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# Effects of Pesticides on Haematology, Thyroid Stimulating Hormone (TSH) and Tri-iodothyronine (T3) Hormones of Agricultural Workers in Swat, Pakistan

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**Abstract**

Swat valley is famous for its high production of agriculture crops. These crops are under constant attacks by different pests and use of pesticides has become very crucial for the control of these pests. Pesticides are toxic substances used to kill organisms which are competing humans for food and may also pose risk to humans. The current study was performed to investigate the effects of pesticides on blood circulating hormones (Thyroid stimulating hormone [TSH], Tri-iodothyronine [T3], and haematological parameters of agriculture workers. A total of 30 blood samples were collected from male agriculture workers who have been exposed to pesticides in crop fields for a long period of time, while 30 blood samples have been taken from the group of people in meanwhile who were not dealing with pesticides. The blood samples were collected in a randomized manner. The haematological parameters and hormones levels were determined using standard methods. The mean and standard deviation values for each parameter were calculated using statistical software Minitab version 16. Paired t-test was used to find the significance of data and p-value of $\leq 0.05$ at 95% confidence interval was considered as the significant. The analysis showed a decrease in the level of TSH and an increase in the level of T3 hormone in group of people who have been exposed to pesticides in comparison to the other group of people who have not been exposed to pesticides (control). The haematological parameters did not show a uniform pattern upon exposures to pesticides. The white blood cells and platelets were the most affected parameters. This preliminary study would be helpful to determine the future risk of diseases in agriculture workers.

**Key words:** Pesticides, Haematology, Thyroid stimulating hormone, Tri-iodothyronine, Swat

## 1. Introduction

Pesticides are chemical substances used for the destruction of environmental organisms which are harmful to humans and plants (El-Magd *et al*., 2011). According to the definition of Food and Agriculture Organization (FAO), pesticide is any substance or mixture of substances intended for preventing, destroying or controlling any pest, including vectors of human or animal disease, unwanted species of plants or animals causing harm or otherwise interfering with the production, processing, storage, transport, or marketing of food, agricultural commodities, wood, wood products or animal feedstuffs, or which may be administered to animals for the control of insects, mites/spider mites or other pests in or on their bodies (Bretveld *et al*., 2006). Pests are organisms which are in competition with human for food. Pests damages and sometime destroy food stuffs intended for human consumption. The use of pesticides in agriculture is to control insects, animals, weeds, and vectors of disease. Pesticides also have some risks as it is toxic to humans and have adverse effect on environment and ecosystem.

Pakistan is an agrarian country. The economy of Pakistan largely depends upon crop yields. Pakistan economy is facing a loss of 6.5 billion annually due to the attack of pests and other pathogens on major food and cash crops. The use of pesticides to control these crops pests is continuously increasing. The farmers use pesticides in horticulture (especially for peaches) and in agriculture (vegetables) to save their time and for increase in production (Nafees *et al*., 2008). About 90% of the total pesticides used are insecticides in nature. More than 108 types of insecticides, 30 types of fungicides, 39 types of weedicides, 5 types of acaricides and 6 different types of rodenticides are being used in Pakistan (Ejaz *et al*., 2004).

The exposures to pesticides have some adverse effects, especially on human health, such as producing leukemia, non-Hodgkin's lymphoma and skin cancer. Many neurological disorders, respiratory symptoms, hormonal disturbances and reproductive abnormalities have also been associated to pesticide exposure (Souheila *et al*., 2011). Exposure to pesticides also affects endocrine glands, haematology, immune system, blood coagulation, cardiovascular system, respiration, metabolisms and several other human organs. Exposure to pesticides also has mutagenic and carcinogenic effects (Kumar, 2008; Patil *et al*., 2012). The uptake of pesticides occurs mainly through skin absorption, eyes, inhalation and ingestion. Occupational exposure to pesticides occurs during mixing of pesticides, loading of equipment’s and also during spraying and application of pesticides. The farmer’s poor knowledge about pesticides and pesticide use, less protection against pesticides exposures, minimal understanding of the health risks and inadequate safety warnings on the packages provided by the manufacturers may influence the toxicity of these pesticides (Arafa *et al*., 2013).

Majority of pesticides are not easily degradable. They remain in soil, leach to the ground water and contaminate the environment. They can also enter the body of organism, accumulate in the food chains and affect the human health. Approximately 220,000 deaths are attributed to pesticides worldwide (Sataka *et al*., 1997). Exposure to pesticides can result in both acute and chronic health problems, which may range from short term effects to chronic diseases like cancer, developmental disorders and reproductive disorders (Yassi *et al*., 2001). It has been

estimated that about three fourths of deaths occur due to pesticides in developing countries (Horrigan *et al*., 2002).

