Pesticides Residues in Blood Serum and Their Adverse Effects on Multi- Biomarkers in Occupationally Exposed Greenhouse Workers: A Cross-Sectional Study in Hamadan City, Iran

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Abstract

The cultivation of greenhouse crops is the most severe form of crop production in Iran, and vast quantities of unreasonable pesticides are being utilized to control pests. The residual level of blood pesticides and its correlation with multi-biomarkers were determined to evaluate the adverse health consequences on greenhouse workers in the Hamdan city of Iran. Participants were 180 adult males, including 90 greenhouse workers exposed to pesticides and 90 control individuals. The residue analyses revealed that 64 out of the 90 workers had residues of dichlorvos, diazinon, and chlorpyrifos in their blood serum; and 56.25% out of these workers were exposed for >15 years period. The residues of all three pesticides were higher than the no observable adverse effect level (NOAEL) in all the exposure duration categories. Comparisons of hematological parameters showed that mean monocyte \((P < 0.001)\), lymphocyte \((P < 0.001)\) and platelet counts \((P = 0.001)\), mean platelet volume \((P = 0.002)\), and plateletcrit (PCT) \((P < 0.001)\) were significantly higher in workers than in controls, whereas hemoglobin (HB) \((P = 0.028)\), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentrations (MCHC) \((P < 0.001)\) were lower in workers than controls. These results show that exposure to different pesticides was associated with alterations in some biochemical and hematological parameters. Prevention and intervention programs can be effective in eliminating pesticide-related alterations among agricultural workers.

Introduction

Agriculture has been an important practice to sustain the life-support systems of human civilizations since ancient times. Modern agriculture has included several innovative ideas to enhance crop production such as the applications of chemical fertilizers and pesticides in farming (Srivastav 2020). Pesticides play a significant role in sustainable agriculture and public well-being; however, the safety of farmers’ exposure to pesticides is a serious concern (Ramos et al. 2010, Rani et al. 2020). In particular, greenhouses are very specific agricultural environments compared to open field farming because of their limited space, high relative humidity and high temperature. In order to increase crop yields, they need to use both high inputs of energy (heating, cooling and ventilation) and chemical inputs (fertilizers and pesticides). It is estimated 2 million tons of different types of pesticide are consumed yearly, mostly in farming in order to enhance productivity (De et al. 2014). There are about 1.3 billion farmers in the world, 80% of whom work in Asia (Rice 2000). In Iran, 17.6% of the total workforce is involved in agriculture. Across all regions studied in Iran (2012–2014), an average of about 14,000 tons of pesticides are used annually, with herbicides accounting for the large quantities (48%), followed by insecticides (33%), and fungicides (19%) (Morteza et al. 2017).

In occupational settings, an estimated 25 million workers are exposed to agrochemicals, mostly accidentally via inhaling or skin absorption. Chronic exposure to pesticides has been linked with allergies, cancer, Parkinson’s disease, diabetes, nervous system damage, birth defects, and reproductive problems, among other effects. Besides dose, other factors may also affect the toxicity of pesticides, such as exposure to pesticides at certain routes, its exposure frequency, type of pesticide, their metabolites,
accumulation, and persistence in the organism. The individual’s health condition, dehydration, and malnutrition also affect the toxic effects of pesticides. WHO (2001) classifies pesticides as category I (extremely hazardous) to category III (slightly hazardous) according to their toxic effects. Unfortunately, limited research has been conducted on blood pesticide residues and the hematological and biochemical changes in populations who are occupationally exposed to pesticides. Thus, in this study, pesticide residues in blood and their association with the hematological and serum biochemical profile among greenhouse workers who were in regular contact with pesticides were examined.

**Materials And Methods**

**Study population and data collection**

The study group was 90 male workers at vegetable greenhouses located within a 21-km radius of the town of Hamadan in western Iran. All participants had been chronically exposed (i.e., for more than 6 months) to a complex mixture of pesticides. A group of individuals not employed in farming served as the control group of 90 healthy workers with no occupational or non-occupational exposure to pesticides, who were randomly selected from urban green space workers in Hamadan city. According to self-reported work history, none of the participants in the control group had any occupational exposure to pesticides during the last 5 years. Greenhouse workers who were potentially exposed to pesticides were selected from greenhouses based on their full-time active participation in the preparation, mixing, loading and application of pesticides. The exposed workers and the controls were balanced by age between 20 and 50 years. All participants provided their informed written consent before being enrolled in the study. Because pesticides are associated with a variety of chronic disorders, individuals with neurological diseases, liver disorders, diabetes, or other chronic disorders were omitted from the target population.

A structured questionnaire was used to obtain information from the study participants. Trained investigators collected all anthropometric and behavioral data. The first section of the questionnaire concerned background characteristics including employment status, educational level, age, weight, height, and place of residence. The second section of the questionnaire contained items about work experience (years), average daily work (hours), names of pesticides used, work experience, history of exposure to pesticides and use of personal protective equipment (PPE) including overalls, boots, masks, gloves, and safety glasses.

