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*The levels of IL-6 and IL-8 cytokines in blood plasma of hop growers  
and farmers in conditions of long-term exposure to pesticides*

The influence of pesticides on immune response and their immunomodulatory effect have been noticed relatively recently. Health risk associated with pesticides application is mainly connected with their toxic effect manifested by, among other things, immunotoxicity, which leads to disorders in the functioning of the immune system.

Pesticides show modulatory effect on the immune system: suppressive and stimulatory according to the type and dose of the compound and the type of antigen stimulus. Immunity suppression is often observed at application of various types of plant protection means. Immunostimulatory effect is connected with an increase in the development of auto-immunity and allergic diseases (2, 12, 14). Immune response is generally dependent on the interaction of antigen with immunocompetent cells as lymphocytes and macrophages. In order to consider immunotoxicity of pesticides they must modulate immunological response at low concentrations. The effect of pesticides, expressed by their immunotoxicity, find reflection in the modulation of the first intrasystemic immunological barrier, manifested by the mobilization of neutrophils during the seasons of intensive chemical treatment. Immunocompetent cells, after a direct contact with chemical compounds (pesticides), may change their reactivity which can be expressed as cytokine production (10, 11, 15). IL-6 i IL-8 cytokines mediate activation of many cells as lymphocytes, neutrophils, macrophages and immune reactions.

The aim of our study was to determine IL-6 and IL-8 concentration in serum of farmers in conditions of long-term exposure to pesticides.

#### MATERIAL AND METHODS

The area of Wilków near Puławy was selected for the study. In this region, hops and fruit culture are dominant, which require intensive chemical treatment. The study was conducted at the end of June and the beginning of July, directly after the period of intensive application of pesticides on hop plantations and in orchards. The area of Witoszyn (Lublin Region) was selected as an area of minimum pesticides use.

Survey data were collected from 238 hop growers, males aged 25–70 from the area of Wilków near Puławy. Based on an environmental survey, a division was made into 3 age groups: 25–40 (60 people); 41–55 (116 people); and 56–70 (62 people). The control group were farmers, from the area of Witoszyn (Lublin Region), aged 25–70. Based on an environmental survey conducted among 53 men, the respondents were divided into 3 age groups: 25–40 (18 people); 41–55 (22 people); and 56–70 (13 people).

Blood for the determination of selected hematologic parameters was taken into syringes with EDTA. Hematologic tests were performed with the use of Coulter STKS (Couter Corporation, Miami, Florida, USA). The amount of erythrocytes, leukocytes and their sub-populations, as well as platelets, were evaluated. Blood for the determination of the level of IL-6, IL-8 was taken into

EDTA laboratory tubes, circulated within 6 h of sampling (3,000 r/min. for 10 min. at the temperature of 4°C). Plasma was stored until the determinations at the temperature of -20°C. The levels of IL-6 and IL-8 in serum was determined with the use of immunoenzymatic test ELISA (OptEIA™ Human IL-6 Set and Human IL-8 Set, PHARMINGEN, USA). Tests were performed according to the procedure stipulated by the producer.

## RESULTS

During the season of the most intensive chemical treatment on hops plantations and in the orchards, the mean values of the number of erythrocytes among the hop growers were slightly higher compared to those obtained in the control group of farmers. These values, however, were not statistically significant.

The number of leukocytes in the general population of hop growers, as well as in the age groups analysed, was slightly higher compared to the control group of farmers; however, these differences were not significant statistically. Within individual sub-populations of leukocytes, only the mean values of the number of basophils and lymphocytes were significantly higher statistically than those obtained in the control group. Mean values of the number of basophils were significantly higher statistically ( $p < 0.05$ ) in hop growers, compared to farmers. An analysis of mean values of the number of lymphocytes also showed a statistically significant increase ( $p < 0.05$ ) in the group of hop growers, compared to farmers. In all the age groups analysed, mean values of the number of basophils and lymphocytes were higher among hop growers, compared to the values obtained for farmers. These values, however, were statistically insignificant.

