Factors influencing plasma butyrylcholinesterase activity in agricultural workers

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Abstract. We studied the influence of some factors on plasma butyrylcholinesterase activity (BChE) and we assessed the health status of agricultural workers and looked for the effects of chronic exposure to pesticides, in the aim to determine factors that must be considered for interpretation of BChE, for occupational monitoring of workers exposed to pesticides. The study was carried out in 110 workers from the Sahel region of Tunisia exposed to pesticides and in 97 controls. All individuals have undergone clinical examination. BChE activity was measured by spectrophotometric method. The mean of BChE activity of workers was significantly lower than that of control group ($P < 0.001$). Among the 44% of farmworkers with at least one neurologic or neuropsychic symptom, 29% had BChE activity lower than 6000 IU/L. Headache was observed in 20% of workers. Ancientness of exposure equal to 10 years or more, last exposure dates back to 60 days or less, exposure for 2 days or more per week, exposure exceeding 150 h per year and to organophosphorus-carbamates mixture were implicated in significant decreases of BChE activity and can be considered as risk factors of cholinergic toxic effects. Thus, BChE activity is a useful indicator to monitor workers chronically exposed to pesticides. However, it should be accompanied by periodical health examination, taking into account risk factors, for an early identification of workers at increased risk.

Key words: pesticides, butyrylcholinesterase, carbamates, occupational exposure, organophosphorus

Résumé. Nous avons étudié l’influence de certains facteurs sur l’activité de la butyrylcholinestérase (BChE), évalué le statut sanitaire des travailleurs agricoles et l’effet de l’exposition chronique aux pesticides, dans le but de déterminer les facteurs à prendre en considération lors de l’interprétation de l’activité de la BChE dans le cadre de surveillance professionnelle systématique de ces travailleurs agricoles. Notre étude a été réalisée chez 110 travailleurs agricoles et 97 témoins. Chaque individu a bénéficié d’un examen clinique. La détermination de l’activité de BChE a été réalisée par une méthode cinétique spectrophotométrique. L’activité moyenne de la BChE est apparue significativement plus faible chez les travailleurs par rapport aux témoins ($p < 0.001$). Parmi les 44 % des travailleurs rapportant au moins un signe neurologique ou neuropsychique, 29 % avaient une activité de BChE inférieure à 6000 UI/L. Les céphalées représentent le symptôme neurologique le plus souvent mentionné (20 %). La variation de l’activité de la BChE est non seulement liée à la nature du pesticide utilisé, mais aussi aux différentes modalités d’exposition. Une ancienneté d’exposition excédant dix ans, une exposition dépassant 150 heures par an à des mélanges de pesticides organophosphorés-carbamates interviennent dans la diminution significative de cette enzyme et peuvent être considérées comme des facteurs de risque des effets toxiques cholinergiques. L’activité de BChE est...
Pesticides constitute a heterogeneous category of chemicals specifically designed for protection of crops against pests, weeds or plant diseases [1]. Their application is still the most effective and accepted mean for the protection of plants from pests, and has contributed significantly to enhanced agricultural productivity and crop yields. All people are inevitably exposed to pesticides, though environmental contamination or occupational use. Agricultural workers are chronically exposed to a combination of agricultural chemicals; therefore, they are at increased risk of acute and chronic toxicity. While acute toxic effects are easily recognized, the effects resulting from long-term exposure to low doses of pesticides are often difficult to assess. Several studies have demonstrated the harmful effects of these compounds on human health such as an increase in endocrine, developmental, immune and neuropsychological disorders [2, 4]. Epidemiological studies have indicated that exposure to pesticides can induce chronic effects on the central and peripheral nervous system, either after acute intoxication or as result of low-level long term exposure [5-7]. Other studies have reported that repeated low level exposure to pesticides increases the risk of neurodegenerative diseases particularly Parkinson’s and Alzheimer’s diseases [8-10] as well as an increase in some types of cancer and several other genetic diseases [2, 3, 11-15]. Bolognesi has reviewed that occupational exposure to mixtures of pesticides is associated with an increase in genetic damage and induces cumulative cytogenetic effects [1]. Therefore, the monitoring of farmworkers’ health as well as the assessment of the individual risk of handling pesticides are necessary to give an indication about the extent of exposure and help to detect the effects of pesticides before adverse clinical health effects occur.