**Sample collection**

Venous blood specimens (approx. 10 mL) were drawn from the antecubital vein of all subjects in the morning hours after a 12-hour overnight fast. Calcium EDTA for CBC analysis was added in the first tube. The blood sample in the other tube was centrifuged to extract clear serum for biochemical tests at 3000 rpm for 15 min. Samples were stored under refrigeration for transportation to the laboratory. In the laboratory, serum samples were stored at −60 °C in the deep freezer until the time of testing (Kongtip et al. 2019).
Pesticide concentration analysis

Exposure to organophosphate pesticides can be measured by determination of urinary alkylphosphates and residue levels in blood serum. So, the blood residue of pesticides was measured in both workers and controls. The pesticide residues were analyzed using gas chromatography with a flame ionization detector (GC, Agilent 7890 N, Agilent Co., Wallbronn, Germany) with an Agilent HP-5 capillary column (30 m × 0.32 mm × 0.25 μm). The consistent approach was developed by USEPA (Method 8141A) (EPA 2004) for evaluation of organophosphate compounds. Analytical standards for organophosphate pesticides were purchased from Sigma- Aldrich (St. Louis, MO, USA). All solvents used were of analytical grade and used without additional refinement. The standard pesticide stock solution was prepared with a 1 mg/mL concentration in Acetone. The dilution of the stock solution with n-hexane was used to prepare effective solutions. The data on the serum residue analysis were analyzed based on the categorization of the farmers on their years of exposure duration.

Determination of Hematological Biomarkers

Hematological parameters included count of red blood cell (RBC), white blood cell (WBC), WBC differential, hemoglobin (HB) and hematocrit (HCT) levels, mean corpuscular volume (MCV), plateletcrit (PCT), mean corpuscular hemoglobin (MCH), mean platelet volume (MPV), platelet count (PLT), MCH concentration (MCHC), red blood cell distribution width (RDW), platelet distribution width (PDW), and hemoglobin A1c (HbA1c). Cell counts were recorded with a hematology cell counter (Mindray BC-3000 autoanalyzer, Shenzhen, China).

Determination of Biochemical Biomarkers

Serum levels of glucose, triglycerides (TG), insulin, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), calcium, phosphorus, and lactate dehydrogenase (LDH) were analyzed with an auto analyzer (Mindray BS-200). Additionally, as an indicator of OP exposure, the activity of Acetylcholinesterase (AChE) with the Ellman et al method was determined (Ellman et al. 1961). The expression level of two tumor markers, i.e., total prostate-specific antigen (TPSA) and carcinoembryonic antigen (CEA), was measured in serum samples with an ELISA method.

Statistical analysis

Statistical analyses were done with STATA version 14.2 software (Stata Corp, College Station, TX, USA). Descriptive quantitative data were expressed as mean ± SD. The independent sample t-test conducted a comparison of the mean biochemical and hematological changes in exposed workers and checks. The comparison of the mean of biochemical and hematological changes in exposed workers and controls were performed by the independent sample t-test. Chi-square tests were done for categorical dependent variables. The multivariate regression was used for relationship between various pesticide concentrations in blood and changes in biomarkers in pesticide exposed group \( (P < 0.05) \).
Results

Our literature search located no studies designed to investigate multibiomarkers and serum pesticide residues in greenhouse workers. Moreover, we were unable to find earlier studies that aimed to investigate farmers’ exposure to pesticides in Iran. To the best of our knowledge, the present study is one of the first to focus on multibiomarkers in this population.

Sociodemographic and occupational characteristics

We recruited 90 greenhouse workers to participate in this study. Ninety urban green space workers were selected as the control group. The sociodemographic characteristics of the study participants are provided in Table S1. Mean age, body mass index (BMI), and body weight in the control group were 36 years, 22.17 kg/m$^2$, and 75.18 kg, respectively. More than 70% of participants in both groups were males. For the exposed group, mean age, BMI, and body weight were 36 years, 23.21 kg/m$^2$, and 70.25 kg, respectively. Overall, there were no significant differences between the two groups in terms of age, gender, BMI, body weight, blood pressure, monthly income, marital status, or smoking habits. More than half of the participants in the exposed group had received elementary school education or were illiterate. Generally, most participants in the control group had higher levels of education. The average years of pesticide use by greenhouse workers was 6.75 years, ranging from 5 to 12 years. Regarding the distances between the home and the greenhouse, most people in the exposed group (93.18%) lived close to the greenhouse (within 15 m), and only 6.82% of them lived far from the workplace. None of the subjects were alcoholic; therefore, this variable was excluded from the analysis. The systematic use of PPE was reported by 31% of greenhouse workers. On the other hand, nearly 70% of greenhouse workers reported potentially unsafe behavior in the use of PPE. Masks and gloves were the most commonly used protective items, whereas overalls and safety glasses were rarely or never used.

Pesticide residues in occupationally exposed subjects

The grouping of greenhouse workers whose blood samples were taken for pesticide residue determination is presented in Table 1. About half (51.1%) of the workers had been involved in pesticide application for over 15 years, while 7-15% had been similarly involved for variable periods ranging from 5 to 14 years. The Table also shows the distribution of the workers that had pesticide residues in their blood, on the basis of their years of involvement in pesticide application (i.e. duration of exposure). A relatively high percentage (56.25%) of the workers was in the over 15 years exposure duration period; 14.06% and 23.43% were in the 10–14 years, and the 5–9 years exposure periods, respectively. Only 6.25% belonged in the 1-4 years exposure period. In the control group, the pesticides residues were found in only three blood samples: diazinon in two samples and dichlorvos in one sample, all of which were below NAOEL values.

