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Effects of Exposure to a Glyphosate-Based Herbicide on Haematological Parameters, Plasma Biochemical Indices and the Microstructure of Selected Organs of the Common Carp (*Cyprinus carpio* Linnaeus, 1758)

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Original article

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Contamination of water environments with herbicides is a common problem nowadays. The aim of the current study was to evaluate the effects of Roundup on common carp (*Cyprinus carpio*) after 1, 3 and 10 days of exposure. The used concentrations corresponded to 1 and 5 mg/l of the active ingredient (glyphosate potassium salt). The haematological analysis performed showed a decrease of the RBC count, as well as an increase of the other erythrocyte indices (Hb, MCV, MCH, MCHC). Most of these changes were dependent on the concentration and time. An increase of the WBC count and the percentage of immature neutrophils occurred, thus indicating the presence of inflammation. In the studied blood biochemical parameters, only minor and temporary changes were observed. The histopathological analysis revealed no alterations in the gills, liver and kidney. Thus, the results of the present study suggest that the haematological parameters are more sensitive and reliable markers of the common carp's exposure to Roundup than the other parameters that were tested.

Key words: pesticide, Roundup, toxicity, fish, blood, histology.

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Pesticides are synthetic or natural chemical compounds (or mixtures thereof) that are used to control harmful or undesirable organisms, especially in the cultivation of plants. Pesticides are applied to the soil and can be transported over long distances through evaporation and precipitation. They also reach water bodies by surface runoff and by percolation through the soil and into the groundwater (DE SOUZA *et al.* 2020). The occurrence of pesticides in water ecosystems, which is a serious ecological problem associated with the chemicalisation of agriculture, has been confirmed in many countries, e.g. Poland (TUZIMSKI

2008), USA (STONE *et al*. 2014), Portugal (PALMA *et al*. 2014), Greece (PAPADAKIS *et al*. 2015), Spain (MASIA *et al*. 2013; AGUILAR *et al*. 2017), Burkina Faso (LEHMANN *et al*. 2018) and Italy (MASIOL *et al*. 2018; MONTUORI *et al*. 2020). Since the contamination of aquatic ecosystems with pesticides is a common phenomenon, the toxicological effects of the exposure of fish to these substances is a subject of interest to many researchers and scientific laboratories around the world. According to ULLAH & ZORRIEHZAHRA (2015), the exposure of fish to pesticides can result in various effects, including changes in the behaviour, microstructure of various organs, haematological parameters, plasma biochemical indices, antioxidant defence system, nutrient profile, oxygen consumption, etc. According to BOJARSKI & WITESKA (2020), anaemia, stress, inflammation and immunosuppression are typical consequences of exposure to pesticides.

The most widely-used pesticides are herbicides, i.e. weed control agents. They represent about 50% of all crop-protection chemicals used throughout the world (COOPER & DOBSON 2007; DE *et al*. 2014). Glyphosate (the active ingredient in Roundup – a commonly used herbicide formulation) is a broad-spectrum herbicide used in a wide range of cropping, utility and industrial situations for the broad-spectrum control of weeds and grasses (PPDB: Pesticide Properties DataBase, 2022). It is highly soluble in water. Glyphosate is moderately toxic to humans and is a skin and eye irritant, as well as being moderately toxic to birds, most aquatic organisms, earthworms and honeybees (PPDB: Pesticide Properties DataBase, 2022). The presence of glyphosate in freshwater ecosystems is a common phenomenon in many countries. BROVINI *et al*. (2021) analysed scientific articles regarding the glyphosate concentrations in the surface freshwater systems of 21 countries and demonstrated that it may pose a moderate to high risk in 95% of the countries investigated, reaching a maximum concentration of 105 mg/l. According to CHUTIMA *et al*. (2020), glyphosate causes adverse effects on aquatic organisms and is bio-accumulated and bio-magnified through the food chain, finally reaching people as consumers.