Biological monitoring is a useful tool for assessing exposure to pesticides and for the evaluation of potential health risks. Among biological indicators, Butyrylcholinesterase activity (BChE) is still a useful biomarker to predict and prevent health hazards of workers chronically and occupationally exposed to pesticides [16-18]. To the best of our knowledge, no studies have examined pesticides effects among farmworkers in the Sahel region of Tunisia. In this work, we studied influence of some factors (main occupation, ancientness of exposure, type of pesticides used, duration of exposure, spraying mode, smoking habits, and alcohol consumption) in the aim to determinate those that must be considered for interpretation of BChE for the occupational monitoring of workers exposed to pesticides.

**Subjects and methods**

**Study population**

This study was carried out in a group of 110 agricultural workers from the Sahel region of Tunisia: 96 males and 14 females aged from 18 to 78 years (42.5 ± 14.8, mean ± SD), chronically exposed to pesticides (organophosphorus [OP], carbamates [CB], pyrethroids, thiocarbamates . . .). They had been using pesticides for an average of 19 ± 11 years. These workers were mostly exposed to a mixture of pesticides from different families in a wide range of formulations, either simultaneously or successively. Workers were randomly selected based on an oral questionnaire consisting on both open and closed questions. Information was obtained about the sex of the workers, age, weight, smoking habits, alcohol consumption, level of education, medical and occupational histories (times of exposure, main occupation, use of personal protective equipment), trade name of most used pesticides, date of last exposure and name of pesticides used at that time.

The control group consisted of 97 healthy individuals (85 males and 12 females) aged from 24 to 65 years (39.1 ± 9.5), without previous occupational exposure to pesticides or any other industrial chemicals. Control group was matched with exposed workers on age and sex.

All individuals have undergone clinical examination. Only neurological and neuropsychic symptoms were described in this part of study: headache, tremor, asthenia, irritability, paraesthesia, anxiousness, limb numbness, sleep disturbance and vertigo.

**Sample collection**

Blood samples were collected in heparinized tubes by venipuncture at the time of clinical examination. The samples were stored in a portable refrigerator and taken to the
Results

The general informations and plasma BChE activities of control group and exposed workers are summarized in Table 1. BChE activity of agricultural workers (7564 ± 2200 IU/L) was significantly lower ($P < 0.001$) than the one of control group (8947 ± 2863 IU/L).

Among the 44% of farmworkers that reported at least one toxic neurological or neuropsychic symptom, 29% had BChE activity lower than 6000 IU/L. Headache was observed in 20% of workers. Numbness or paraesthesia of limbs (14%) as well as asthenia (13%) were also among the most observed symptoms in the group of workers.

Common name and World Health Organisation (WHO) classification of pesticides frequently used by studied population are presented in Table 2. Mancozeb, methomyl and chlorpyriphos-ethyl were the most used pesticides, with frequencies of use equal to 84, 73 and 71% respectively. Table 3 summarizes BChE mean activities of agricultural workers according to some occupational features. Among workers, 63% were owners, 77% were permanent and 90% were versatile. Although BChE mean activity of sprayers was lower when compared with the versatile workers, no significant difference was found between them. Among these workers, 27% were illiterate and 55% had primary school education.

In this study, illiterate workers had a noteworthy low BChE activity when compared with farmers that had primary school education ($P = 0.01$). 79% of workers respected scrupulously the dilution procedures that must be performed, while 5% did not and almost all workers prepared their dilutions in the field and they hadn’t a shelter local. Ninety-seven percent used more than one pesticide in the same intervention. These pesticides were often from different classes. Of the workers, 15% use portable pumps, 50% use trailed sprayers and 35% use other types (atomizers or stabilized sprayers) or a combination of two types at the same time. Among the workers, 90% reported an irregular moment of pulverization and sometimes, as needed, they pulverized several times per day. Regarding personal protective equipment (PPE), it was generally limited to the use of masks and/or rubber gloves and from all the workers; only 15% used PPE during pulverization but their usage was occasional and often they didn’t wear masks especially when pulverizing in greenhouses. No significant difference was found between BChE activity of workers using PPE and the one of workers no using any protective devices whatsoever.