The distribution of the workers and the mean concentration of pesticide residues in their blood serum based on duration of exposure showed in Table 2. About 38% of the workers had dichlorvos with a mean concentration of 0.35 mg kg$^{-1}$, 26% diazinon (mean = 0.11 mg kg$^{-1}$), 36%, and chlorpyrifos (mean = 0.14
mg kg\(^{-1}\)), in their blood. All farmers in the 5-9 years exposure group had no residue of lindane in their blood. All workers in the 1-4 years exposure group had no residue of dichlorvos in their blood. The residues of all three pesticides were higher than NOAEL values in all the exposure duration categories.

**Determination of toxic effects**

**Hematological parameters**

Table 3 shows the hematological parameters in the two groups. According to bivariate analyses, significantly higher values for monocytes \((P < 0.001)\), lymphocytes \((P < 0.001)\), PLT \((P = 0.001)\), MPV \((P = 0.002)\), and PCT \((P < 0.001)\) were observed in exposed workers compared to the control group, whereas granulocyte count, HGB, MCH, and MCHC values were significantly lower. Although there were no significant differences in WBC count between groups, 11.11\% of greenhouse workers were found to have leukocytosis.

Fig. 1A shows the percent frequencies of abnormalities in blood samples from the exposed and control groups. Ten (11.11\%) of the greenhouse workers had an above-normal WBC count, while no participants in the control group had values outside the normal range for WBC. Also, 5 (5.55\%) greenhouse workers had a below-normal WBC count. Other parameters along with the percentage frequencies of anomalies are shown in Fig. 1A. Table 4 shows multivariate regression analysis of variance (ANOVA) for hematological and biochemical parameters. Regression was significant \((F \text{ calculated}>F \text{ significance})\) for all parameters except RBC and MCH. It can be indicated from the analysis that pesticide concentrations in the blood posed significant effect on almost all the hematological and biochemical parameters of blood. The effect from other factors was non-significant such as the smoking habit even the number of smokers in all the studied group was constant.

**Biochemical parameters**

The biochemical markers tested in the two groups are presented in Table 3. AChE in the exposed group and controls is showed in Fig. 2. In the exposed group, mean AChE was lower than in the control group. The results of bivariate analyses showed significantly higher levels of serum glucose and LDL-C in the exposed group \((P < 0.001)\). Mean serum phosphorus concentration in the exposed group was significantly lower than in the control group \((P < 0.001)\). No significant differences between groups were found in other biochemical parameters including total cholesterol, insulin, TG, HDL, LDH, HbA1c, and calcium levels. Table 4 shows multivariate regression analysis of variance (ANOVA) for biochemical parameters. Regression was significant \((F \text{ calculated}>F \text{ significance})\) for all biochemical parameters including blood glucose, AChE, TG, Total cholesterol, HDL-C, LDL-C, Phosphorus, TPSA, and CEA.

The data for mean serum TPSA and CEA showed no significant differences between exposed workers and controls (Table 3). In all participants, tumor marker levels were within the normal range, i.e., <4.0 ng/mL for TPSA, and <2.5 ng/mL for adult nonsmokers or <5.0 ng/mL for adult smokers for CEA.
only exception was one 50-year-old participant in the exposed group with a PSA level higher (5.2 ng/mL) than the normal range.

Exposed workers had significantly higher cholesterol levels than control participants. LDL-C was also higher in the exposed group than in the control group (Table 3). Fig. 1B illustrates the abnormalities in biochemical parameters. Eight (54.54%) of the greenhouse workers had elevated serum glucose levels, whereas in the control group no cases of above-normal glucose levels were observed. Also, in 21 workers (38%) the LDL-C level was above normal, whereas in the control group this parameter was abnormal in only 4 participants (7.27%). Other parameters along with the percentage frequencies of anomalies are shown in Fig. 1B.

Discussion

Pesticide residues in occupationally exposed individuals

Comparisons of serum pesticide residues in the exposed and control groups showed that greenhouse workers had multiple pesticides residues above the NOAEL values. Most of the greenhouse workers had OP residues consisting of diazinon, dichlorvos and chlorpyrifos residues above the NOAEL (Table 2). The frequency and quantity of detected pesticide residues in this study might be related to poor work place conditions. It was observed that all individuals did not use personal protection equipment (PPE) regularly. The use of PPE during pesticide application has not yet been generally accepted in developing countries. The lack of safety plans has also been reported in countries such as the United Arab Emirates (Gomes et al. 1999), Egypt (Stewart 1996), Brazil (Recena et al. 2006), and Ethiopia (Mekonnen &Agonafir 2002). In this study, finding a small number of pesticides in a control sample may be due to food chain exposure. Tariq et al. (2007) (Tariq et al. 2007) collected unique statistics on various pesticide-polluted foodstuffs, such as fruits, veggies and fish, collected over past 20 years, from various parts of Pakistan. These authors additionally determined variations in the data reported, relying on the different climatic prerequisites of the country, depending on the varieties and sorts of fruits and vegetables. After a range of preliminary research by means of Masud and Farhat (1985)(Masud &Farhat 1985), Cheema and Shah (1987) (Cheema 1987) and Masud and Hassan (1995) (Masud 1995), concentrations of OCs, OPs and different pesticides were very excessive.