Many investigators have demonstrated that exposure to Roundup/glyphosate results in various functional (pathophysiological) changes in fish. For example, Roundup exposure led to haematological alterations and biochemical changes in the brain, liver and muscles of the piava *Leporinus obtusidens* (Valenciennes, 1837) (GLUSCZAK *et al*. 2006), as well as haematological, haematopoietic and blood biochemical changes in the common carp (KONDERA *et al*. 2018). Furthermore, GHAFFAR *et al*. (2021) showed that rohu *Labeo rohita* (Hamilton, 1822) treated with glyphosate exhibited haematological and blood biochemical alterations, as well as oxidative stress in the gills, liver and kidneys. Similarly, a response related to antioxidant defences was observed in matrinxã *Brycon amazonicus* (Spix & Agassiz, 1829) after being treated with Roundup Transorb® (BLASCO *et al*. 2021). MODESTO & MARTINEZ (2010) demonstrated that Roundup caused oxidative stress in the liver and inhibited acetylcholinesterase activity in the muscles and brain of the streaked prochilod *Prochilodus lineatus* (Valenciennes, 1837). Oxidative stress was also observed in Roundup-exposed silver catfish *Rhamdia quelen* (Quoy & Gaimard, 1824) (DE MENEZES *et al*. 2011). The biochemical changes that occur in intoxicated fish may also be related to changes in their behaviour. FARIA *et al*. (2021) revealed that glyphosate led to neurotoxic effects and behavioural changes in zebrafish *Danio rerio* (Hamilton, 1822). It was also observed that exposure to Roundup formulations affected the behavioural patterns of *Jenynsia multidentata* (Jenyns, 1842) (SANCHEZ *et al.* 2021).

It has been shown that Roundup/glyphosate causes structural (histopathological) changes in different organs of fish belonging to various species. The study performed by AYOOLA (2008) demonstrated that glyphosate-exposed African catfish *Clarias gariepinus* (Burchell, 1822) exhibited histopathological changes in the brain, gills, liver and kidney. Moreover, histopathological lesions were observed in the gills, liver and kidney of Roundup-exposed Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) (JIRAUNGKOORSKUL *et al*. 2002). According to MESHKINI *et al*. (2019), histopathological alterations were also noted in the case of the gills, liver and kidney of Roundup-exposed rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Histopathological changes in Roundup/glyphosate-treated fish have also been observed by other researchers (e.g. DO CARMO LANGIANO & MARTINEZ 2008; RAMIREZ-DUARTE *et al*. 2008; CHANDRASEKERA & WEERATUNGA 2011; BRAZ-MOTA *et al*. 2015; MA *et al*. 2015; MOUSTAFA *et al*. 2016; GHAFFAR *et al*. 2021).

It has been demonstrated that the reproductive functions can also be disturbed. HUED *et al*. (2012) revealed that Roundup affected the male sexual activity of *J. multidentata*. According to DAVICO *et al*. (2021), Roundup WG® adversely affected the ovarian maturation in zebrafish, which can lead to reproductive toxicity, compromising the population dynamics. The study conducted by UREN WEBSTER *et al*. (2014) showed that the exposure of adult zebrafish to glyphosate reduced the egg production and increased the rate of embryo mortality, while premature hatching was also noted. Increased mortality, developmental delays and abnormalities were noted in the case of embryos from the control (unexposed) parental individuals after the treatment was applied during embryogenesis. Moreover, SOCHA *et al*. (2021) showed that Roundup affected the early life stages of the common carp. It should be added that Roundup/glyphosate has been found to result in genotoxic effects in

many fish species, e.g. the streaked prochilod (CAVALCANTE *et al*. 2008), European eel *Anguilla anguilla* (Linnaeus, 1758) (GUILHERME *et al*. 2010), peppered corydoras *Corydoras paleatus* (Jenyns, 1842) (DE CASTILHOS GHISI & CESTARI 2013) and the rohu (GHAFFAR *et al*. 2021).

