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Hematological Alterations in Common Carp (*Cyprinus carpio* L.) Exposed to Herbicides: Pendimethalin and Ethofumesate Tested Separately and in Mixture*

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Herbicides are used in large amounts in agriculture and the evaluation of their toxic effects is of major concern to environmental safety. The aim of the present study was to investigate common carp hematological alterations caused by herbicide exposure. Fish were treated with pendimethalin and ethofumesate tested separately and in mixture administered to aquarium water. Peripheral blood of treated fish was collected after 1, 3 and 7 days of exposure and compared to control. The total number of erythrocytes (RBC), total number of leukocytes (WBC), hematocrit value (Hct), total hemoglobin concentration (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and leukograms were determined at once. The results indicate that herbicide exposure caused different changes in the hematological profile of the fish. In the case of exposure to individual herbicides, short-term fluctuations of various hematological indices were noted. Moreover, a significant increase in RBC and Hct after a short period of exposure (1-3 days) in fish exposed simultaneously to both tested herbicides was observed. Exposure to herbicides affected the leukocyte profile after 3 and 7 days of duration. Fluctuations of hematological parameters are a typical change in fish exposed to pesticides.

Key words: Herbicides, hematology, common carp, toxicity.

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Using pesticides to control unwanted species has become a common practice worldwide. Herbicides are often used in modern agriculture and landscape management. In Poland, they account for about 60% of all agricultural pesticide use (POLISH MINISTRY OF AGRICULTURE AND RURAL DEVELOPMENT 2014). The herbicide pendimethalin is a representative of the dinitroaniline class. It is widely used to control annual grasses and certain broadleaf weeds. Pendimethalin protects crops like wheat, corn, soybeans, potatoes, cabbage, peas, carrots and asparagus. Its primary mode of action is inhibiting the growth of roots and shoots of weeds by preventing plant cell division and elongation in susceptible species (GILLIAM *et al.* 1993). Ethofumesate belongs to the group of benzofuran herbicides. It affects several different weed species, but it has predominantly been used

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for annual bluegrass control in cool-season grasses and on bermudagrass greens, tees, and fairways overseeded with perennial ryegrass (HAGGAR & KIRKHAM 1981). Pendimethalin and ethofumesate are commonly used herbicides in Polish agriculture. They enter the aquatic environment mainly as a result of transport by air flow, leaching and runoff (DINELLI *et al.* 1996; PÄTZOLD *et al.* 2007). Pendimethalin is a water pollutant classified as moderately toxic to aquatic organisms. It can cause various physio- and histopathological changes in fish (ABD-ALGADIR *et al.* 2011). It may interfere with endocrine processes via hormone receptors, leading to feminization (NGAMNIYOM & PANYARACHUN 2011). Pendimethalin can also induce hematological abnormalities in fish, such as abnormal nuclei of erythrocytes (SHAO *et al.* 2002). To our knowledge there is no available data on the mechanisms of toxic action of ethofumesate on aquatic animals. Herbicides quickly penetrate into peripheral blood of fish via gill epithelium. The hematological profile is frequently utilized for the detection of physiopathological changes under different stress conditions such as exposure to various pesticides. The aim of this study was to determine the influence of pendimethalin and ethofumesate tested separately and in mixture on common carp hematological parameters.

Material and Methods

Animals and experimental conditions

The experiments were approved by the First Local Ethics Committee in Kraków, Poland (No. 124/2010). Individuals of common carp (*Cyprinus carpio* L.) with a mean weight of 80 ± 5.0 g were supplied by the Department of Ichthyobiology and Fisheries, University of Agriculture in Kraków (Poland). Fish were divided into 7 equinumerous experimental groups and the control group, and acclimated for two weeks in 800 l glass tanks. In each tank 36 individuals (one experimental group) were kept. Aquarium water was dechlorinated and constantly aerated. Many studies have revealed that several environmental factors (temperature, O_2 availability, water pH, photoperiod and season) can influence hematological parameters in fish (PEDRO *et al.* 2005; SOPIŃSKA 1983; SRIVASTAVA & CHOUDHARY 2010). Other factors which can affect the hematological profile include species of fish, age and nutritional state (SOPIŃSKA 1983; SVETINA *et al.* 2002; SHOEMAKER *et al.* 2003). Therefore, the physical and chemical parameters remained at the same level throughout the experiments: temperature 20.0°C; pH 7.0, dissolved oxygen 7.0 mg O_2 l^{-1}, and hardness 300 mg $CaCO_3$ l^{-1}. Fish were fed at 24 h intervals with a commercial fish feed. Experiments were carried out in accordance with the European Communities Council Directive (86/609/EEC).