Blood transports food and oxygen to all cells of the human body and removes their waste products. It also helps to maintain body temperature, transports hormones, and fights infections. Two main constituents of blood are the blood cells or corpuscles (45%) and plasma (55%) (Dainty, 2006). The pale-yellow liquid part of the blood which holds the blood cells in suspension form is known as blood plasma. The blood plasma without clotting factors is known as blood serum. There are three main types of blood cells, the red blood cells (RBC) or erythrocytes, the white blood cells (WBC) or leukocytes and platelets (PLT) or thrombocytes (Dainty, 2006). Haematopoiesis is the process by which these cells are produced and regulated. These cells perform various functions such as transporting oxygen, repairing damaged cells, and fighting against infections. So, the body must cautiously regulate their production. For example, there are about $3.5 \times 10^{11}$ WBC and $3 \times 10^{9}$ RBC per kilogram (kg) of body weight which are regulated by growth factors, hormones and by several oxygen ($O_{2)}$ sensitive receptors (Adimy *et al.*, 2005). Haemopoiesis is the formation of blood cells which is determined by the interaction of multiple genes and it involves cytokines and some other factors of protein (Reinhold *et al.*, 2007).

Haematological analysis is very much important in many fields of research such as fish farming, toxicology and monitoring of environmental pollution. This is used as an indicator for pathological and physiological changes in fishery management and disease investigation. Several studies have been reported on the effects of pesticides on haematology of various fish species in different parts of the world (Khatun *et al.*, 2014).

Thyroid gland is one of the largest endocrine glands in the body, consisting of a pair of lobes (left and right), the size of each lobe is about the egg of a small hen and are located behind the larynx (Kirsten, 2000). Two principal hormones are produced by thyroid gland: thyroxin (T4) and tri-iodothyronine (T3). These hormones have many effects on the body but the most important function is to stimulate metabolism. The pituitary gland (a small gland at the base of the brain) regulates the secretion of these thyroid hormones produced by the thyroid gland. The pituitary gland secretes the thyrotropin or thyroid stimulating hormone (TSH) which stimulates the thyroid gland for the secretion of T4 and T3. The secretion of TSH in the pituitary gland is regulated by the hypothalamus (a discrete area of the brain, which is located above the pituitary gland). The hypothalamus secretes a hormone called thyrotropin releasing hormone (TRH). The TRH directs the pituitary gland to secrete TSH (King, 2005).

A total of 91 pesticides have been reported which can affect the endocrine glands. However, the disorders that are generated by the endocrine disruptive pesticides can be temporary or permanent. It may produce reproductive abnormalities or congenital malformations (Souheila *et al*., 2011). It has previously been shown that the farmers and allied people who have been exposed to the pesticides possess more risks for thyroid cancer (Sohail *et al*., 2004).

Peach (*Prunus persica*) is one of the most important cash crop of Northern areas of Pakistan and is grown on an area of 4543 hectares with the annual production of 48284 tones. Swat valley is one of the main growing areas of peach. Due to its high cash return it has become

one of the main sources of income for the residents of Swat valley where its area of production has increased considerably in the last two decades. One of the main problems being faced by the growers is regarding insect/pest attack and their main method of control is chemical application of insecticides. The rate of cancer is incredibly high in Northern areas of Pakistan. This rate is particularly high in farmers which are supposed to be associated with their exposures to the pesticides (Zeb *et al*., 2008).

The current study was carried out to find the effects of pesticides on TSH and T3 hormones of agriculture workers of peach growing areas in District Swat (North-west of Pakistan). The effect of pesticides on their haematology (blood parameters) was also investigated. The study will help to determine the future risks of diseases in the agriculture workers of this area.

## 2. Materials and Methods

### *2.1 Sample Collection and Experimental Design*

A total of 60 individuals were selected as a sample size from different areas of District Swat (peach growing areas), in which 30 blood samples were collected from those male agriculture workers who have been routinely exposed to pesticides for a considerably long period of time but they did not had the previous history for any infectious diseases or other environmental exposures. The individuals for the study were enrolled during February, 2014 and the blood samples were collected from approximately the same age people ( 20-40 years) who have been working in the crop fields for an average of last 5 years. Similarly 30 blood samples were collected in the meanwhile from the group of individuals (university students) within the same area ( 20-40 years) but they did not possess any current/previous history for infectious diseases and exposer to pesticides or other environmental exposures (as a control). Informed written consent was signed from each participant of the study and the study was approved by ethical committee of Swat University, Pakistan. The samples were collected in a randomised manner. Blood specimens (5 mL) were taken from each individual and blood was collected in ethylene diamine tetraacetic acid (EDTA)-coated sterile vacutainer tubes. The blood specimens were immediately shifted to Sultan clinical laboratory, Saidu Sharif, Swat and were stored in a freezer until utilized. The blood samples were processed in clinical laboratory to find out the levels of TSH and T3 hormone and haematological parameters in circulating blood.