Several other factors were studied to explain those implicated in the variation of BChE activity for these agricultural workers. The variation curve of BChE mean activity according to years of work was performed. A significant negative correlation was noted between the decrease in mean BChE activity and the ancientness of exposure ($r = -0.251$, $P = 0.02$). Significant decrease was noted in BChE activity of workers which had history of exposure less than or equal to 10 years ($7481 \pm 2267$ IU/L), as compared to control group ($P = 0.01$). Besides, the activity of plasma cholinesterase was significantly lower ($P < 0.051$) in workers whose years of work exceeded 20 years ($6840 \pm 2146$ IU/L) as compared with those with a history of exposure less than or equal to 20 years ($7919 \pm 2125$ IU/L). The inhibition of BChE activity was also pronounced with daily duration of exposure to
pneutopsesticides, and activity of this plasma enzyme of workers exposed for 7 h per day (6532 ± 2326 IU/L) presented lower BChE activity, near the significance (P = 0.052), when compared with those exposed for only 2-3 h per day (7805 ± 1548 IU/L). A significant correlation was noted between BChE mean activity and the last exposure to pesticides when the Spearman rank test was applied (r = 1, P < 0.01).

In fact, BChE mean activities were significantly lower in workers whose exposure dates back to 60 days or less when compared with those whose last exposure goes back to more 60 days (figure 1).

The number of workdays per week was among factors that were implicated in the variation of BChE activity. Boxplot of BChE levels in pesticides exposed farmers based on the number of work days per week are illustrated in figure 2. Farmers who worked 3 days or more per week have a BChE activity significantly lower when compared with those who

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**Table 2.** Chemical class, common name, activity and World Health Organisation (WHO) classification of pesticides frequently used by studied population.

<table>
<thead>
<tr>
<th>Chemical class</th>
<th>Common name</th>
<th>Activity</th>
<th>Class (WHO)</th>
<th>User (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organophosphorus</td>
<td>Chlorpyriphos-ethyl</td>
<td>I</td>
<td>II</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Dimethoate</td>
<td>I, Ac</td>
<td>II</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Metamidophos</td>
<td>I, Ac</td>
<td>Ib</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Methidathion</td>
<td>I</td>
<td>Ib</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Cadusafos</td>
<td>Ne</td>
<td>Ia</td>
<td>19</td>
</tr>
<tr>
<td>Carbamates</td>
<td>Methomyl</td>
<td>I</td>
<td>lb</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Propamocarb</td>
<td>F</td>
<td>NC</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Carbofuran</td>
<td>I</td>
<td>lb</td>
<td>23</td>
</tr>
<tr>
<td>Pyrethroides</td>
<td>Cypermethrin</td>
<td>I</td>
<td>II</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Deltamethrin</td>
<td>I</td>
<td>II</td>
<td>53</td>
</tr>
<tr>
<td></td>
<td>Bifenthrin</td>
<td>I, Ac</td>
<td>II</td>
<td>25</td>
</tr>
<tr>
<td>Othersb</td>
<td>Mancozeb</td>
<td>F</td>
<td>III</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>Maneb</td>
<td>F</td>
<td>III</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>Fenarimol</td>
<td>F</td>
<td>III</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Amitraz</td>
<td>I, Ac</td>
<td>III</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Imidacloprid</td>
<td>I</td>
<td>III</td>
<td>66</td>
</tr>
</tbody>
</table>

I: insecticide; Ac: acaricide; F: fungicide; Ne: nematicide. * WHO classification of pesticides: Ia: extremely hazardous, Ib: highly hazardous, II: moderately hazardous, III: slightly hazardous, NC: not classified. * Other chemical classes: including thiocarbamates, pyrimidin, amidines...