Test chemicals

Two pure herbicide chemicals were used in the experiment: pendimethalin (certified analytical standard IPO 192, CAS No. 40487-42-1) and ethofumesate (certified analytical standard IPO 530, CAS No. 26255-79-6). The tested substances were obtained from the Institute of Industrial Organic Chemistry (IPO), Branch Pszczyna (Poland). Tested concentrations of pendimethalin were chosen on the basis of LC_{50} values and available environmental studies. The lower tested concentration of pendimethalin (2.5 μg l^{-1}) was detected in samples of river water in Poland (SADOWSKI & KUCHARSKI 2007) and it is also equal to 1/100 of the averaged 96 hour LC_{50} value (KAMRIN 1997; PESTICIDE PROPERTIES DATABASE 2014). The second tested pendimethalin concentration (25 μg l^{-1}) was 10 times higher in relation to the lower one. To our knowledge there is no available data on environmentally detected concentrations of ethofumesate. Thus, the tested concentrations of ethofumesate were chosen only on the basis of the LC_{50} value. The lower tested concentration of this herbicide (0.11 mg l^{-1}) was 1/100 of 96 hour LC_{50}, and the higher one (1.1 mg l^{-1}) was 1/10 of 96 hour LC_{50} (PESTICIDE PROPERTIES DATABASE 2014). Thus, the second tested concentration of ethofumesate was 10 times higher than the lower one as in the case of pendimethalin. During the exposure (duration of 7 days) the test medium was renewed twice to sustain the nominal concentration of herbicides. Each herbicide was tested in lower and higher concentrations – separately or mixed as illustrated in Table 1.

Hematological parameters

Blood samples were collected from 12 (n) individuals from each group after 1, 3, and 7 days of exposure. Before blood collection, experimental fish were anesthetized using Propiscin (KAZUŃ & SIWICKI, 2001) and next eliminated from the group. The total number of erythrocytes (RBC), the total number of white cells (WBC), hematocrit (Hct) value, and the total hemoglobin concentration (Hb) were determined at once. An aliquot of the blood was transferred to microcapillary tubes, which were centrifuged (2000 g, 10 min) using an MPW-212 centrifuge (MPW Med. Instruments). The hematocrit value was calculated as the percentage of red blood cell pellet in the total blood column. Hemoglobin contents were determined in total blood by the standard method using cyanmethemoglobin

Table 1

The concentration of tested herbicides in the experimental groups

Experimental group	Herbicide	Concentration	Number of fish
Control	none	not applicable	36
P1	pendimethalin	2.5 μg l^{-1}	36
P2	pendimethalin	25 μg l^{-1}	36
E1	ethofumesate	0.11 mg l^{-1}	36
E2	ethofumesate	1.1 mg l^{-1}	36
P1E1	pendimethalin + ethofumesate	2.5 μg l^{-1} + 0.11 mg l^{-1}	36
P2E2	pendimethalin + ethofumesate	25 μg l^{-1} + 1.1 mg $^{-1}$	36

with Drabkin's solution by a BioTek Eon spectrophotometer (BioTek® Instruments) at 540 nm (SVOBODOVÁ *et al.* 1991). The RBC count and WBC count were manually obtained using a Bürker hemocytometer with Natt-Herrick's diluting solution (Sigma Aldrich Corporation, USA). Additionally, smears were prepared in triplicate and percentages of different kinds of white blood cells (lymphocytes, monocytes, mature neutrophils, promyelocytes, myelocytes, and metamyelocytes) were determined. Next, erythrocyte indices as MCH – mean corpuscular hemoglobin, MCV – mean corpuscular volume, and MCHC – mean corpuscular hemoglobin concentrations were estimated using the following equations (BOMSKI, 1995):

$$\text{MCV } (\mu\text{m}^3) = \frac{\text{Hct } (\%) \times 10}{\text{RBC } (\text{mln } \mu\text{l}^{-1})}$$

$$\text{MCH (pg)} = \frac{\text{Hb } (\text{g dl}^{-1}) \times 10}{\text{RBC } (\text{mln } \mu\text{l}^{-1})}$$

$$\text{MCHC } (\text{g dl}^{-1}) = \frac{\text{Hb } (\text{g dl}^{-1}) \times 100}{\text{Hct } (\%)}$$

