicity. The chronic effects can be mental disorders such as depression [1], alterations in the peripheral nervous system, immune system, liver, and in electrolytes [2]. There are also acute symptoms such as headache, mood alterations, fatigue in the legs, blurred vision, sleep alterations, memory diminution, and dizziness [3].

Exposure to these substances can also generate biochemical alterations, that is, the presence of biological markers of effect, which show harmful effects of exposure to pesticides. Liver enzymes are used as markers of intoxication by pesticides, because these substances are absorbed and subsequently metabolized by the liver [4,5]. It is also important to evaluate markers of renal function, knowing that the kidneys play an important role in the biotransformation and excretion of toxic substances [6,7]. In order
to monitor a possible intoxication by agrochemicals, we use the dosage of acetylcholinesterase (AChE) and/or butyrylcholinesterase (BuChE) enzymes, which are strongly inhibited by agricultural pesticides of the classes of organophosphates and carbamates, which can generate a cholinergic crisis that can lead to death [8,9].

To identify parameters that characterize the appropriate lifestyle, one can use tools such as the questionnaire "Fantastic Lifestyle", originally developed by Wilson and Ciliska. This instrument is a self-administered questionnaire that considers the behavior of individuals in the last month and whose results allow us to determine the association between lifestyle and health [10].

Aiming at a better understanding of biological events in the face of chronic exposure to pesticides, and considering that the monitoring of health issues of family workers is directly reflected in agricultural development, it is essential to conduct studies that allow the evaluation of the health of rural workers. Therefore, the aim of this study is to evaluate possible changes in pesticide intoxication markers, liver, kidney and behavioral profiles resulting from exposure to agricultural pesticides in rural workers in the far west of the state of Santa Catarina.

2 MATERIAL AND METHODS

2.1 STUDIED POPULATION

Participation was voluntary, and after receiving detailed explanations of the intervention protocol, the participants signed the Informed Consent Form. The protocol was approved by the Ethics and Human Research Committee of the Universidade do Oeste de Santa Catarina (UNOESC - CAAE no. 55809116.0.0000.5367), opinion.

Volunteers who took continuous medication or food supplements, had liver or kidney diseases, cancer, thyroid disorders, diabetes, angina, alcoholism, smoking and morbid obesity were excluded from the study. Participants who did not follow the researchers' instructions correctly were also excluded.

2.2 EXPERIMENTAL DESIGN

A cross-sectional research model was carried out. The study population was composed of farmers, residents of the municipality of Guarujá do Sul - SC, located in the Far West of the state of Santa Catarina, chosen by convenience, aged over 18 years, who had direct or indirect contact with agricultural pesticides in the last 30 days, not carriers of hepatopathies, nephropathies and other diseases that could interfere with the results of
biochemical tests. Participation in the study was voluntary and after receiving detailed explanations of the intervention protocol, the participants signed the Informed Consent Form (ICF).

2.3 LIFESTYLE ASSESSMENT

In order to assess the individual lifestyle of the volunteers, a questionnaire was applied. The questionnaire "Fantastic Lifestyle" which is an instrument developed in the Department of Family Medicine at McMaster University in Canada by Wilson and Ciliska [10], with the purpose of helping physicians who work with prevention, so that they can better know and measure the lifestyle of their patients. It consists of an adaptation from the work of Añez, Reis, and Petroski [11]. This tool considers the behavior of individuals in the last month and its results allow the determination of the association between lifestyle and health. The instrument has 25 questions divided into nine domains which are: 1) family and friends; 2) physical activity; 3) nutrition; 4) smoking and drugs; 5) alcohol; 6) sleep, safety belt, stress and safe sex; 7) type of behavior (anger, hurry and hostility); 8) introspection; 9) work.

Each question presents five alternative answers, with the alternative on the left being the one with the lowest value (0) or least relation to a healthy lifestyle, and the one on the right with the highest value (5), related to a healthy lifestyle. The coding of the questions is done by points, and the sum of the points leads to a score that classifies the individuals into five categories Excellent (E); Very Good (MB); Good (B); Regular (R); Needs Improvement (NM). In order to monitor contact with agricultural pesticides and the use of PPE, a simple questionnaire was applied where the volunteer had to choose the option that best fit him.

