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Peripheral Blood Changes of the Japanese Quail *Coturnix coturnix japonica* Following Repeated Small Doses of Trichlorfon

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The problem of chronic intoxication due to pollution of soil, water and plants by pesticides used in agriculture has become of ever-increasing importance in recent years. Poisoning of this kind often affects a very large number of animals, causing serious economic losses. Such intoxication is also dangerous on account of the fact that it may take place accompanied by indistinct clinical symptoms, making diagnosis and treatment difficult and being capable of leading to irreversible pathological, morphological and physiological changes in various tissues, and also in the peripheral blood.

As can be seen from a review of relevant literature, studies so far made on the effect of long-lasting administration of trichlorfon on the blood picture have been carried out on mammals only (Gibel et al., 1973; Machemer, 1981).

It therefore appeared useful to examine changes in the blood morphology of quails repeatedly intoxicated with small doses of this insecticide.

MATERIAL AND METHODS

Studies were made on 108 birds at the age of 6 weeks, weighing from 100 to 130 g. The quails were given trichlorfon per os daily for 20 days, in the form of a solution in Ringer's fluid, in doses of 5 mg/kg body weight. Hematological determinations were made on the 5th, 10th, 15th and 20th day of intoxication and on the 5th, 10th, 15th and 30th day after arrest of the pesticide treatment. The control group were given physiological saline solution.

Three hours after administering the pesticide or fluid, the number of erythrocytes (RBC) and leucocytes (WBC) were determined in blood taken from the brachial vein,

using the chamber method with Natt-Herrick diluting fluid (1953), while hemoglobin level (Hb) was determined by the cyanmethemoglobin method, and hematocrit value (Ht) by the micromethod. Mean corpuscular volume (MCV) was calculated in accordance with the formula given by Wintrobe (1956). The percentage of leucocytes was defined on smears stained by Pappenheim's method. The number of erythroblasts per 1000 erythrocytes was determined on the same preparations. Osmotic resistance of erythrocytes was determined by the method given by Pawelski (1971).

The results obtained were statistically analyzed, using the Student-t test for independent data. The value $p < 0.05$ was taken as a statistically significant difference (Oktaba and Niedokos, 1980).

RESULTS AND DISCUSSION

It was found that administration of trichlorfon in doses of 5 mg/kg of body weight/24 hours significantly lowered the number of erythrocytes up to the 10th day of studies. On the fifth day the decrease in the number of erythrocytes was 20.8%, and on the 10th day 21.0%. In successive measurements, on the 15th and 20th day, an insignificantly lower value of this parameter was found, respectively by 11.0% and 7.7%.
After arrest of the pesticide treatment the number of erythrocytes exhibited only slight fluctuations in relation to the number of these blood parameters in the control animals (Table 1, fig. 1).

Hemoglobin level fell to a statistically significant degree on the fifth day of studies, by 14.4% ($p < 0.02$). In successive measurements hemoglobin content in the experimental birds was respectively 6.8%, 18.6% and 21.1% higher than in the control birds ($p < 0.001$ in all cases). After arrest of the pesticide treatment, hemoglobin level was 15.9% higher on the fifth day ($p < 0.01$) than in the control. No significant changes in this index were found in further determinations.

Hematocrit values were significantly lower only on the 5th day of administering the preparation, that is, by 21.1% ($p < 0.001$). Changes in hematocrit values did not exceed 5.5% and were not statistically significant from the 10th day to the end of the experiment.

A significant increase in erythroblast content was found on the 5th and 10th day of intoxication, of respectively 221.7% ($p < 0.05$) and 147.4% ($p < 0.01$). On successive days of studies the contents of these