Statistical analysis

Statistical analyses were carried out using one way ANOVA, after testing for homogeneity of variance (Levene test) followed by a post-hoc Tukey's test. The level of significance was set at $\alpha = 0.05$. The data were presented as means ± SEM. Results were analyzed using the STATISTICA 10 program.

Results

Exposure to herbicides affected the hematological parameters of common carp. The observed changes in the number of erythrocytes and leukocytes, hemoglobin concentration, hematocrit level, mean corpuscular hemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration are depicted in Tables 2-4.

After one day of exposure, no alterations were noted in groups with lower concentrations of herbicides used separately (P1 and E1 groups). The higher concentration of pendimethalin (P2 group) caused a significant drop in the hemoglobin level. In experimental groups in which a mixture of pen-

Table 2

Derived hematological parameters in common carp affected by herbicide exposure after 1 day of experiment duration ($n = 12$). Superscript asterisks indicate significant differences

Experimental group	RBC (10^6 μl^{-1})		WBC (10^3 μl^{-1})		Hct (%)		Hb (g dl^{-1})		MCV (μm^3)		MCH (pg)		MCHC (g dl^{-1})	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Control	1.47	0.2	43.83	6.8	24	1.0	10.25	0.8	176.57	13.5	78.77	10.0	44.61	4.4
P1	1.57	0.2	36.50	6.9	25	0.8	8.99	0.8	192.46	29.4	70.68	16.3	36.80	3.5
P2	1.35	0.2	39.00	8.2	20	0.9	6.78*	0.3	186.32	25.5	65.62	11.4	34.78	3.0
E1	1.44	0.2	29.33	5.3	23	0.6	10.08	0.8	195.55	25.8	87.77	14.4	44.11	3.6
E2	1.37	0.1	32.50	4.0	24	0.6	11.32	0.5	195.37	20.0	90.91	8.9	46.86	2.0
P1E1	2.34*	0.1	31.67	4.9	32*	1.2	12.10	0.6	136.36	6.5	52.58	3.6	38.53	1.9
P2E2	2.56*	0.1	20.50	3.8	32*	0.9	9.85	0.6	125.53	2.8	38.72	2.5	30.86 *	1.9

Table 3

Derived hematological parameters in common carp affected by herbicide exposure after 3 days of experiment duration ($n = 12$). Superscript asterisks indicate significant differences

Experimental group	RBC ($10^6\ \mu l^{-1}$)		WBC ($10^3\ \mu l^{-1}$)		Hct (%)		Hb (g dl^{-1})		MCV (μm^3)		MCH (pg)		MCHC (g dl^{-1})	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Control	1.53	0.1	13.00	0.7	27	0.8	7.53	0.2	178.84	10.3	50.84	2.7	28.65	0.9
P1	1.48	0.1	14.00	1.2	26	1.2	8.07	0.3	183.00	12.3	56.40	3.7	31.37	1.8
P2	0.97	0.1	21.50	4.2	22*	0.8	7.51	0.2	261.30	28.8	89.75*	9.1	34.79	1.6
E1	1.57	0.2	36.17*	5.0	26	0.8	7.22	0.2	220.82	37.0	61.64	10.6	28.29	1.2
E2	1.47	0.1	22.33	4.0	26	0.6	9.68*	0.5	192.38	15.0	72.03	7.3	37.15*	1.8
P1E1	2.41*	0.1	28.00	5.2	33*	0.7	8.57	0.4	141.85	11.2	38.27	4.9	26.42	1.2
P2E2	2.20*	0.2	22.83	4.4	33*	1.3	8.98*	0.3	164.26	17.7	44.07	4.3	27.39	1.5