### *2.2 Thyroid Hormones Analysis*

The quantitative measurement of TSH and T3 hormone in human blood was done using the enzyme linked fluorescent assay (ELFA) (Biomerieuxmini VIDAS®). The blood specimens were centrifuged for 2 minutes at 4000 rpm for the separation of serum. The serum (200 µL) was transferred into clean sterilized tubes and was used for each individual test. The levels of both TSH and T3 hormones were analysed by calibrated automated machine according to the manufacturer's instructions (Biomerieuxmini VIDAS®). The TSH and T3 hormone reagent strips were labelled with appropriate sample identification numbers. The sera (approximately 200 µL) were put into the sample well for each of TSH and T3 reagent strip. The TSH and T3 reagent strips, and solid phase reagents (SPRs) or probes were loaded into appropriate instrument section positions and were checked to ensure that color labels with three letter assay

on SPRs match with the reagent strips. All the assay steps were performed automatically in the calibrated machine and the assay was completed in approximately 40 minutes (Beck, 1986; Caldwell *et al*., 1985). The accuracy of the results was confirmed by regular checking of positive and negative controls. The results obtained were stored in a computer for further analysis.

### *2.3 Analysis of Haematological Parameters*

The blood parameters such as erythrocyte count, leukocyte count, lymphocyte count, platelets (PLT) count, haemoglobin (HGB) level, mean corpuscular volume (MCV), standard deviation of red cells distribution (RDW-SD), coefficient variation in red cells distribution (RDW-CV), platelet distribution width (PDW), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean platelet volume (MPV), platelet crit (PCT) and haematocrit (HCT) levels were determined according to the manufacturer's instructions (Haematology analyser BC 1800, USA). Midulocites (monocytes and eosinophil) percentage (Mid %), granulocytes percentage (Gran %) and lymphocytes percentage (Lymph %) were also calculated on the same apparatus (Emam *et al*., 2012). The results obtained on automated machine were analysed on computer.

### *2.4 Statistical Analysis*

The mean and standard deviation (SD) values were calculated for the group of samples exposed to pesticides and also for control samples group (Table 1), and a comparison was made between the two groups. Statistical software Minitab version 16 was used for analysis of the data. Paired t-test was used to find the significance of data. A p-value of $\leq 0.05$ at 95% confidence interval was considered as the significant.

## 3. Results

The current study was conducted to evaluate the effects of pesticides on farmer's health by determining their levels of blood circulating hormones (TSH and T3) and other biochemical parameters. The results obtained show that farmer's exposure to pesticides significantly changed their circulating blood TSH and T3 hormone levels in comparison to the control group of people who have not been exposed to pesticides. The level (mean value) of TSH was decreased while the level of T3 hormone was increased in group of individuals who have been exposed to pesticides in comparison to control group. Thus, pesticides had significant, but opposing effects on the levels of TSH and T3 hormone (Table 1).

The levels of some of the haematological parameters such as WBC, HGB, HCT, MCV, RDW-SD, Mid % and Gran % were increased in agriculture workers who have been exposed to pesticides when compared to control group. Remarkably, there was a dramatic increase in the levels of WBC in the pesticides exposed samples when compared to control samples group (Table 1). In contrast, the levels of some of the haematological parameters such as RDW-CV, PLT, PDW and Lymph % were decreased in agriculture workers who have been exposed to the pesticides in comparison to control group. The most dramatic decrease was noted in PLT count (Table 1). However the exposure to pesticides did not alter the levels of RBC, MCH, MCHC, MPV and PCT effectively (Table 1). The statistical analysis showed that a significant

association was found between the exposure to pesticides of agriculture workers and the count of WBC, HGB, HCT and MCV (Table 1).