The common carp is widely distributed in almost all countries of the world, and is considered to be a very important aquaculture species in many Asian and some European countries (RAHMAN 2015). Moreover, it is commonly used in toxicological research (e.g. FOROUHAR VAJARGAH *et al*. 2018; TODOROVA *et al*. 2019; KONDERA *et al*. 2020), including experiments with pesticides (e.g. MA *et al*. 2015; STOYANOVA *et al*. 2015; WANG *et al*. 2018; MA *et al*. 2019; YANCHEVA *et al*. 2019; STOYANOVA *et al*. 2020a; GEORGIEVA *et al*. 2021; SOCHA *et al*. 2021; YALSUYI *et al*. 2021; YANCHEVA *et al*. 2022a). Moreover, our experience has shown that the size of the common carp allows for the collection of sufficient blood for haematological and biochemical analyses, which may not be possible in the case of fish of a significantly smaller size. The efficacy of the common carp as a sentinel species has been proven in laboratory experiments, field studies and biomonitoring programmes (YANCHEVA *et al*. 2022b).

A haematological analysis is commonly used to evaluate the health and welfare of fish in aquaculture and scientific research (FAZIO 2019; WITESKA *et al*. 2022). It is used, among other things, to evaluate the influence of various chemical substances on fish, and articles regarding this issue were previously reviewed (BURGOS-ACEVES *et al*. 2019; BOJARSKI & WITESKA 2020). Haematological tests are commonly supplemented by an analysis of the biochemical parameters determined in the full blood, plasma or serum. The haematological and blood biochemical indices can provide extensive information about the oxygen transport capacity, immune status, level of stress, nutritional status, health status, etc. (WITESKA *et al*. 2022). A no less important tool for assessing the impact of xenobiotics on fish is a histopathological analysis – a visual assessment of the microstructure of the organs, sometimes supplemented with calculations of the histopathological indices and their statistical analysis (e.g. YANCHEVA *et al*. 2019; STOYANOVA *et al*. 2020a; STOYANOVA *et al*. 2020b; YANCHEVA *et al*. 2022a). In our opinion, the simultaneous use of a haematological analysis, blood biochemical analysis and a histopathological evaluation allows for a comprehensive assessment of the influence of toxic agents on the fish organism. Thus, the aim of the current study was to assess the influence of Roundup (a commonly used herbicide formulation) on the haematological parameters, blood biochemical indices and microstructure of vital organs (gills, liver and kidney) of the common carp after 1, 3 and 10 days of exposure.

Material and Methods

Experiment design

The study was conducted after obtaining the approval of the Local Ethics Committee for Animal Testing at the Medical University of Silesia in Katowice (Resolution No. 28/2021 of 19.05.2021). Clinically healthy and sexually mature common carp, which had always been kept under laboratory conditions, were used: weight, 71.27 ± 7.98 g (mean ± SD); length, 16.95 ± 0.84 cm (mean ± SD). The experiment was performed at the Institute of Ichthyobiology and Aquaculture of the Polish Academy of Sciences in Gołysz (Zaborze, Poland). The fish were placed in nine tanks, with 11 individuals per tank (a total of 99 fish were used in the experiment). Each tank had a volume of 300 litres and was filled with 200 litres of water. The acclimation lasted for 14 days. After this period, three equinumerous groups were established; each group consisted of 3 tanks with 11 fish per tank. The control fish (C group) were kept in water without the addition of the herbicide. The fish in group R1 were exposed to the commercial herbicide Roundup® 360 Plus (Monsanto; USA) at a lower tested concentration, corresponding to 1 mg/l of the active ingredient (glyphosate in the form of potassium salt); while the fish in group R2 were exposed to the same herbicide formulation at a higher tested concentration, corresponding to 5 mg/l of the same chemical. The concentrations of glyphosate used in the experiment were based on the study performed by KONDERA *et al*. (2018), who exposed common carp to a herbicide containing glyphosate isopropylammonium salt as an active compound for 7 days. The tested concentrations corresponded to 0.1, 0.5 and 5.0 mg/l of the active ingredient. According to the authors, the applied concentrations did not lead to disease symptoms or mortalities. Due to the fact that no analytical measurements of the actual concentrations were carried out in the present study, the concentrations provided above are nominal. The exposure lasted for 1, 3 or 10 days. After the exposure (at each sampling time), 11 fish from each group (C, R1 and R2) were used for the collection of biological material (blood was collected from 11 individuals, while the gills, liver and kidney were collected from 5 animals). The physicochemical parameters of the water (Table 1) were measured every two days during both the acclimation and exposure. The temperature and oxygen concentration were measured using an Oxi 3310 oximeter (WTW, Poland), while the pH was determined using a pH meter produced by Mettler Toledo (Switzerland). Colorimetric kits produced by Zoolek (Poland) were used to test the ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations, as well as the general hardness (GH) and carbonate hardness (KH). During the exposure, the water and the solution of the applied herbicide were changed twice a day (every