Table 4

Derived hematological parameters in common carp affected by herbicide exposure after 7 days of experiment duration ($n = 12$). Superscript asterisks indicate significant differences

Experimental group	RBC ($10^6\ \mu l^{-1}$)		WBC ($10^3\ \mu l^{-1}$)		Hct (%)		Hb (g dl^{-1})		MCV (μm^3)		MCH (pg)		MCHC (g dl^{-1})	
	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
Control	1.52	0.1	16.18	1.4	27	0.8	10.34	0.5	176.80	9.2	69.26	4.8	39.13	1.7
P1	1.57	0.1	16.83	1.6	28	0.4	13.93*	0.5	176.62	6.0	89.38	4.0	50.85*	2.2
P2	2.44*	0.1	14.67	1.7	33*	1.1	13.13*	0.5	134.42	6.1	54.39	2.6	40.62	1.4
E1	2.58*	0.1	21.50	2.7	33*	1.0	12.67*	0.4	127.43	6.4	49.54	2.2	39.20	1.3
E2	1.53	0.1	15.50	1.2	28	1.0	11.18	0.4	180.55	4.9	73.20	2.8	40.71	1.6
P1E1	1.56	0.1	19.17	2.4	28	1.1	11.70	0.6	186.47	11.9	77.12	4.3	42.63	3.2
P2E2	0.71*	0.1	25.67	5.8	22*	0.9	8.87	0.7	371.78*	39.7	145.75*	17.3	39.73	3.7

dimethalin and ethofumesate was administered (P1E1 and P2E2) significant increases in red blood cell count and hematocrit value were observed in comparison to the control. A significant decrease in MCHC level was noted only in the P2E2 group. The remaining indices (WBC, MCV and MCH) in all experimental groups were similar to the control group.

After three days of exposure to herbicides there were still no changes of hematological parameters in the P1 group. In the E1 group a significant increase in WBC was noted. In fish of the P2 group a significant decrease in Hct as well as a significant increase in MCH were observed. The level of hemoglobin and the MCHC value increased significantly in the E2 group. Compared to the control specimens, fish after 3 days of exposure to mixed herbicides (P1E1 and P2E2 groups) had a significantly higher erythrocyte count and hematocrit value. In the P2E2 group the level of hemoglobin was also significantly increased.

The end of exposure (after seven days) showed a significant increase of hemoglobin level and MCHC value in the P1 group. A significant increase in RBC, Hct, and hemoglobin level occurred in fish of the E1 group. The same alterations were observed in the P2 group. No changes were noticed in E2 and P1E1 groups. In the P2E2 group, RBC and Hct values significantly decreased while MCV and MCH values were significantly higher. The number of WBC was similar amongst exposed and control fish.

Leukocyte profiles of fish of control and experimental groups are shown in Table 5. Percentage values recorded for all different leukocyte types were comparable in control and experimental groups after 1 day of exposure.

Longer (three days) exposure to pendimethalin, ethofumesate and mixed herbicides resulted in a significant increase in lymphocyte percentage and a decrease in mature neutrophil percentage. A significant decrease in promyelocyte percentage was also observed in all experimental groups except for the P1 group. A significant increase in metamyelocyte percentage was noted only in groups of fish exposed to the mixture of tested herbicides (P1E1

Table 5

Leukocyte differential count in common carp affected by herbicide exposure ($n = 12$). Superscript asterisks indicate significant differences