Mean platelets values in hop growers were significantly lower statistically ( $p < 0.01$ ) than in the control group. An analysis conducted within age groups showed statistically lower ( $p < 0.01$ ) numbers of platelets in hop growers aged 41–55 (207.7), compared to the group of farmers at the same age interval (236.5). Also in the remaining age groups (25–40 and 56–70), the number of platelets in hop growers was lower compared to farmers. These values, too, were not statistically significant. Table 1 shows the detailed data.

Table 1. Morphotic elements in blood in the population examined

Hematologic parameters examined	Hop growers		Farmers	
	N	$\bar{x} \pm SE$	N	$\bar{x} \pm SE$
Erythrocytes	238	5 370 000 $\pm$ 25 000	53	4 840 000 $\pm$ 33 000
Leukocytes	238	7860.1 $\pm$ 1800.0	53	7320.1 $\pm$ 1900.0
➤ Neutrophils	238	4198.6 $\pm$ 1391.6	53	4061.6 $\pm$ 1341.8
➤ Eosinophils	238	277.7 $\pm$ 160.6	53	289.8 $\pm$ 179.7
➤ Basophils	238	66.4 $\pm$ 6.4**	53	35.4 $\pm$ 2.6
➤ Monocytes	238	680.5 $\pm$ 90.5	53	638.0 $\pm$ 96.1
➤ Lymphocytes	238	2666.7 $\pm$ 905.1**	53	2349.8 $\pm$ 688.6
Platelets	238	209.6 $\pm$ 50.0*	53	232.3 $\pm$ 56.1

N – number of people

\*\*  $p < 0.05$  \*  $p < 0.01$  compared to the control group of farmers

Table 2. Mean values of IL-6 level (pg/ml) in plasma among hop growers and farmers

Groups examined	N	IL-6 level (pg/ml) $\bar{x} \pm SE$
Hop growers	238	4.7 $\pm$ 0.7 *
Farmers	53	2.7 $\pm$ 0.6

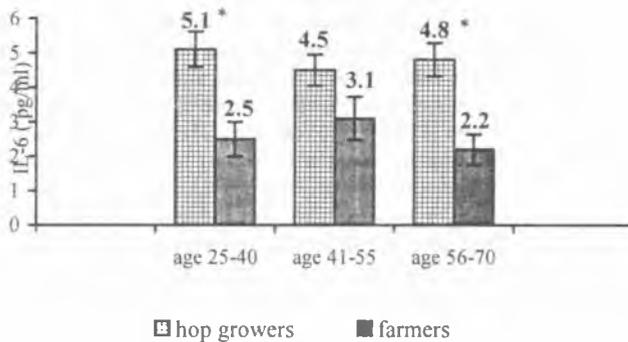
N – number of people, \*  $p < 0.001$  compared to the control group of farmers

Table 3. Mean values of IL-8 level (pg/ml) in plasma of hop growers and farmers

Groups examined	N	IL-8 level (pg/ml) $\bar{x} \pm SE$
Hop growers	238	7.1 $\pm$ 0.9 *
Farmers	53	2.4 $\pm$ 0.3

N – number of people, \*  $p < 0.001$  compared to the control group of farmers

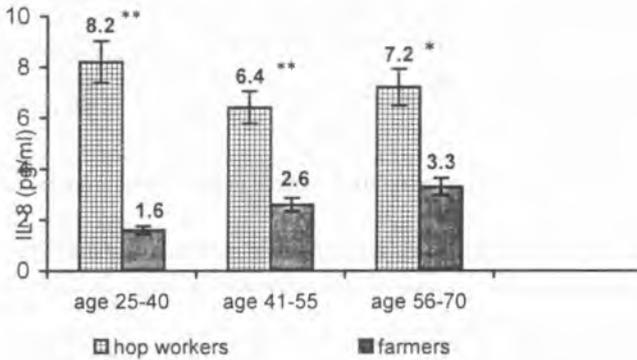
The analysis of the level of IL-6 (pg/ml) in serum showed statistically significant increased values of the level of this cytokine in hop growers, compared to the group of farmers (control group). In the group of hop growers, mean values of 4.7 pg/ml were obtained, while among farmers 2.7 pg/ml with statistical significance of  $p < 0.001$  (Tab.2). The analysis of the results according to age showed significantly higher statistically values ( $p < 0.05$ ) of the level of IL-6 in hop growers aged 25–40 and 56–70, compared to 2 equivalent age groups of farmers (Fig.1). In the age group 41–55, mean values of plasma IL-6 were higher in hop growers (4.5 pg/ml), compared to the control group (3.1 pg/ml). These values were not statistically significant.