## 4. Discussion

Agriculture is one of the main sources of income for the people of Swat, Pakistan. Unfortunately this industry is under constant attacks from different pests and as a result the use of pesticides has increased sufficiently. The exposure to pesticides has become a serious problem around the world (Souheila *et al*., 2011), and particularly in developing countries like Pakistan where it has a significant impact on public health (Horrigan *et al*., 2002). The exposure to pesticides has previously been reported to have a negative effect on human health and produces several disorders and complex diseases such as cancer (Kumar, 2008; Yassi *et al*., 2011; Patil *et al.*, 2012).

District Swat is located in Khyber Pakhtunkhwa (North-west province of Pakistan) and has a population size of approximately 1.7 million. This district is amongst the peach growing areas of Pakistan where the use of pesticides is on its peaks. The rate of human cancer is incredibly high in Northern areas (including Swat) of Pakistan. This may be due to the frequent exposure of agriculture workers to various pesticides (Zeb *et al*., 2008). The current study aimed to investigate the impact of pesticides on thyroid hormones and other haematological parameters in the circulating blood of agriculture workers in District Swat.

The current study investigated the effects of pesticide exposure on TSH and T3 hormone level of agriculture workers. The function of both of these hormones is to stimulate the metabolism. The disturbances in the production of these hormones can impair metabolism and can lead to several developmental disorders and diseases. It has already been shown that exposures to pesticides adversely affect human health, producing hormonal disorders (Souheila *et al*., 2011). The exposure to pesticides has also been shown to enhance the chances for thyroid cancer (Sohail *et al*., 2004). It has been suggested that pesticides may produces such abnormalities in humans by interfering with their hormones (Watt, 2002). The exposures to pesticides of agriculture workers in this study significantly affected their levels of blood circulating TSH and T3 hormones. The level of TSH was decreased dramatically while T3 hormone level was slightly increased in agriculture workers who have been exposed to pesticides when compared to control group. Thus pesticides have significant but opposing effects on the levels of TSH and T3 hormone (Table 1). However, the results of Toft *et al*. (2006) are not in agreements with our current findings. They showed a decrease in T3 hormone level and an increase in TSH level of group of people who have been exposed to pesticides. This variation in the results may be due to changes in environmental factors, differences in immunity of the selected population and differences in the use of pesticides.

The effects of pesticides on human haematology are not studied well. However, some preliminary studies have indicated that occupational exposure to pesticides can significantly affect the blood constituents (Khan *et al*., 2013). Similarly, several studies have been conducted in animals which report that pesticides alter the haematology of animals (Khatun *et al*., 2014). The part of current study regarding the effects of pesticides on haematology of agriculture workers showed an increase in the levels of WBC, HGB, HCT, MCV, RDW-SD,

Mid % and Gran % in agriculture workers who have been exposed to pesticides in comparison to control group, while the levels of RDW-CV, PLT, PDW and Lymph % were decreased in pesticide exposed samples in comparison to control samples group. However, the exposure to pesticides did not dramatically alter the levels of RBC, MCHC, MCH, MPV and PCT (Table 1). Interestingly, WBC and PLT were the most affected parameters (Table 1). Shahi and Singh (2011) notions are also in agreement with the current findings who reported an increase in the WBC count in response to the pesticides exposure. Similarly, an increase in the levels of Mid %, Gran %, MCV and WBC has also been reported by Dunstan *et al.* (1996), which again justify our findings. Khan *et al*. (2013) observations are also in consistency with the present finding who revealed a significant increase in the count of HGB and RBCs upon exposure of the individuals to pesticides. No significant differences were noted in the MPV count in response to pesticides exposure of the studied field workers. However, in controversy, Voral *et al*. (2012) and Edem *et al*. (2012) demonstrated a decrease in the quantity of MPV in response to the pesticide exposures of agriculture workers. Edem *et al*. (2012) also reported a decrease in the count of PDW, HCT and PLT in such exposed individuals. These observations are in agreement with the current findings where a similar decrease was noted in the quantity of PDW and PLT in the pesticides exposed samples. The results of Shalaby *et al*. (2013) also support the current findings where they reported low platelets count in the people who have been exposed to pesticides for long period of time. However, in controversy to the findings of Edem *et al*. (2012), a significant increase was observed in the present pesticides exposed field workers. Such variations in the results may be due to the differences in the use of pesticides in the study areas and differences in immune responses to pesticides.

The study revealed that occupational exposure of agriculture workers to pesticides significantly changes some of their haematological parameters and thyroid hormones levels. Thus, exposure to pesticides may pose a serious threat for human health. The careful application of pesticides in agriculture and other practices may be highly recommended. However, the limited number of samples analyzed and the limited study area, the results may remain tentative. It may be desirable to carry out some further studies taking large population size and large study area, and also to determine the pesticides residues in the circulating blood of the agriculture workers who have been exposed to pesticides.