Determination of hemotoxic effects

In the present study, significantly higher values for some hematological parameters, i.e., monocytes, lymphocytes, PLT, MPV, and PCT, along with lower granulocyte, HGB, MCH, and MCHC values, were observed in exposed workers compared to our reference group (Table 3). Our pesticide hemotoxicity findings are in line with recent reports of changes in CBC parameters linked to pesticide exposure (Fareed et al. 2013). In contrast to our study, Sudjaroen (2015) (Sudjaroen 2017) found no significant differences in hematological parameters, e.g., WBC, MCV, MCHC, RDW, HB, or RBC, between exposed workers and the control group. In a similar study by Hayat et al. (2018) (Hayat et al. 2018), hemoglobin-HB and MCH concentrations below average values were reported in the control group, in consonance with the results of
the present study. In another study by Araoud et al. (2002) (Araoud et al. 2012), lower HCT and higher MCHC values were reported in agricultural workers. The results of a metaanalysis showed that pesticide exposure was associated with hematopoietic cancers, including non-Hodgkin lymphoma (Merhi et al. 2007). In contrast, short-term exposure to pesticides has been associated with changes in hemoglobin, monocyte, and platelet levels, indicating a direct toxic impact on peripheral blood cells (Hu et al. 2015).

From regression analysis of variance ANOVA (Table 4), all hematological parameters had been considerably exclusive except RBC and MCH. WBC, HB, HCT, MCV, MCHC, PLT, MPV, and PCT were also appreciably (P < 0.05; Multivariate regression ANOVA) altered with pesticide concentrations detected in their blood. Bhalliet al. (Bhalli et al. 2006) determined that PCV and Hb concentrations were typically affected by pesticide exposure in exposed individuals, while MCH and MCHC were ineffective in the Multan-Punjab region of Pakistan. Varol et al. (Varol et al. 2014) and Edem et al. (Edem et al. 2012) confirmed a similar trend in blood parameters in response to pesticide exposure by farmers. Edem et al. (Edem et al. 2012) also stated decreased PCV of exposed individuals. Quraishi et al. (Quraishi et al. 2015) showed an increase in WBC, Hb, PCV and MCV levels of farmers exposed to pesticides compared to the control group, but the platelet count in samples exposed to pesticides decreased compared to the controls. This change may be due to exposure to several different pesticides.

**Biochemical parameters**

The biochemical markers tested in the two groups are presented in Table 3. As seen in Table 4, regression was significant (F calculated>F significance) for all biochemical parameters including blood glucose, AChE, TG, Total cholesterol, HDL-C, LDL-C, Phosphorus, TPSA, and CEA.

For some time, AChE plasma activity has been used as a biomarker of OP pesticide exposure and toxicity. WHO has recommended AuChE activity lower than 50% of the reference value as the critical value (WHO 1986). We observed that plasma AChE activity was considerably lower in exposed workers than in controls, suggesting the presence of residues in the body of workers exposed to OPs. Similar results were found in agricultural workers in Tunisia (Wafa et al. 2013) and tobacco farmers in Pakistan (Hodgson and Levi 1996). Aside from activity, AChE inhibition can also be used as an indicator of risk in evaluations of OP exposure. However, several studies have shown that cholinesterase inhibition alone is insufficient to explain all the toxicological effects reported after exposure to OP pesticides (Amitai et al. 1998).

Exposure to pesticides, particularly organochlorine and OP classes, has been found to increase the risk of cardiovascular disease. Evidence for this association was reported in work by (Zamzila et al. 2011) and supported by (Lasram et al. 2009), whose animal study confirmed that chronic exposure to OP pesticides can reduce paraoxonase activity. In addition, several studies in humans confirmed the association of exposure to endocrine-disrupting chemicals with diabetes, obesity, and associated metabolic disorders (Alonso-Magdalena et al. 2011). Electrolytes such as inorganic phosphorus, sodium and potassium were also affected by chronic or acute exposure to pesticides (Legler et al. 2015), although the mechanisms
underlying these effects and their importance have not yet been studied. According to our literature review, only two studies have investigated electrolyte (phosphorus) changes following pesticide exposure; the results of one of these studies (Hu et al. 2015) differed from the present findings, while the results of the other study were consistent with our data.

With regard to the tumor markers TPSA and CEA, serum levels were normal in all participants except for one exposed worker whose TPSA level was above normal. Some studies have assessed prostate cancer risks in relation to years of farming, types of agriculture, and pesticide application (Fleming et al. 1999). Krstev et al. (1998) studied the correlation of the risk of prostate cancer in African Americans with years of employment in agriculture and forms of farming. African American men were found to have a higher prevalence of prostate cancer than any other racial and ethnic group in the world.