\*  $p < 0.05$  compared to the control group of farmers

Fig. 1. Mean values of IL-6 level (pg/ml) in plasma of hop growers and farmers with consideration of the division into age groups

Comparing the levels of plasma IL-8 in hop growers and farmers, statistically significant differences were observed on the level  $p < 0.001$ . Mean values of IL-8 (pg/ml) in the group of hop growers were 7.1 pg/ml, whereas in farmers 2.4 pg/ml (Tab. 3). In hop growers aged 25–40, IL-8 level was significantly higher statistically (8.2 pg/ml), compared to the control group (1.6 pg/ml). The level of plasma IL-8 in hop growers in the age groups 41–55 and 56–70 was 6.4 pg/ml and 7.2 pg/ml respectively, while for farmers of analogous age groups – 2.6 pg/ml and 3.3 pg/ml, respectively. In these cases, the differences were statistically significant (Fig. 2).



\*  $p < 0.05$  \*\*  $p < 0.001$  compared to the control group of farmers

Fig. 2. Mean values of IL-8 level (pg/ml) in plasma of hop growers and farmers according to age groups

## DISCUSSION

Contact of the rural population with various chemical preparations applied on farms exerts an influence on the state of health of this large population. Chemical compounds applied in agriculture are absorbed through the skin, airways and alimentary tract, and induce pathological changes of a toxic, irritative and allergic character. There are reports which confirm that pesticides applied in hops growing cause pathological symptoms manifested by dermal changes, headaches and dizziness, nausea and vomiting, as well as sensation disorders (1, 3, 13).

In the own studies, no differences were noted in the absolute number of white morphological elements in hop growers, compared to the control group of farmers. The analysis of individual subpopulations of leukocytes, however, showed a significantly higher statistically number of basophils ( $p < 0.05$ ) and lymphocytes ( $p < 0.05$ ) in hop growers, compared to farmers. Basophilic granulocytes are engaged in the course of allergic reactions. The natural and most frequent mechanism activating basophils is bonding the antigen (allergen) by cellular membrane through the proper receptor – the antibody. Activation of basophils may also take place with omitting immune mechanisms, i.e. irrespectively of allergen. The activators inducing the release of basophil mediators, such as histamine are, among others: IL-8 and factors secreted by platelets and lymphocytes. The above-mentioned mediators participate in the immediate allergic response to allergens, causing, among other things, an increased permeability of the vessels leading to oedema and contraction of smooth muscles (5, 7, 8).

The studies conducted by Daniel showed that pentachlorofenol, which is a component of many pesticides, affects the function of lymphocytes and the level of cytokines produced. People who had a high level of pentachlorofenol in blood also had increased proportions of monocytes ( $p < 0.05$ ), and an increased level of IL-8 in plasma ( $p < 0.02$ ). After the exposure to pesticides containing pentachlorofenol, a weakening was noted in the stimulation of lymphocytes, and an increase of the CD4/CD8 ratio (4).

An exposure to chemical compounds may exert an influence on respiratory condition causing chronic bronchitis and chronic upper airways disorders. Keman et al. investigated the relationship between cytokines in blood and symptoms of the respiratory system in employees of a chemical plant. The production of IL-8 was significantly elevated in workers with acute bronchitis ( $p < 0.05$ ) and chronic bronchitis ( $p < 0.01$ ), compared to the staff who had no such symptoms. The people in the study also had a decreased level of cytokines IL-4, IL-5, IL-6 and IFN- $\gamma$ , compared to the healthy employees (6).