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**Table 3.** Buthyrylcholinesterase (BChE) mean (SD) activities of agricultural workers according to some occupational features.

<table>
<thead>
<tr>
<th>Frequency (%)</th>
<th>BChE mean activity (IU/L)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Status of farmer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Worker</td>
<td>37</td>
<td>7593 ± 2117</td>
</tr>
<tr>
<td>Owner</td>
<td>63</td>
<td>7269 ± 2497</td>
</tr>
<tr>
<td>School education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illiterate</td>
<td>27</td>
<td>6842 ± 1766</td>
</tr>
<tr>
<td>Primary</td>
<td>55</td>
<td>8148 ± 2112</td>
</tr>
<tr>
<td>Secondary</td>
<td>18</td>
<td>7374 ± 2655</td>
</tr>
<tr>
<td>Occupational situation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Occasional</td>
<td>23</td>
<td>7861 ± 2129</td>
</tr>
<tr>
<td>Permanent</td>
<td>77</td>
<td>7353 ± 2369</td>
</tr>
<tr>
<td>Occupational exposition</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprayer</td>
<td>10</td>
<td>7084 ± 2083</td>
</tr>
<tr>
<td>Versatile</td>
<td>90</td>
<td>7521 ± 2332</td>
</tr>
<tr>
<td>Pulverization mode</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Portable pump</td>
<td>15</td>
<td>6963 ± 2225</td>
</tr>
<tr>
<td>Trailed sprayer</td>
<td>50</td>
<td>7849 ± 1911</td>
</tr>
<tr>
<td>Others</td>
<td>35</td>
<td>7559 ± 2547</td>
</tr>
<tr>
<td>Moment of pulverization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irregular</td>
<td>90</td>
<td>7484 ± 2301</td>
</tr>
<tr>
<td>Regular</td>
<td>10</td>
<td>7533 ± 2513</td>
</tr>
<tr>
<td>PPE use</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>15</td>
<td>7768 ± 2214</td>
</tr>
<tr>
<td>Yes</td>
<td>85</td>
<td>7558 ± 2042</td>
</tr>
</tbody>
</table>

PPE: personal protective equipments.

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**Figure 1.** Comparison of buthrylcholinesterase (BChE) mean activity between agricultural workers based on last exposure (day) (° P = 0.02).
Butyrylcholinesterase activity in agricultural workers

Day(s)/week

BchE activity (IU/L)

14 000
12 000
10 000
8 000
6 000
4 000
2 000

*  

Figure 2. The boxplots of butyrylcholinesterase (BChE) for agricultural workers studied based on the number of work days per week (*$P=0.002$ versus workers exposed one day/week).

worked 1 day ($P < 0.01$) or 2 days per week ($P=0.046$). A significant correlation was found between this enzyme level and number of days per week ($r = -0.404$, $P < 0.01$).

In order to better investigate the variation of plasma cholinesterase activity, we studied the influence of the yearly duration of exposure on this enzyme (figure 3). The activity of BChE was negatively correlated with the number of exposure hours to pesticides for these agricultural workers ($r = -0.390$, $P < 0.01$).

The plasma cholinesterase activity of workers whose the number of exposure hours was between 200 and 300 h per year ($6115 \pm 1910$ IU/L) was significantly lower when compared with those exposed to pesticides for less than or equal to 50 h per year ($8388 \pm 1285$ IU/L) ($P < 0.05$).

The influence of smoking habits and alcohol consumption on the variation of BChE activity was studied, for all agricultural workers. Smokers had lower BChE activity (-10%) ($7180 \pm 2332$ IU/L) than nonsmokers ($7985 \pm 2209$ IU/L). However, the BChE activity was higher (+2%) in drinkers ($7620 \pm 2041$ IU/L) when compared with nondrinkers ($7446 \pm 2399$ IU/L), but no significant difference was found between these groups. All smokers (drinkers and non drinkers) had usually the lower BChE activity, and all drinkers (smokers and non smokers) had usually the highest one.