To date, several studies have used CEA and other tumor markers to evaluate the prevalence of cancer in people exposed to chemicals. For example, Pluygers and Gourdin (1978) (Pluygers et al. 1993) evaluated the cancer risk related to pesticide use, and found that CEA levels were associated with a definite increase in risk among all agricultural workers.

Our findings show that long-term exposure to pesticides results in detectable pesticide residues in serum, where their cytotoxic effects contribute to blood and biochemical changes. These results underline the importance of prevention and intervention programs in eliminating pesticide-related alterations among agricultural workers. We recommend the following interventions to improve pesticide use and safety behaviors: (1) increase the use of educational programs such as documentaries and talks as well as dissemination through radio, television, and newspapers to raise awareness about good safety behaviors and the long-term consequences of pesticide use; (2) make protective safety devices more accessible and modify them according to local needs; and (3) strengthen monitoring mechanisms to reduce the illegal import of banned pesticides.

Conclusions

The findings of this study show that workers exposed to pesticides had serum residues above the NAOEL for several pesticides. Chronic exposure to different pesticides was associated with alterations in some biochemical and hematological parameters, and these alterations correlated positively with serum pesticide residue levels. To reduce exposure and potentially harmful effects, appropriate methods of pesticide application in agriculture, increased information on safe behaviors for farmers, better monitoring, and more widespread risk assessments of crops are strongly recommended.

Limitations

Although, the present study examined several health indicators, a few limitations should be noted. First, despite known associations between the negative effects of pesticides on human health and risk factors
such as dietary habits, source of drinking water, insecticide use in the home, and heavy exercise in the past, the number of potentially influential variables investigated in this study was relatively limited, and the possible effects of some variables on health status in our participants were not investigated here. Second, given the sampling variation associated with the small sample size, some findings are difficult to interpret. Therefore, further research is needed with larger sample sizes and longer follow-up periods. Nevertheless, because human and animal studies have related pesticides to an increased risk of cancer and other health problems, these findings suggest the need for careful monitoring of farmworkers’ exposure to agricultural chemicals.

**Declarations**

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**Authors’ contributions** ML: Conceptualization, Methodology, Investigation, Resources, Writing - original draft, Writing-review & editing, Supervision, Funding acquisition, Project administration; AGh: Methodology, Validation, Writing-review & editing; JP: Methodology, Statistical analysis, Writing-review & editing; FS: Conceptualization, Methodology, Investigation, Statistical analysis, Writing - original draft, Writing-review & editing; MTS: Methodology & Validation, AB: Methodology, Writing-review & editing. All authors read and approved the final manuscript.

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**Data availability** All data generated or analyzed in this study are included in this published article (and its supplementary information files).

**Ethics approval and consent to participate** The protocol and ethics of this study were approved by the Ethics Committee of Hamadan University of Medical Science (Ethic Code of IR. UMSHA.1398.558), and all the participants provided their informed written consent, agreed to provide samples, and received no payment for their participation.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Consent for publication** The manuscript does not contain data from any individual person so consent for publication is “not applicable”.

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**Tables**

**Table 1**

Number and probable exposure periods of greenhouse workers, that have pesticide residues in their blood serum

| Duration of exposure (years) | No. of Workers | Proportion (%) | No. of Workers with residue | Proportion (%) |
|-----------------------------|----------------|----------------|----------------------------|----------------|
| 1-4                         | 7              | 7.77           | 4                          | 6.25           |
| 5-9                         | 23             | 25.55          | 15                         | 23.43          |
| 10-14                       | 14             | 15.55          | 9                          | 14.06          |
| >15                         | 46             | 51.1           | 36                         | 56.25          |

**Table 2**

Mean concentration of pesticides residues in blood sample of greenhouse workers based on duration of exposure.
| Duration of exposure (years) | No. of Workers with residue | Number of workers with residue (Mg kg\(^{-1}\)) |
|-----------------------------|-----------------------------|------------------------------------------------|
|                             |                             | dichlorvos diazinon Chlorpyrifos                |
| 1-4                         | 4                           | 0 (0.0) 2 (0.10) 2 (0.15)                       |
| 5-9                         | 15                          | 5 (0.36) 6 (0.11) 4 (0.13)                      |
| 10-14                       | 9                           | 3 (0.27) 2 (0.07) 4 (0.11)                      |
| >15                         | 36                          | 16 (0.43) 7 (0.17) 13 (0.19)                     |