2.4 LABORATORY EVALUATIONS

Blood samples were collected from the participants after a fast of at least 8 hours. The analyses of Alanine aminotransferase (AST/TGO); Aspartate aminotransferase (AST/TGP); Gamma glutamyl transferase (GGT); Alkaline phosphatase (ALP); Urea; Creatinine and Cholinesterases were measured in the BIO-2000IL analyzer (BIO PLUS), using the respective commercial kits (Labtest Diagnostics®-MG-Brazil), according to the manufacturer's instructions. The values of AST/TGP; ALT/TGO; ALP; GGT were expressed in U/L; GGT expressed in U/L; Urea expressed in mg/dL; Creatinine expressed in mg/dL; Cholinesterases expressed in U/L. The value of the Glomerular Filtration Rate
(GFR) was estimated by the formula of the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) study [12]; its results were expressed in mL/min/1.73m².

2.5 STATISTICAL ANALYSES

The results of the biochemical tests were tabulated and expressed by means of descriptive statistics, with the aid of Microsoft Excel 2016 Software. The farmers' biochemical and cholinesterase test data will be grouped, according to the farmers' score on the fantastic questionnaire and an analysis of variance (ANOVA) will be performed to check for statistical difference between the groups, considering p-values < 0.05 as significant.

3 RESULTS

Lifestyle evaluation of the study population

A questionnaire was applied in order to evaluate the lifestyle and behavior of the participants. The questionnaire used was an adaptation of the "Fantastic Lifestyle" tool [10] as recommended by Añez, Reis, and Petroski [11]. The values related to physical activity, nutrition, type of behavior, and work were highlighted. The results of the questionnaire were compiled and explained in Table 1.

<table>
<thead>
<tr>
<th>Rated Item</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family and Friends</td>
<td>7.24 (±1.05)</td>
</tr>
<tr>
<td>Physical Activity</td>
<td>3.97 (±2.21)</td>
</tr>
<tr>
<td>Nutrition</td>
<td>7.35 (±2.66)</td>
</tr>
<tr>
<td>Cigarette and Drugs</td>
<td>14.24 (±1.18)</td>
</tr>
<tr>
<td>Alcohol</td>
<td>10.15 (±2.03)</td>
</tr>
<tr>
<td>Sleep, Seatbelts, Stress, and Safe Sex</td>
<td>15.88 (±2.77)</td>
</tr>
<tr>
<td>Type of Behavior</td>
<td>4.41 (±1.99)</td>
</tr>
<tr>
<td>Introspection</td>
<td>9.24 (±1.81)</td>
</tr>
<tr>
<td>Work</td>
<td>3.74 (±0.62)</td>
</tr>
<tr>
<td>Sum FANTASTICO questionnaire</td>
<td>76.21 (±6.35)</td>
</tr>
</tbody>
</table>

Fantastic questionnaire score on each item evaluated. The higher the score the better the relationship between the item and the participant.

Contact with agricultural pesticides by the study population

Figure 1 shows that most of the farm workers (64.7%) participate in the whole production process, especially in the preparation and application of agricultural pesticides in the plantation, whether they are insecticides, fungicides, or herbicides. Only 14.7% of
farmers reported not using agricultural pesticides on their crops, while 20.5% of farmers reported not coming into direct contact with these substances, but they come into indirect contact by living and working near pesticide application areas.

Figure 1 - Types of contact with agricultural pesticides expressed in percentage (%)

Use of agricultural pesticides by farmers on crops in the far west region of the state of Santa Catarina.

Use of PPE and information about agricultural pesticides

As shown in figure 2 it shows that most volunteers had technical guidance on the proper use of pesticides (54.4%), however, they hardly use PPE (17.1%), even though they have access to this type of equipment (65.7%), this reason is possibly the cause of many farmers reporting problems after application of agricultural pesticides (75.5%). Most still say they seek health care and do routine exams (60.4%).

Figure 2 - Guidelines and agricultural practices related to the use of agrochemicals

Guidelines and agricultural practices related to the use of agricultural pesticides performed by farmers in the far west region, in the state of Santa Catarina.

Division according to the lifestyle of the population studied

From the sum of each category evaluated with the "Fantastic Lifestyle" questionnaire, Scores were obtained. Where they were divided into three groups,
following the evaluation criteria: 70.59% of the individuals had their quality of life classified as "Very Good", 17.65% as "Good", and 11.76% as "Excellent" (Figure 3).

Division of the volunteers according to the scores of the fantastic questionnaire. They were divided into three groups: Good, Very Good, and Excellent.

Laboratory and biochemical analysis

The data from the "Fantastic Lifestyle" questionnaire were classified and correlated with the results of the biochemical tests (Figure 4). It is observed that only the ALT values had a statistically significant difference (p=0.0123). It was also observed that some cholinesterases, AST, ALP, GGT, urea, creatinine and GFR values were outside the reference threshold.