As mentioned above, the agricultural workers of the studied population were selected randomly. We had neither limited the number of pesticides used during the last period prior to our study, nor specified some kinds or names of pesticides to be used during this period. Depending on the oral questionnaire, these workers used various pesticides often in combination, some commercially available and others made by workers themselves. Results described above were performed for all workers regardless of pesticides used in last period of exposure. In order to investigate the effect of each pesticide classes (OP, CB) every one alone or in mixture, workers were clustered depending on pesticides used during last exposure. Among farmers

Figure 3. Variation of plasma cholinesterase means activity in agricultural workers according to number of hours per years.
who used OP or CB pesticides alone or in mixture, 30% reported at least one neurologic symptom. The total clusters of workers who used whatever classes of OP, CB alone or OP-CB in mixture, presented a significant decrease in BChE activity when compared with those who used other pesticide classes during last exposure \((P < 0.01)\). A significant decrease was also noted for BChE activities of workers who used OP-CB mixture compared with those who used other pesticides classes (pyrethroids, thiocarbamates...) \((P < 0.05)\), as well as when compared with those who used CB pesticides alone \((P < 0.05)\) or OP alone \((P < 0.05)\) (table 4).

Although there were no significant difference between BChE of workers who used OP pesticides and those used CB \((P = 0.4)\), only BChE activity of groups exposed to CB were significantly lower as compared to those exposed to others pesticides \((P < 0.05)\). Even, the BChE mean activity of workers using pesticides classes other than OP and CB was significantly lower compared with control group \((P < 0.05)\).

### Discussion

BChE activity of agricultural workers was significantly lower than the one of control group. Our finding is in accordance with other similar studies that evaluated the cholinesterase activity whether in plasma or in erythrocytes [17-20]. The significant decrease in BChE for these workers was an indicator of exposure to OP and/or CB pesticides. Ohayo-Mitoko et al. reported that many Kenyan agricultural workers have considerable changes in acetylcholinesterase inhibition and low acetylcholinesterase activities due to exposure to pesticides during the high exposure period [21].

Headache appears to be the most common neurological manifestation attributable to pesticides exposure, and it could probably be the consequence of chronic effects of most pesticides on the central nervous system. The same finding was reported by Parron et al. who indicated that the most frequent symptom of workers with low rate of exposure was headache [4]. This result was also specified in the study of Strong et al. [21].

It seems that school education could, even in part, be implicated in the general knowledge (warning labels, measure of handling, storage of pesticides...) and especially, in the workers’ awareness of the risks posed by pesticides usage. Illiterate workers had a BChE activity significantly lower when compared with those who had primary school education. These latter were probably more careful when handling pesticides.

Most of workers did not wear PPE and their usage was occasional for those who wear it. Cost, general lethargy and discomfort are the main reasons why such devices are not used. Therefore, we failed to find a significant difference between workers’ BChE using PPE and those no using any protective devices whatsoever. However, Cateno et al. reported that the utilization of PPE was associated with a marginal increase in plasma cholinesterase activity near the significance level [19]. This result is consistent with those of other previous studies reporting that sprayers without protective clothing showed marginal reduction in their blood cholinesterase activity, during the exposure period [22-24]. A negative correlation was noted between the BChE mean activity and ancientness of exposure. Our finding is in agreement with that of Kamal et al. [25]. However, other studies reported that there is a positive correlation between BChE activity and years of work [19, 26]. The BChE activity was significantly lower in workers whose years of work exceeded twenty as compared with those with a history of exposure less than or equal to twenty years. Besides, the BChE activity of workers, whose number of exposure hours to pesticides was between two and three hundred per year was significantly lower when compared with those exposed for less than or equal to 50 hours per year. These results can illustrate the cumulative effects of long-term exposure to these toxic compounds that could lead to neurological disorders not yet or partially occur for agricultural workers. Similar results were found by He et al., which reported that there is a high correlation between the inhibition of erythrocyte cholinesterase activity and the duration of high exposure to pesticides, in a group of farmers from two rural areas of eastern China [16].