**Table 3**

Comparison of hematological and biochemical parameters between exposed greenhouse workers and controls (n=180).
| Parameter (unit)          | Exposed workers (n=90) | Reference (n=90) | P-value  |
|--------------------------|------------------------|------------------|----------|
|                          | Mean ± SD              | Mean ± SD        |          |
| Hematological            |                        |                  |          |
| WBC (*10^9/L)            | 6.80 ± 2.33            | 6.18 ±1.14       | 0.122    |
| Lymphocyte count (*10^9/L) | 3.52 ± 1.20          | 2.38 ± 0.45      | <0.001   |
| Granulocyte count (*10^9/L) | 2.52 ± 1.40          | 3.23 ± 0.60      | 0.003    |
| Monocyte count (*10^9/L) | 0.76 ± 0.41            | 0.51 ± 0.13      | <0.001   |
| HB (g/dL)                | 14.61 ± 1.28           | 15.26 ± 1.44     | 0.028    |
| RBC (*10^{12}/L)         | 5.44 ± 0.59            | 5.27 ± 0.49      | 0.140    |
| HCT (%)                  | 44.94 ± 3.25           | 44.96 ± 3.77     | 0.981    |
| MCV (fL)                 | 86.02 ± 6.88           | 85.34 ± 4.09     | 0.059    |
| MCH (pg)                 | 26.93 ± 2.65           | 28.88 ± 1.57     | <0.001   |
| MCHC (g/dL)              | 32.44 ± 0.90           | 33.88 ± 0.84     | <0.001   |
| PLT (*10^9/L)            | 278.18 ± 66.05         | 233.34 ± 56.04   | 0.001    |
| MPV (fL)                 | 10.18 ± 0.59           | 9.76 ± 0.67      | 0.002    |
| PDW (%)                  | 15.19 ± 0.49           | 15.10 ± 1.08     | 0.625    |
| PCT (%)                  | 0.275 ± 0.05           | 0.225 ± 0.04     | <0.001   |
| Biochemical              |                        |                  |          |
| AChE (U/L)               | 5896.371 ± 1116.47     | 6893.121 ± 1122.87 | 0.010   |
| Blood glucose (mg/dL)    | 99.30 ± 13.50          | 89.69 ± 10.33    | <0.001   |
| INS (µlU/mL)             | 4.75 ± 3.38            | 5.71 ± 4.18      | 0.346    |
| TG (mg/dL)               | 126.08 ± 74.03         | 127.41 ± 82.23   | 0.936    |
| Total cholesterol (mg/dL) | 182.65 ± 41.55        | 168.73 ± 31.01   | 0.086    |
| LDL-C (mg/dL)            | 113.92 ± 31.44         | 106.97 ± 29.12   | <0.001   |
| HDL-C (mg/dL)            | 37.24 ± 9.70           | 44.71 ± 7.90     | 0.288    |
| LDH (U/L)                | 323.02 ± 70.66         | 325.44 ± 70.66   | 0.867    |
| Parameter                  | Value 1          | Value 2          | p-value |
|----------------------------|------------------|------------------|---------|
| HbA1c (%)                  | 4.62 ± 0.38      | 4.72 ± 0.33      | 0.228   |
| Calcium (mg/dL)            | 9 ± 0.46         | 8.95 ± 0.35      | 0.588   |
| Phosphorus (mg/dL)         | 2.94 ± 0.46      | 4.72 ± 0.38      | <0.001  |
| TPSA* (ng/mL)              | 0.63 ± 0.91 (n=60) | 0.62 ± 0.65 (n=59) | 0.927   |
| CEA** (ng/mL)              | 1.94 ± 1.68      | 1.76 ± 1.40      | 0.590   |
| Smokers                    | 2.29 ± 3.25 (n=14) | 2.13 ± 2.11 (n=10) | 0.996   |
| Nonsmokers                 | 1.60 ± 0.99 (n=76) | 1.28 ± 1.21 (n=80) | 0.496   |