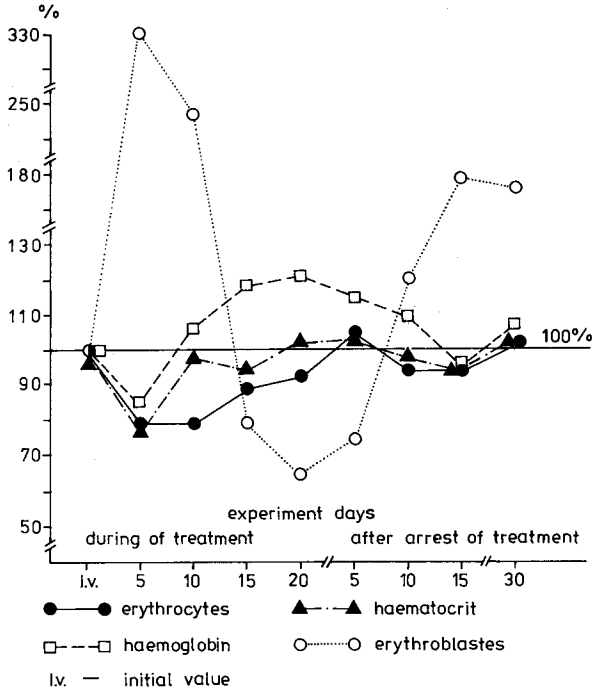

Figure 1. Changes in the erythrocyte system in Japanese quail poisoned by small doses of trichlorfon.

blood cells decreased insignificantly below the control value and rose again on the 15th and thirtieth day after arrest of the pesticide treatment, by respectively 79.4% and 75.4% (in both cases $p < 0.05$).

Mean corpuscular volume exceeded control value by 18.8% only on the 10th day of intoxication, while no significant changes in the value of this index were found in the other determinations made.

Minimum osmotic resistance of erythrocytes in the experimental animals was lower than in birds in the control group. The lowest resistance was shown by erythrocytes on the 10th day of intoxication (0.75% NaCl). After arrest of the pesticide treatment the osmotic resistance of erythrocytes remained slightly lowered for the whole period of the experiment.

A significant increase of 108.0% in the number of leucocytes was not found until the 10th day of trichlorfon administration. On successive days of intoxication the number of leucocytes increased by respectively 165.0%

Table 1. Erythrocyte system in Japanese quail poisoned by small doses of trichlorfon

Days		Erythrocytes mln/mm^3 $\bar{x} \pm$ SE	Hemoglobin g% $\bar{x} \pm$ SE	Hematocrit % $\bar{x} \pm$ SE	Erythroblasts ‰ $\bar{x} \pm$ SE	MCV μ^3 $\bar{x} \pm$ SE	Osmotic resistance of erythrocytes % NaCl
initial value		3.458 ± 0.118	12.27 ± 0.302	43.22 ± 0.709	5.89 ± 0.872	133.21 ± 11.767	0.59
during treatment	5	2.740^c ± 0.674	10.50^b ± 2.434	34.08^d ± 7.231	19.54^a ± 5.304	127.87 ± 25.181	0.64
	10	2.724^d ± 0.295	13.11 ± 1.611	42.58 ± 3.294	14.57^d ± 2.330	158.27^b ± 22.637	0.75
	15	3.076 ± 0.266	14.55^d ± 1.715	40.93 ± 4.221	4.60 ± 0.558	133.18 ± 10.103	0.71
	20	3.191 ± 0.321	14.86^d ± 1.805	44.46 ± 3.203	3.80 ± 0.745	140.28 ± 12.875	0.71
post treatment	5	3.624 ± 0.418	14.22^c ± 1.507	44.81 ± 3.693	3.71 ± 0.934	124.82 ± 13.581	0.62
	10	3.267 ± 0.258	13.38 ± 1.193	42.53 ± 3.582	7.10 ± 1.748	130.52 ± 10.365	0.61
	15	3.280 ± 0.142	11.84 ± 0.822	41.36 ± 2.837	10.57 ± 2.000	126.34 ± 10.464	0.64
	30	3.538 ± 0.108	13.18 ± 1.429	44.21 ± 4.454	10.33 ± 1.730	124.93 ± 12.167	0.61

a - $p < 0.05$; b - $p < 0.02$; c - $p < 0.01$; d - $p < 0.001$

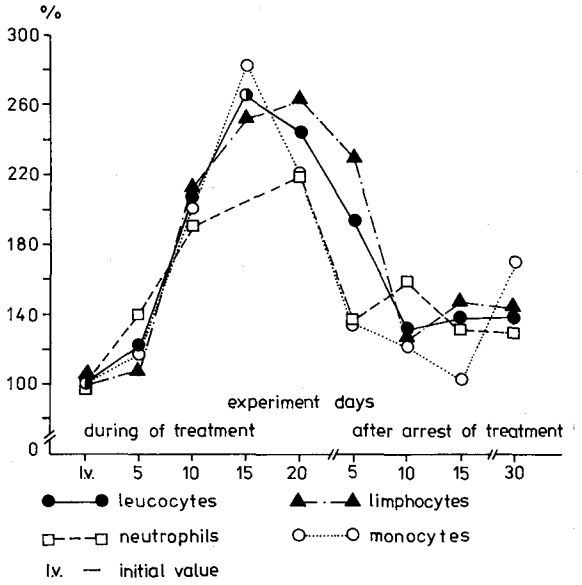

Figure 2. Changes in leucocyte system in Japanese quail poisoned by small doses of trichlorfon.