Exposure	Experimental group	Lymphocytes (%)		Monocytes (%)		Mature neutrophil granulocytes (%)		Promyelocytes (%)		Myelocytes (%)		Metamyelocytes (%)	
		mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM	mean	SEM
1 day	Control	95.92	0.4	1.58	0.5	0.58	0.2	0.83	0.2	0.92	0.5	0.33	0.1
	P1	93.42	1.3	2.50	0.5	0.25	0.2	2.00	0.6	0.75	0.2	0.58	0.2
	P2	96.25	0.7	0.83	0.3	0.33	0.2	1.42	0.4	1.00	0.2	0.33	0.1
	E1	94.92	1.2	0.92	0.3	0.42	0.3	1.92	0.4	1.42	0.5	0.42	0.2
	E2	94.92	1.1	0.92	0.3	0.75	0.3	2.17	0.5	0.58	0.3	0.67	0.2
	P1E1	92.17	1.9	1.58	0.4	1.50	0.5	2.67	0.7	1.17	0.3	0.92	0.3
	P2E2	93.67	1.0	1.50	0.3	1.00	0.3	1.67	0.4	0.92	0.3	1.08	0.3
3 days	Control	83.50	3.5	2.17	0.6	4.33	1.0	5.58	1.3	2.67	0.7	1.75	0.4
	P1	91.17*	1.3	1.33	0.3	2.25*	0.4	3.33	0.5	2.50	0.7	1.00	0.3
	P2	91.17*	1.3	0.92	0.2	1.50*	0.3	2.92*	0.5	1.92	0.3	1.58	0.4
	E1	91.17*	1.4	1.75	0.4	1.67*	0.4	2.75*	0.5	1.67	0.3	1.08	0.3
	E2	93.00*	0.9	2.58	0.6	1.75*	0.4	1.42*	0.4	0.75	0.2	0.83	0.2
	P1E1	94.83*	1.2	1.17	0.5	1.25*	0.3	1.33*	0.4	0.92	0.3	0.33*	0.1
	P2E2	91.08*	1.2	0.58	0.2	2.25*	0.5	2.83*	0.6	3.00	0.7	0.25*	0.2
7 days	Control	91.58	1.0	0.50	0.2	0.92	0.2	4.25	0.8	2.08	0.3	0.83	0.3
	P1	24.58*	3.5	2.08	0.7	13.92*	3.5	41.00*	4.4	16.50*	2.4	1.92	0.6
	P2	71.33*	1.7	0.75	0.5	1.75	0.4	16.83*	2.5	7.58	1.4	1.50	0.3
	E1	83.50	2.7	0.92	0.4	1.67	0.7	9.17	2.2	3.50	0.5	1.25	0.4
	E2	27.17*	4.2	1.67	0.4	6.33	1.4	26.08*	2.9	25.17*	1.9	13.67*	1.8
	P1E1	90.00	1.0	1.00	0.2	0.58	0.2	5.25	0.7	2.67	0.4	0.58	0.2
	P2E2	90.75	1.2	0.67	0.2	0.42	0.2	6.42	1.0	1.42	0.3	0.33	0.2

and P2E2 groups). The percentage of monocytes and myelocytes was similar to that in the control group.

After seven days of exposure a significant decrease in lymphocyte percentage in P1, P2, and E2 groups was noted. Lymphocyte percentage in the other exposed groups (E1, P1E1, and P2E2) did not change. A significant increase in mature neutrophil percentage was noted only in the P1 group. Among non-mature neutrophils, a significant increase in promyelocyte percentage in P1, P2, and E2 groups was observed, while a significant increase in myelocyte percentage was noticed in P1 and E2 groups. A significant increase in metamyelocyte percentage was noted only in the E2 group.

Discussion

Exposure of carp to herbicides elicited different types of changes in hematological profiles. Individual hematological parameters changed differently in different experimental groups. No characteristic tendency for changes was observed for exposure to single herbicides. There were only short fluctuations in different hematological parameters (Tables 2-4). A slightly different situation occurred when fish were exposed to both herbicides (pendimethalin and ethofumesate) at the same time. In this case, a sudden and sharp increase in RBC and Hct was observed during the short exposure period (1-3 days) as well as a considerable decrease of these parameters during the last stage of exposure (7 days). Therefore it appears that the presence of greater amounts of herbicides in waters may exert a greater and more lasting effect on the hematological profile of fish. The results may also be indicative of the synergistic action of the studied herbicides. The effect of the herbicides under study was also manifested in the leukocyte profile observed in blood smears. In all the exposed groups, an upward tendency was observed after three days of exposure for lymphocyte percentage and a downward tendency for mature neutrophils and promyelocytes. At the end of exposure (after 7 days) a downward tendency was noted for lymphocyte percentage as well as an upward tendency for promyelocyte percentage (Table 5). The strongest effect of the mixture of both herbicides was manifested in the changes of two parameters – RBC and Hct.