In our study, BChE activities were significantly lower in workers whose exposure times back to sixty days or less when compared with those whose last exposure goes back to more 60 days. Hence, we suggest that the regeneration of plasma cholinesterase activity requires at least 60 days. According to Cocker et al. a regular monitoring of formulation workers with two high exposure incidents shows a
long recovery period for plasma cholinesterase activity as well as erythrocyte cholinesterase activity. This period has been suggested as being sixty days [27]. BChE was influenced by cigarette smoking and alcohol consumption. Smokers had usually the lower activity while all drinkers had usually the higher one. Therefore, we suggest that the increase of BhE activity by drinking or the inhibition of this activity by smoking can occur. Moreover, we suggest that there are interactions between cigarette smoking and alcohol consumption, i.e. alcohol consumption can modulate the effect of cigarette smoking on BChE activity, but other investigations must be performed to confirm these findings. According to the study of Naravanaeni and Jamil, smoking habit had no significant effect on plasma BChE activity [14]. Whereas, other studies concluded that tobacco consumption inhibits cholinesterase activity and hence increases the risk of toxicity by pesticides for workers occupationally exposed to these harmful compounds [25, 28]. Smoking increases the effect of pesticides most probably by increasing its inhalation [25].

The significant decrease noted for BChE activity in workers who used OP or CB pesticides alone and the no significant differences between BChE of workers who used OP pesticides and those used CB indicate the similar effect of these two kinds of pesticides. The decrease in BChE activity was more and more noted for workers who used OP-CB mixture. This result suggests that the inhibitory effect on BChE activity shown in subjects with combined OP-CB exposure was more potent than of every kind alone. According to He et al., the cholinesterase inhibition in groups with combined OP-pyrethroids exposure is mainly induced by the OP components of the pesticides mixture and the toxicity of OP insecticides is more potent than pyrethroids and other insecticides [16]. BChE mean activity of workers using pesticides classes other than OP and CB was significantly lower when compared with control groups. This finding is in accordance with that of Hernandez et al., who reported the usefulness of cholinesterase activity as biomarker of exposure in the monitoring of workers long-term exposed to pesticides other than OP [26].

Our results are in agreement with several studies, which reported that it is difficult to assess and predict the final toxic effect when individuals are exposed to many classes and mixture of pesticides, in different doses and times of exposure, [4, 18, 19, 26]. Besides, because of the interaction that could occur among the involved agents, a potentiating or antagonizing the effect, toxic manifestation could not be specific for each compound or particular class and so, the pattern of health effects expected in the case of mono-exposures could be affected. Interactions that may occur between classes of pesticides in mixture remain to be investigated, in order to predict the final hazard after long-term exposure to pesticides. Despite limits, it was possible to draw conclusions about the overall health risk of exposure to these complex compounds.

Conclusion

In this study, several neurological and neuropsychic symptoms were reported by agricultural workers, which could probably be the consequence of long-term exposure to pesticides. Ancientness of exposure equal to 10 years or more, last exposure dates back to 60 days or less, exposure 2 days or more per week, exceeding one hundred fifty hours per year and to various types of pesticides, especially organophosphorus-carbamates mixture, were implicated in a significant variation of BChE activity and can be considered as risk factors of exposure to pesticides. Hence, BChE activity is a useful indicator to monitor workers chronically exposed to pesticides. However, it should be accompanied by periodic health examination, taking into account risk factors, for an early identification of workers at increased risk. An appropriate educational program about the harmful effects of pesticides should be performed for agricultural workers to minimize the risk of incorrect pesticides use. It could be an ideal approach including recommendations on the more appropriate health and safety measures and on the importance of the use of correct personal protective equipment during pesticides preparation and application. This program must also provide sufficient information for these workers on the appropriate use of these harmful compounds.

To investigate the chronic effects of pesticides, we are studying other enzymes and biological parameters related to organ function, such as liver and kidney, which may also be affected by chronic exposure to these harmful compounds.

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References

Original article