and 144.0% (in all cases $p < 0.001$). After arrest of the pesticide treatment an increased number of leucocytes was maintained, by respectively 92.9% on the 5th day ($p < 0.001$), 32.1% on the 10th day (NS), 37.2% on the 15th day ($p < 0.001$) and 38.4% on the thirtieth day of studies ($p < 0.001$)(Table 2, fig. 2).

The number of neutrophils increased significantly from the 10th day of intoxicating the birds, and was 89.9% greater during this time ($p < 0.01$). In the other two measurements the value of this parameter exceeded the value for the control group by 165.7% and 120.4% ($p < 0.01$; 0.02).

A significant increase in the number of eosinophils was also noted on the 10th, 15th and 20th day of studies, by respectively 152.5%, 246.0% and 65.5% ($p < 0.02$; 0.02; 0.05).

As regards the number of basophils it was found that there was a tendency to increase during the course of intoxication and the number was maintained on a higher level after arrest of the pesticide treatment.

Table 2. Leucocyte system in Japanese quail poisoned by small doses of trichlorfon

Days		The number of leucocytes and their forms in thoushands/mm^3					
		Leucocytes $\bar{x} \pm SE$	Neutrophils $\bar{x} \pm SE$	Eosinophils $\bar{x} \pm SE$	Basophils $\bar{x} \pm SE$	Lymphocytes $\bar{x} \pm SE$	Monocytes $\bar{x} \pm SE$
initial value		10.600 ± 0.732	3.582 ± 0.519	0.461 ± 0.083	0.012 ± 0.007	6.032 ± 0.329	0.563 ± 0.073
during treatment	5	12.860 ± 1.893	4.981 ± 0.607	0.640 ± 0.170	0.038 ± 0.012	6.570 ± 1.150	0.662 ± 0.103
	10	22.046^d ± 0.879	6.804^c ± 0.642	1.164^a ± 0.228	0.176 ± 0.072	12.774^d ± 0.618	1.130^c ± 0.142
	15	28.091^d ± 0.921	9.518^c ± 1.269	1.595^b ± 0.335	0.181 ± 0.085	15.195^d ± 1.566	1.592^c ± 0.288
	20	25.867^d ± 0.967	7.896^b ± 1.301	0.749^a ± 0.092	0.033 ± 0.020	15.833^d ± 0.861	1.236^c ± 0.139
post treatment	5	20.450^d ± 1.356	4.863 ± 0.886	0.395 ± 0.098	0.064^a ± 0.023	13.843^d ± 1.498	0.753 ± 0.122
	10	14.000 ± 2.377	5.667 ± 1.240	0.277 ± 0.070	0.060 ± 0.033	7.325 ± 1.193	0.699 ± 0.117
	15	14.543^d ± 0.363	4.679 ± 0.728	0.661 ± 0.161	0.036 ± 0.017	8.786^c ± 0.696	0.579 ± 0.080
	30	14.667^d ± 0.409	4.613 ± 0.540	0.542 ± 0.070	-	8.601^c ± 0.540	0.954^a ± 0.165

a - $p < 0.05$; b - $p < 0.02$; c - $p < 0.01$; d - $p < 0.001$

The number of lymphocytes increased significantly by 111.8% on the 10th, by 151.9% on the 15th and 162.5% on the 20th day of intoxication (in all cases $p < 0.001$). After arrest of the pesticide treatment the number of lymphocytes in different measurements continued to exceed the control value, by respectively 129.5% ($p < 0.001$), by 21.4% (NS), by 45.6% and 42.6% ($p < 0.01$; 0.01).

Significant increase in the number of monocytes was found from the 10th day of intoxicating the birds with the pesticide: the figure was respectively 100.7%, 182.8% and 119.5% (in all cases $p < 0.01$). After arrest of the pesticide treatment the number of monocytes did not differ significantly from the control value up to the 15th day, but on the 30th day the number of these blood cells was found to be 69.4% higher ($p < 0.05$).

Intoxication over a period of twenty days with trichlorfon in doses of 5 mg/kg body weight caused a large number of changes in the quails' erythrocyte system.