Acute (96 h) exposure of common carp to the herbicide metribuzin at a concentration of 175.1 mg l^{-1} caused a significant decrease in Hct, Hb, MCV, and WBC values (VELISEK *et al.* 2009c). According to VELISEK *et al.* (2010) sub-chronic exposure of common carp to the herbicide terbutryn (2, 20, and 40 μg l^{-1}) caused a significant increase in RBC, while MCV and MCH values were significantly decreased. CRESTANI *et al.* (2006) revealed that the herbicide clomazone caused a significant decrease in Ht level after 96 h of exposure at a concentration of 0.05 mg l^{-1} and after 192 h at concentrations of 0.05 mg l^{-1} and 1.0 mg l^{-1} in *Rhamdia quelen*. After 192 h of recovery period, the Hct level in experimental groups (0.5 and 1.0 mg l^{-1}) returned to normal. GLUSCZAK *et al.* (2006), KREUTZ *et al.* (2011) and MODESTO and MARTINEZ (2010) studied the influence of glyphosate-based herbicides on hematological parameters of fish blood. Short-time (96 h) exposure of *Leporinus obtusidens* to the herbicide Roundup (3, 6, 10, and 20 mg l^{-1}) decreased RBC, Hct, and Hb content. According to the investigators, these observations indicated anemia of experimental fish (GLUSCZAK *et al.* 2006). KREUTZ *et al.* (2011) revealed that total erythrocyte and leukocyte counts were significantly lower in the blood of *Rhamdia quelen* from the herbicide glyphosate exposed group (96 h; 0.73 mg l^{-1}) relative to the same type of cells counted in the blood of non-exposed fish, while Hct level was not changed. On the other hand, experiments conducted by MODESTO & MARTINEZ (2010) showed a significant increase in hematocrit and number of erythrocytes and leukocytes in *Prochilodus lineatus* exposed to Roundup Transorb® after 24 and 96 h of exposure.

Hematological changes in fish may also be a result of the action of other types of pesticides. The main hematological response of fingerling European catfish (*Silurus glanis*) to an acute exposure to the insecticide diazinon (2-32 mg l^{-1}) was a significant decrease of erythrocyte, leukocyte, hemoglobin, hematocrit, MCV, MCH, and MCHC values compared to the control specimens (KÖPRÜCÜ *et al.* 2006). ADEDEJI *et al.* (2009) reported that a diazinon-exposed group (9.4 mg l^{-1} of Diazintol®) of African catfish (*Clarias gariepinus*) showed significantly lower values of erythrocyte count, leukocyte count, hemoglobin content, and hematocrit compared to the control group after 96 h of exposure. According to the authors, changes in erythrocyte profile after exposure to diazinon may be associated with disruption of hematopoiesis. The insecticide endosulfan changed hematological parameters in Asian swamp eel (*Monopterus albus*) (SIANG *et al.* 2007). Endosulfan toxicity caused a significantly lower value of erythrocyte count, leukocyte count, hemoglobin and hematocrit. Changes in basic hematological parameters led to slightly lower MCH and MCHC values of the test organisms as compared to the control. However, the MCV value of the test organisms was generally higher. The authors noticed that the degree of change was not significantly correlated to the exposure time (24-96 h) or endosulfan concentration (0.05-10 mg l^{-1}) (SIANG *et al.* 2007). Nevertheless, the results obtained by GIRÓN-PÉREZ *et al.* (2008) showed that endosulfan did not affect the hematological parameters (RBC, Hb content, Hct, MCV, MCH, and MCHC) of Nile tilapia (*Oreochromis niloticus*) for neither of two tested concentrations (4 and 7 μg l^{-1}) after 96 h of exposure. Acute (96 h) exposure of common carp to the insecticide deltamethrin (0.13 mg l^{-1} of Decis flow 2.5) led to a significant decrease in RBC, hemoglobin content and hematocrit (SVOBODOVÁ *et al.* 2003). Similar results were obtained by SETH and SAXENA (2003) in *Channa punctatus* exposed to different concentrations (0.15-0.55 mg l^{-1}) of the insecticide fenvalerate. The reduction in RBC count and Hb concentration indicated the occurrence of anemia of experimental fish. A decrease in the levels of RBC, Hb, and Hct was reported in common carp after poisoning with the insecticide cypermethrin (0.02 ppm) (DÖRÜCÜ & GIRGIN 2001), and a decrease in total leukocyte count was noticed in carp following acute poisoning with the insecticide permethrin (0.03 and 1.1 μg l^{-1}) (SOPIŃSKA & GUZ 1998). SAXENA and SETH (2002) revealed that the RBC count, Hb content and Hct value decreased abruptly after 5 days of exposure to cypermethrin and declined further with the time of exposure (up to 30 days) and increase in insecticide concentration (0.1-0.35 ppm). Significant decreases in Hb percentage and Hct level were also observed. On the other hand, the hematological parameters in jundiá (*Rhamdia quelen*) exposed to cypermethrin (0.08-0.12 ppm; from 2 to 4 days) were generally unchanged with the exception of Hb level and MCHC value. These parameters increased with the exposure time and concentration level (BORGES *et al.* 2007). *Labeo rohita* exposed to sublethal levels of two different insecticides, cypermethrin (0.16-0.80 μl l^{-1}) and carbofuran (0.06-0.30 μg l^{-1}), elicited a significant time- and dose-dependent decrease in RBC, hemoglobin content, and Hct value during the exposure period (28 days) (ADHIKARI *et al.* 2004). Acute (96 h) exposure of common carp to low concentration of the insecticide bifenthrin (57.5 μg l^{-1} of Talstar EC 10) did not affect the erythrocyte profile, Hb content and Hct level of the tested animals (VELISEK *et al.* 2009a). While in rainbow trout (*Oncorhynchus mykiss*) exposed to the same insecticide (at a concentration of 14.7 μg l^{-1} of Talstar EC 10) decreased MCV and MCH values were observed (VELISEK *et al.* 2009b).