**Table 4**

Multivariate regression analysis for hematological and biochemical parameters in blood samples of greenhouse workers and controls.
| Parameter       | Covariates (X)             | Coefficients | Standard Error | P-value | F (Cal) | F (Sig) |
|-----------------|----------------------------|--------------|----------------|---------|---------|---------|
| **Hematological** |                            |              |                |         |         |         |
| WBC             | Intercept                  | 13.57        | 0.08           | 0.00    | 12.04   | 0.00    |
|                 | Smoking duration (years)   | -0.12        | 0.05           | 0.01    |         |         |
|                 | pesticide exposure (years) | -0.11        | 0.07           | 0.20    |         |         |
|                 | Education (years)          | 0.12         | 0.77           | 0.10    |         |         |
|                 | Age (years)                | 0.02         | 0.03           | 0.71    |         |         |
|                 | Total insecticide conc. (mg L⁻¹) | -58.13     | 11.23          | 0.00    |         |         |
| HB              | Intercept                  | 12.35        | 7.26           | 0.09    | 7.75    | 0.00    |
|                 | Smoking duration (years)   | 0.78         | 0.48           | 0.11    |         |         |
|                 | pesticide exposure (years) | 0.05         | 0.75           | 0.94    |         |         |
|                 | Education (years)          | -0.42        | 0.63           | 0.53    |         |         |
|                 | Age (years)                | 0.14         | 0.27           | 0.58    |         |         |
|                 | Total insecticide conc. (mg L⁻¹) | 442.08     | 112.04         | 0.00    |         |         |
| RBC             | Intercept                  | 6292.13      | 764.24         | 0.00    | 0.46    | 0.88    |
|                 | Smoking duration (years)   | 5.02         | 52.32          | 0.92    |         |         |
|                 | pesticide exposure (years) | -36.24       | 85.25          | 0.66    |         |         |
|                 | Education (years)          | 62.31        | 63.24          | 0.36    |         |         |
|                 | Age (years)                | -16.12       | 24.32          | 0.51    |         |         |
|                 | Total insecticide conc. (mg L⁻¹) | -6414.13    | 11600.79       | 0.57    |         |         |
| HCT             | Intercept                  | 3.00         | 0.47           | 0.00    | 1.75    | 0.14    |
|                 | Smoking duration (years)   | 0.06         | 0.03           | 0.11    |         |         |
|                 | pesticide exposure (years) | -0.05        | 0.02           | 0.27    |         |         |
|                 | Education (years)          | 0.02         | 0.05           | 0.70    |         |         |
|                 | Age (years)                | 0.01         | 0.04           | 0.30    |         |         |
|                 | Total insecticide conc. (mg L⁻¹) | -31.15      | 7.12           | 0.00    |         |         |
| Parameter | Covariates (X) | Coefficients | Standard Error | P-value | F (Cal) | F (Sig) |
|-----------|----------------|--------------|----------------|---------|---------|---------|
| MCV       | Intercept      | 293158.12    | 64324.12       | 0.00    | 1.88    | 0.14    |
|           | Smoking duration (years) | 6331.20 | 4264.31 | 0.14 |
|           | pesticide exposure (years) | 371.32 | 6933.21 | 0.97 |
|           | Education (years) | -6180.21 | 5765.14 | 0.29 |
|           | Age (years)      | 267.06      | 2157.32       | 0.91    |         |         |
|           | Total insecticide conc. (mg L⁻¹) | 1037094.01 | 977324.17 | 0.10 |
| MCH       | Intercept      | 29.03        | 16.65          | 0.01    | 0.43    | 0.75    |
|           | Smoking duration (years) | 3.87 | 1.13 | 0.65 |
|           | pesticide exposure (years) | -0.85 | 1.74 | 0.73 |
|           | Education (years) | -0.07 | 1.46 | 0.01 |
|           | Age (years)      | 0.19         | 0.55           | 0.00    |         |         |
|           | Total insecticide conc. (mg L⁻¹) | -613.07 | 254.70 | 0.48 |
| MCHC      | Intercept      | 77.00        | 3.71           | 0.69    | 0.81    | 0.47    |
|           | Smoking duration (years) | -0.16 | 0.25 | 0.67 |
|           | pesticide exposure (years) | -0.16 | 0.40 | 0.38 |
|           | Education (years) | 0.14 | 0.31 | 0.23 |
|           | Age (years)      | 0.10         | 0.14           | 0.00    |         |         |
|           | Total insecticide conc. (mg L⁻¹) | 64.25 | 56.12 | 0.27 |
| PLT       | Intercept      | 27.63        | 3.87           | 0.46    | 0.74    | 0.42    |
|           | Smoking duration (years) | -0.27 | 0.26 | 0.13 |
|           | pesticide exposure (years) | -0.26 | 0.54 | 0.00 |
|           | Education (years) | -0.16 | 0.35 | 0.00 |
|           | Age (years)      | 0.19         | 0.14           | 0.21    |         |         |
|           | Total insecticide conc. (mg L⁻¹) | 186.78 | 56.32 | 0.86 |

Note: The table represents the coefficients for different parameters along with their standard errors, p-values, and F values.
|                      | Smoking duration (years) | pesticide exposure (years) | Education (years) | Age (years) | Total insecticide conc. (mg L$^{-1}$) |
|----------------------|--------------------------|----------------------------|-------------------|-------------|--------------------------------------|
|                      | -0.14                    | 0.11                       | 0.61              |             | -7.75                                |
|                      |                         | 0.03                       | 0.17              | -0.08       |                                      |
|                      |                         | 0.06                       | 0.28              | -0.03       |                                      |
|                      |                         | 0.61                       |                   | -0.33       |                                      |

|                      | PCT                      |                            |                   |             |                                      |
|----------------------|--------------------------|----------------------------|-------------------|-------------|--------------------------------------|
| Intercept            | 12.57                    | 15.35                      | 0.13              | 0.75        | 0.50                                 |
| Smoking duration (years) | -0.19                    | 1.23                       | 0.58              |             |                                      |
| pesticide exposure (years) | -0.13                    | 1.54                       | 0.76              |             |                                      |
| Education (years)     | 0.11                     | 2.27                       | 0.14              |             |                                      |
| Age (years)           | 0.04                     | 0.23                       | 0.00              |             |                                      |
| Total insecticide conc. (mg L$^{-1}$) | -72.11                   | 278.32                     | 0.00              |             |                                      |