Neutrophils participating in the process of phagocytosis reach the site of initiated inflammatory response due to chemotactic factors, e. g. IL-8. IL-8 affects neutrophils causing chemotaxis, stimulation of respiratory processes, degranulation and release of lysosome enzymes. Because IL-8 is a strong phagocytosis stimulator, its growth in plasma may be directly associated with the stimulation of neutrophils for phagocytic processes. In our own studies, a clear increase was observed in the level of IL-8 in plasma among hop growers (7.1 pg/ml), compared to farmers (2.4 pg/ml).

IL-6 plays a special role in the stimulation of B cells for differentiation into cells producing antibodies. The elevated level of this cytokine in plasma, noted in our own studies, in hop growers (4.7 pg/ml, compared to farmers (2.7 pg/ml) may be associated with an intensification of the humoral response in conditions of intensive exposure to pesticides. Studies conducted by Omahony in people at age groups 20–40 (mean age 29), and over 62 (mean age 73), confirmed that both in T cells and monocytes an increase was noted in the level of pro-inflammatory cytokines, which was correlated with age. The level of IL-6 and TNF- $\alpha$  in T cells was considerably higher among the elderly. A similar, although statistically insignificant increase in TNF- $\alpha$ , IL-6, IL-1b in monocytes was observed in the same group of people (9).

Summarising we can conclude that changes noted with relation to the hematologic parameters examined, such as: platelets, basophils and lymphocytes, may be an exponent of the potential exposure to pesticides. During the season of intensive chemical plant treatment, a high level of IL-6 and IL-8 in plasma observed in hop growers may be a sensitive indicator of a complex activation of the mechanisms of humoral and cellular immune response.

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## SUMMARY

The objective of the study covered: determination of the usefulness of hematologic tests in the evaluation of exposure to pesticides and study of the levels of IL-6 and IL-8 cytokines in blood plasma of hop growers and farmers in conditions of long-term exposure to pesticides. Data were collected from 238 males, hop growers aged 25–70 from the area of Wilków near Puławy. The control group were 53 males from the area of Witoszyn (Lublin Region), aged 25–70, who were occupied mainly with land cultivation. Laboratory tests covered the determinations of selected morphological parameters and the levels of IL-6 and IL-8 in blood plasma in hop growers and farmers. A significant decrease was observed in the number of platelets in the general population of hop growers, compared to the control group. The analysis of sub-populations of leukocytes showed a statistically higher number of basophils and lymphocytes in hop growers. An increase was noted in the plasma level of IL-8 in hop growers (7.1 pg/ml) compared to farmers (2.4 pg/ml). An increase was also observed in the level of plasma IL-6 in people growing hops (4.7 pg/ml) compared to farmers (2.7 pg/ml).

Poziom cytokin IL-6, IL-8 w osoczu krwi plantatorów chmielu i rolników  
w warunkach długotrwałego narażenia na pestycydy

Założenia badań obejmowały: określenie przydatności badań hematologicznych w ocenie narażenia na pestycydy, zbadanie poziomów cytokin IL-6, IL-8 w osoczu krwi plantatorów chmielu i rolników w warunkach długotrwałego narażenia na pestycydy. Dane ankietowe zebrano od 238 mężczyzn w wieku od 25 do 70 lat z okolic Wilkowa k/Puław. Grupę kontrolną stanowili mężczyźni z okolic Witoszyna (woj. lubelskie) w wieku od 25 do 70 lat, zajmujący się głównie uprawą roli. Badania laboratoryjne obejmowały wykonanie oznaczeń wybranych parametrów morfologicznych krwi, oznaczenie w osoczu poziomów IL-6, IL-8 u plantatorów chmielu i rolników. Obserwowano znaczące obniżenie liczby płytek krwi w populacji generalnej w porównaniu z grupą kontrolną. Analiza subpopulacji leukocytów wykazała statystycznie wyższą liczbę bazofili i limfocytów u plantatorów chmielu. Wzrost poziomu IL-8 i IL-6 w osoczu zanotowano u plantatorów chmielu odpowiednio 7,1 pg/ml i 4,7 pg/ml w porównaniu z grupą kontrolną: 2,4 pg/ml i 2,7 pg/ml.