Result biochemical tests in relation to the fantastic questionnaire score. Liver profile (AST (A), ALT (B), ALP (C) and GGT (D)) expressed in U/L; Acute poisoning profile (cholinesterases (E)) expressed in U/L; Renal profile (creatinine (F), urea (G)) expressed in mg/dL and GFR (H) mL/min/1.73m2. Data were presented as mean ± SD. Data were treated by ANOVA test.
4 DISCUSSION

The present work correlated the lifestyle of farmers, with markers of hepatic, renal homeostasis in addition to possible intoxication by pesticides. The prevalence of the participants was male, with 76.4%, as well as different studies related to the use of agricultural pesticides [13, 14], with a mean age of 50.2 (± 13.7) years, which shows an aging of the farming population [15, 16].

Analyzing the aspects related to the lifestyle of the participants by applying the questionnaire "Fantastic Lifestyle" [11], it is observed that, most individuals (70.59%) had their quality of life classified as "Very Good", and the rest as "Good" (17.65%) and "Excellent" (11.76%). None of the volunteers fell into the "Fair" and "Needs Improvement" groups, according to the evaluation criteria. These factors are related to the financial situation of the family, according to Frigotto [17], greater financial gains often induce less healthy lifestyle habits, due to access to food and leisure that are more pleasurable, but harmful to health, which includes items such as food [18], which had a result of 7.35 (±2.66), which shows that the diet of farmers is not balanced, consisting mainly of fats and carbohydrates, in line with other data in the literature [19]. A healthy diet is based on a good relationship between macros and micronutrients, as well as other substances beneficial to our body, is related to a good development and a good quality of life [20-22], whereas an unbalanced diet is related to the increase of chronic noncommunicable diseases (NCDs), such as hypertension and diabetes [23, 24].

Still on this panorama, some factors consistent with risk behaviors were analyzed, such as the consumption of alcohol, cigarettes and other drugs, besides the use of seat belts, stress control, quality of sleep, safe sex and physical activity. The results in the areas of alcohol consumption (10.15 ± 2.03), cigarette smoking and other drugs (14.24 ± 1.18), show that the population studied does not have an addictive character or dependence on such substances, even though alcohol is one of the most used substances by this class [25]. Regarding sleep, safety belt, stress and safe sex, the participants had a good performance (15.88 ± 2.77), the great majority explained that they have safety habits, and do not have risky habits. Regarding the practice of physical activity, the population studied was not very active (3.97 ± 2.21), even before this data, it was not observed any rate of sedentary lifestyle, considering that farmers are usually very active in their daily tasks, but do not practice anything beyond their routine obligations, either by exhaustion of the day-to-day or even by lack of time [26].
Still in this scope, the results in relation to the relationship with family and friends (7.24±1.05), type of behavior (4.41±1.99), introspection (9.24±1.81) and relationship with work (3.74±0.62), show a certain vulnerability of this population. Most of the volunteers reported having a hurry behavior, feelings of disappointment and depression, besides being dissatisfied with their work, but they claim to have someone to talk to relatively often. Two hypotheses were raised for this result: the first is related to the long working hours, prolonged exposure to sunlight and ultraviolet light, stress and other situations at work and in life [27], where the individual facing these working conditions, financial situation and the uncertainty of the future tend to acquire depressive characteristics and isolation [28-30].

Another hypothesis that can explain this sense, is that exposure to agricultural pesticides is causing the change in behavior. These data are in line with the scientific literature, which shows a higher incidence of symptoms of irritability, depression, anxiety, cases of suicide, and family estrangement in populations exposed to agricultural pesticides [14, 31-34]. This effect has been attributed to the neurotoxic activity and endocrine deregulation caused by pesticides, confirmed by experimental studies [1], mainly of the organophosphate [34] and carbamate [35] classes. This hypothesis can be taken into account, considering that when asked about the use of PPE, the vast majority of volunteers said they did not use this type of equipment (82.9%), which is in line with other studies, such as Araújo et al. The non-use of this equipment is often linked to non-availability, high cost and financial difficulties, lack of guidance on the importance of use or discomfort caused by heat [36].

Within the scope of the objective of analyzing the possible differences between the "Good", "Very Good" and "Excellent" lifestyle groups, relating them to markers of hepatic and renal homeostasis, the statistical evidence indicates that the lifestyle does not influence the values of AST, ALP, GGT, urea, creatinine and GFR, however, the ALT presented significant variance, where the "Good" lifestyle group presented higher values than the "Very Good" and "Excellent" groups. However, one volunteer from the "Good" group presented altered ALP and GGT; as well as three from the "Very Good" group and one from the "Excellent" group presented increased ALP values. In relation to renal markers, two volunteers from the "Good" group presented alterations in urea and another in creatinine, as well as four from the "Very Good" group presented increased values for urea and eight increased values for creatinine; in the "Excellent" group, no volunteer presented alterations in renal markers. In parallel, when the results of the GFR (CKD-
EPI) were analyzed, only five volunteers presented values above 90 mL/min/1.73m², considered the ideal, one from the "Good" group and four from the "Very Good" group, which may suggest a certain degree of renal deficiency [37], moderate dehydration [38], hyper-protein diet [39] increased muscle mass [40] or even an accumulation of toxic substances in the kidneys in this case, agricultural pesticides [41-43].