|                      | Biochemical               |                            |                   |             |                                      |
|----------------------|--------------------------|----------------------------|-------------------|-------------|--------------------------------------|
| AChE                 |                          |                            |                   |             |                                      |
| Intercept            | 24.23                    | 654.24                     | 0.13              | 1.87        | 0.12                                 |
| Smoking duration (years) | -0.32                    | 63.12                      | 0.11              |             |                                      |
| pesticide exposure (years) | -0.21                    | 84.32                      | 0.92              |             |                                      |
| Education (years)     | -0.12                    | 66.74                      | 0.41              |             |                                      |
| Age (years)           | 0.31                     | 27.64                      | 0.00              |             |                                      |
| Total insecticide conc. (mg L$^{-1}$) | 175.23                   | 1256.60                    | 0.01              |             |                                      |
| Blood glucose         |                          |                            |                   |             |                                      |
| Intercept            | 6.02                     | 0.07                       | 0.12              | 4.22        | 0.00                                 |
| Smoking duration (years) | -41.24                   | 0.04                       | 0.23              |             |                                      |
| pesticide exposure (years) | 56.17                    | 0.11                       | 0.87              |             |                                      |
| Education (years)     | -15.12                   | 0.23                       | 0.44              |             |                                      |
| Age (years)           | -5635.17                 | 0.14                       | 0.00              |             |                                      |
| Total insecticide conc. (mg L$^{-1}$) | 4.12                     | 15.32                      | 0.00              |             |                                      |
| TG                   |                          |                            |                   |             |                                      |
| Intercept            | 4.00                     | 0.09                       | 0.01              | 6.24        | 0.00                                 |
| Smoking duration (years) | 0.07                     | 0.78                       | 0.10              |             |                                      |
| pesticide exposure (years) | -0.14                    | 0.41                       | 0.17              |             |                                      |
| Parameter                        | Covariates (X) | Coefficients | Standard Error | P-value | F (Cal) | F (Sig) |
|---------------------------------|----------------|--------------|----------------|---------|---------|---------|
| Phosphorus                      | Intercept      | 4.00         | 8.36           | 0.41    | 6.47    | 0.00    |
|                                 | Smoking duration (years) | 0.07         | 0.56           | 0.85    |         |         |
|                                 | Pesticide exposure (years) | -0.03        | 0.68           | 0.98    |         |         |
|                                 | Education (years)   | 0.09         | 0.74           | 0.11    |         |         |
| Total cholesterol               | Intercept        | 6.11         | 4.32           | 0.54    | 10.26   | 0.00    |
|                                 | Smoking duration (years) | -27.13       | 0.31           | 0.00    |         |         |
|                                 | Pesticide exposure (years) | 68.21        | 0.64           | 0.21    |         |         |
|                                 | Education (years)   | -13.25       | 0.25           | 0.11    |         |         |
|                                 | Age (years)         | -5432.21     | 0.16           | 0.10    |         |         |
| Total insecticide conc. (mg L⁻¹)| Intercept         | 7.04         | 56.17          | 0.00    |         |         |
|                                 | Smoking duration (years) | -0.41        | 0.02           | 0.32    |         |         |
|                                 | Pesticide exposure (years) | -0.32        | 0.16           | 0.45    |         |         |
|                                 | Education (years)   | -0.17        | 0.28           | 0.68    |         |         |
|                                 | Age (years)         | 0.33         | 0.24           | 0.74    |         |         |
|                                 | Total insecticide conc. (mg L⁻¹) | 154.21     | 17.44          | 0.65    |         |         |
| LDL-C                           | Intercept          | 21.28        | 0.08           | 0.00    | 1.74    | 0.12    |
|                                 | Smoking duration (years) | -0.41        | 0.02           | 0.32    |         |         |
|                                 | Pesticide exposure (years) | -0.32        | 0.16           | 0.45    |         |         |
|                                 | Education (years)   | -0.17        | 0.28           | 0.68    |         |         |
|                                 | Age (years)         | 0.33         | 0.24           | 0.74    |         |         |
|                                 | Total insecticide conc. (mg L⁻¹) | 154.21     | 17.44          | 0.65    |         |         |
| HDL-C                           | Intercept          | 12.27        | 17.32          | 0.10    | 5.54    | 0.00    |
|                                 | Smoking duration (years) | -0.17        | 2.64           | 0.00    |         |         |
|                                 | Pesticide exposure (years) | -0.13        | 4.25           | 0.23    |         |         |
|                                 | Education (years)   | 0.18         | 1.57           | 0.75    |         |         |
|                                 | Age (years)         | 0.09         | 0.44           | 0.97    |         |         |
|                                 | Total insecticide conc. (mg L⁻¹) | -65.32      | 245.35         | 0.02    |         |         |
|         | Intercept   | TPSA          | CEA           |
|---------|-------------|---------------|---------------|
|         |             | 14.32         | 271011.12     |
|         |             | 2.54          | 0.58          |
|         |             | 0.32          | 0.14          |
|         |             | 0.83          | 0.81          |
|         |             | 0.21          | 0.45          |
| Smoking duration (years) | 0.81 | 6544.32 | 365.32 |
| pesticide exposure (years) | 0.07 | 0.07 | 0.87 |
| Education (years) | -0.63 | -6123.22 | -6123.22 |
| Age (years) | 0.24 | 0.24 | 0.24 |
| Total insecticide conc. (mg L\(^{-1}\)) | 223.04 | 1034011.07 | 223.04 |

F (Cal) = F Calculated; F (Sig) = F Significance.

**Figures**
Figure 1

Percentage of abnormalities in hematological (A) and biochemical (B) parameters in greenhouse workers and the control group.
Figure 2

Boxplots of plasma AChE in the exposed group and controls.

Supplementary Files

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