The decrease in the number of erythrocytes observed on the 5th day and maintained up to the 10th day would appear to be caused by the hemolyzing action of successive doses of the pesticide. The oldest erythrocytes and their early developmental forms appeared most predisposed to disintegration, as is indicated by the considerable decrease in erythroblast content. During this period the osmotic resistance of erythrocytes decreased. Increase in the number of erythrocytes and their minimal resistance was observed in successive measurements, which is most probably the result of the appearance in the peripheral blood of a considerable number of young, but already mature, erythrocytes, which are characterized by relatively great resistance to hypotonic fluids. The rapid return of hematocrit value to the control level, despite the still reduced number of erythrocytes, also points to the presence of the young forms of these blood cells, or possibly of swollen erythrocytes as the result of the action of trichlorfon.

Hemoglobin content in complete blood decreased during the initial intoxication period (5 days), then distinctly increased after successive doses of the preparation, and when these were no longer given quickly decreased to a value close to the control. This increase is probably due to the hemolyzing effect, already reffered to, of the pesticide, with simultaneous reduced capacity of the organism to metabolize it. The changes found in hemoglobin level after a single or repeated intoxication with large doses of trichlorfon suggest

that this interpretation is correct. In these cases the insecticide causes the reserve phenomenon, reducing hemoglobin level in both complete blood and in plasma (Gromysz-Kałkowska et al., 1981; Szubartowska, 1983). In acute intoxication, when the insecticide exerts a primarily stress-forming effect on the organism, it also simultaneously causes an increase in haptoglobin (Grzybowski et al., 1968; Richter, 1975) which binds the hemoglobin of the plasma (Laurel and Nyman, 1957). The small doses of trichlorfon given in the present study may be assumed not to have a stress-forming effect and the changes observed in the erythrocyte system are the result only of the toxic effect of this pesticide. This is shown by the results of studies by Ali and Shakon (1981) who obtained a decrease in the level of erythrocytes and hemoglobin with chronic intoxication of rabbits with malathion, and increased mean erythrocyte volume, with absence of changes in other hematological parameters.

Changes in the leucocyte system after repeated administration of small doses of trichlorfon were manifested in the form of leucocytosis, with neutrophilia and lymphocytosis. In turn trichlorfon administered in doses of 15 mg/kg of body weight for two weeks caused leucocytosis as the result only of great neutrophil granulocytosis (Stieglitz et al., 1974). It must be pointed out that this was a dose three times greater than that used in our studies and possibly acted as a chemical stressor, since neutrophil leucocytosis is characteristic of activation of the pituitary-adrenal axis. The increased level of catecholic amines causes disintegration of neutrophils, and the products of their disintegration stimulate granulopoeisis (Bogdanik, 1963).

Chaitov et al. (1975) found, with repeated intoxication also with small doses of the pesticide Anthio, different changes, namely leucopenia.

As stated by Ławkowicz and Krzemińska-Ławkowiczowa (1960) leucocytosis, forming the result of increase of both neutrophils and lymphocytes, occurs in cases of slight increase in adrenaline level. In view of these authors' statement it may be taken that intoxication of quails with small doses of trichlorfon leads to a slight increase in adrenaline level and in consequence to neutrophil and lymphocytic leucocytosis. After arrest of the pesticide treatment the number of leucocytes was maintained on a higher level. During this time the neutrophil content was only slightly greater, but the number of lymphocytes significantly exceeded the control value. The persistently

increased number of lymphocytes in the present experiments is confirmed in studies on chronic intoxication of rats with chlorfenvinfos (Klukowska, 1976). Intoxication for 6 months with this pesticide led to leucocytosis combined with neutropenia and lymphocytosis. The recently described changes in the leucocyte system may be due to stimulation of lymphatic tissue or even to its leucaemic neoplastic proliferation as the result of the long period of treating the animals with the pesticide. The neoplastic changes found in mice by Gibel et al. (1971) after the animals had been treated with trichlorfon, also for a period of six months, would seem to provide support for this interpretation. In chronic pesticide intoxication it is a question of the destructive effect of preparations on the membrane of lysosomes (Giermaziak, 1973; Giermaziak et al., 1975). According to the neoplastic hypothesis put forward by Allison, the enzymes liberated from these structures attack their own cells, causing changes in the chromosome genome. As a result of these processes the normal cell is transformed into a neoplastic cell (Stankiewicz, 1973).

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