## 5. Conclusions

From the current study, it has been concluded that the farmer's exposure to pesticides significantly affect their thyroid hormone level and some of the haematological parameters (particularly WBC). The alternations in the hormones level and blood constituents might negatively impact human health and may be alarming signals in these workers. This study may be helpful to determine the future risks of diseases in people who have been working with pesticides.

## 6. Recommendations

1- Protective measures such as the use of facial masks, socks and gloves must be undertaken when dealing with pesticides.

2- Government agencies must implement strict legislation over selling, purchase and on the use of pesticides.
3- The public should be properly trained and educate about the use of pesticides and its safety measures.
4- Building the capacities and capabilities of the institutions or provision of projects concerned with chemicals management.
5- Establishment of sound chemical management system involving all concerned parties from government, agricultural workers, industry, research institutes, non-governmental organizations and academia through multi-stakeholder committee.
6- Establishment of poison control centres with an adequate clinical and analytical capacities in addition to functions of treatment and prevention.
7- Other methods for pest control like integrated pest management (IPM) should be introduced in the region.

## Conflicts of Interests

The authors declare that they have no conflicts of interests.

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Table 1. Effects of pesticides on haematology, thyroid stimulating hormone (TSH) and Tri-iodothyronine (T3) hormone of agriculture workers

| S. No | Parameters | Samples exposed to pesticides Mean ± SD | Control samples Mean ± SD | P -value |
|---|---|---|---|---|
| 1 | TSH (ng/dl) | 1.13 ± 1.02 | 2.14 ± 0.60 | 0.000* |
| 2 | T3 (nmol/l) | 1.35 ± 0.38 | 1.22 ± 0.25 | 0.05* |
| 3 | WBC (10^3) | 8.90 ± 2.49 | 6.81 ± 1.38 | 0.001* |
| 4 | Lymph % | 31.46 ± 6.94 | 33.96 ± 9.21 | 0.287 |
| 5 | Mid % | 8.73 ± 5.87 | 7.46 ± 2.03 | 0.275 |
| 6 | Gran % | 59.80 ± 9.25 | 58.78 ± 10.14 | 0.731 |
| 7 | HGB (g/dl) | 15.43 ± 1.82 | 14.03 ± 1.98 | 0.014* |
| 8 | RBC (10^6ul) | 4.94 ± 0.57 | 4.74 ± 0.46 | 0.154 |
| 9 | HCT % | 43.49 ± 4.87 | 39.74 ± 5.03 | 0.004* |
| 10 | MCV (fl) | 88.29 ± 4.37 | 78.90 ± 19.23 | 0.012* |
| 11 | MCH (pg) | 31.26 ± 2.20 | 31.91 ± 12.54 | 0.784 |
| 12 | MCHC (g/dl) | 35.41 ± 0.95 | 35.16 ± 2.42 | 0.636 |
| 13 | RDW-CV% | 13.89 ± 3.30 | 16.75 ± 8.87 | 0.125 |
| 14 | RDW-SD (fl) | 41.04 ± 2.90 | 39.76 ± 8.71 | 0.314 |
| 15 | PLT (10^3) | 234.27 ± 59.40 | 242.90 ± 55.71 | 0.593 |
| 16 | MPV (fl) | 8.74 ± 0.93 | 8.32 ± 1.91 | 0.422 |
| 17 | PDW | 15.40 ± 0.40 | 16.47 ± 5.43 | 0.288 |
| 18 | PCT % | 0.20 ± 0.04 | 0.22 ± 0.09 | 0.269 |

Blood samples have been collected from the male agriculture workers (30 individuals) exposed to pesticides and also from the unexposed (30 samples) normal persons (as control). The mean values and standard deviation (± SD) values were calculated for various parameters and comparison was made between the two groups. Paired t-test was used to find the significance of data and a p-value of ≤0.05 at 95% confidence interval was considered as significant (denoted by asteric *). The parameters studied are indicated as thyroid stimulating hormone (TSH), tri-iodothyronine (T3), white blood cells (WBC), Lymphocytes percentage (Lymph %), Midulocites percentage (Mid %), Granulocytes percentage (Gran %), haemoglobin (HGB), red blood cells (RBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), coefficient variation in red cells distribution width (RDW-CV), standard deviation in red cells distribution width (RDW-SD), platelets (PLT), mean corpuscular volume (MCV), platelet distribution width (PDW) and platelet crit (PCT).

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