The altered results of AST and ALT enzymes, occur mainly in injury or destruction of liver cells [44,45], whereas ALP and GGT markers are elevated in hepatobiliary changes [46,47]. The results of the present study indicate that the group with the "Good" lifestyle is more susceptible to liver damage, however, the other groups also suffer from such conditions. The findings, are a strong indication of a chronic intoxication, by pesticides, even though it is difficult to diagnose and has an undefined clinical picture [3, 48]. The data meet the literature, as highlighted by Oga and collaborators [49], in which exposure to agricultural pesticides is widely related to the release of AST and ALT into the blood. In relation to the renal markers, even though there was no variance, there were indications of possible damage, a result similar to that of Figueiredo et al. [50], who found renal alterations in 370 farmers in the region of Campinas, São Paulo state. Therefore, these data suggest that a more detailed evaluation should be performed, with other more specific markers, in addition to a clearer evaluation of the exposure time that may influence liver and kidney alterations [51].

Cholinesterases are specific molecular targets of organophosphate and carbamate pesticides. Therefore, their activity is recognized as a marker related to agricultural pesticide poisoning [52]. When we refer to cholinesterase, we are referring to the family of these enzymes, which is composed of acetylcholinesterase, erythrocyte cholinesterase, and butyrylcholinesterase, plasma cholinesterase [51]. Organophosphates and carbamates have as a mechanism of action, the inhibition of these enzymes [53], thus resulting in an accumulation of acetylcholine or butyrylcholine, in cholinergic synapses, thus generating clinical manifestations of cholinergic syndrome [54]. The present study did not show a significant difference in the activity of cholinesterases between the groups; however, eight decreased values were found, six in the "Very Good" group and two in the "Excellent" group, which contradicts the initial hypothesis, where the group with a worse quality of life would have a higher incidence of intoxication. This fact may be related to the non-use of PPE, many volunteers also complained of headache, nausea and fatigue, soon after the application of pesticides, which is a strong indication of cholinergic syndrome [55]. It is worth remembering that in intoxication by carbamates, the activity
of enzymes is restored in a few days (reversible inhibitor) [56], whereas in exposure by organophosphates the inhibition is irreversible [48].

In general, the results show that, even though no significant differences were found between the groups, changes were found in the liver profile, one being significant, in the kidney profile, and in the intoxication marker in the farmers, which may be related to a low use of PPE by the rural workers. On the other hand, it was evidenced that even in different qualities of life, there can be the occurrence of contamination by pesticides.

We could have chosen another experimental design, comparing exposed and unexposed individuals, and possibly we could have observed a greater difference between the groups studied. It is suggested that in future studies or extension work, it would be interesting to evaluate the oxidative and antioxidant profile, besides the analysis of the lipid profile and glycemic profile of the participants, bearing in mind that the main point to be improved is the diet of the volunteers, besides the education for people to eat better. Knowing that, in the study, no addiction or drug addiction was observed, however, the unbalanced diet ends up being the escape valve of these people.

5 CONCLUSION

Considering the sum of the scores of the "Fantastic Lifestyle" questionnaire, the farmers' quality of life can be classified as satisfactory. However, when one looks at some aspects evaluated in isolation, one notices deficits regarding diet, interaction with family and friends, introspection, and relationship with work. It was not possible to establish a relationship between lifestyle and the biochemical markers used in this study. However, a portion of the population studied showed decreased results in cholinesterase dosages, showing an overexposure, possibly associated with the non-use of PPE, a factor that was confirmed in this study.

The results obtained from this study show that the population living in rural areas is exposed to pesticides, even if many times without showing signs or symptoms, which may, in the long term, trigger a series of complications to human health. The results suggest that the use of agricultural pesticides, especially without the use of PPE, can indeed be harmful to health, altering markers of liver and kidney function, cholinesterases, and possibly others not addressed in this study. Therefore, we conclude that other studies should be conducted to confirm these findings and thus define a sociodemographic profile promoting a greater approach and awareness about the use of these substances.
REFERENCES