The leukocyte profile can also be affected by different kinds of pesticides. Experiments conducted by MODESTO and MARTINEZ (2010) revealed a significant increase in the number of lymphocytes and a reduction in the number of neutrophils after 96 h of *Prochilodus lineatus* exposure to the herbicide Roundup Transorb® (5 mg l^{-1}). ADEDEJI *et al.* (2009) reported that groups of African catfish (*Clarias gariepinus*) exposed to the insecticide diazinon (9.4 mg l^{-1} of Diazintol®) showed a significant decrease in lymphocyte count and a significant increase in the count of developmental forms of neutrophils: myelocytes and metamyelocytes after 96 h of exposure. An increase in neutrophil granulocyte count was noticed in common carp following acute poisoning with the insecticide permethrin (0.03 and 1.1 μg l^{-1}) (SOPIŃSKA & GUZ 1998). The insecticide bifenthrin (57.5 μg l^{-1} of Talstar EC 10) caused significant differences in both relative and absolute monocyte counts between exposed and control groups of common carp after 96 h of exposure (VELISEK *et al*. 2009a). In rainbow trout (*Onchorhynchus mykiss*) exposed to the same insecticide (14.7 μg l^{-1} of Talstar EC 10) an increased count of band neutrophils compared to the control was observed (VELISEK *et al*. 2009b).

A number of authors revealed different hematological changes in fish caused by pesticides. The changes may be manifested by both decreases or increases during exposure time. It seems that the changes depend on fish species, type of pesticide, and intoxication level (i.e. time of exposure and pesticide concentration). Fluctuations of hematological parameters reflect the reaction of the fish organism to stress. Generally, some hematological alterations may also occur in fish exposed to low or even very low concentrations of pesticides, which indicates that these parameters are extremely sensitive to pesticide pollution. This seems crucial because pesticides in low or very low concentrations are commonly noted in surface waters. Since hematological parameters reflect the poor condition of fish more quickly than other commonly measured parameters, and since they respond quickly to changes in environmental conditions, they have been perceived as very good biomarkers of water contamination. However, the reaction of chemical stressors may be temporary due to the compensatory potential of fish. Studies on the influence of different types of pesticides on fish blood parameters should be continued to determine the long-term effects of these compounds on various fish species. In particular, it is necessary to conduct further studies to determine the effects of many pesticides acting simultaneously on fish, due to the possibility of a synergistic effect. To our knowledge, available literature on the mechanisms of toxic action of pesticides on fish is likewise insufficient. This issue also requires scientific study.

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