Table 1

Water parameters (mean ± SD) in the control and experimental groups

Parameter / group	Control	R1 group	R2 group
Temp. [°C]	21.86 ± 0.29	21.88 ± 0.29	21.85 ± 0.30
O_2 [mg/l]	6.62 ± 0.57	6.84 ± 0.31	6.80 ± 0.36
pH	7.51 ± 0.14	7.51 ± 0.16	7.45 ± 0.17
NH_3 [mg/l]	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
NO_2^- [mg/l]	0.07 ± 0.07	0.08 ± 0.07	0.06 ± 0.04
NO_3^- [mg/l]	14.83 ± 5.09	13.62 ± 5.16	14.29 ± 5.56
GH [°GH]	6.03 ± 0.19	6.14 ± 0.35	6.39 ± 0.57
KH [°KH]	3.86 ± 0.69	3.93 ± 0.65	4.00 ± 0.77

twelve hours), in order to keep the concentration of the herbicide as constant as possible and to remove nitrogenous metabolites excreted by the fish. During the acclimation, the water was exchanged in an analogous manner to remove nitrogenous metabolites and to accustom the fish to this procedure. The photoperiod was 14:10 (light to dark). The fish were fed daily with Aller Silver 3 mm (Aller Aqua Polska, Poland) feed at a dose of 0.5% of the body mass. No feed was given only on the day that they were subjected to euthanasia.

Biological material collection

After the exposure (1, 3 or 10 days), sampling of the biological material for the haematological, biochemical and histological analyses was performed. Blood was collected from the caudal vein (*vena caudalis*) with a needle and syringe into heparinised (Heparinum WZF, Polfa, Poland) Eppendorf tubes without the use of anaesthetic, as it is known that anaesthetics can affect the haematological parameters (BISHKOUL *et al*. 2015; WITESKA *et al*. 2017) and plasma biochemical indices (VELISEK *et al*. 2009; VELISEK *et al*. 2011; LEPIC *et al*. 2014). Some of the collected blood was subjected to the haematological analysis; while the rest of the blood was centrifuged (3000 g, 20 min) using a Mikro 200R centrifuge (Hettich, Germany) to obtain plasma for a determination of the biochemical indices. The plasma was stored frozen (-80°C) until the biochemical analyses were performed. After the blood sampling, the fish were euthanised by MS 222 (Merck; USA) overdosing (500 mg/l). After the euthanasia, the gills, liver and trunk kidney were collected for the histological analysis.

Haematological analysis

All haematological determinations were conducted for n = 11. To determine the red blood cell (RBC) count and the white blood cell (WBC) count, the blood was diluted 100 times with Hayem's solution, provided by Chempur (Poland). The numbers of RBCs and WBCs were then counted with a Bürker haemocytometer and a standard optical microscope, using a 100x magnification for the erythrocyte analysis and 200x for the leukocyte counting. The erythrocytes were counted in an area of 0.2 mm^2, while the leukocytes were counted in an area of 4 mm^2. Then, the number of blood cells per microlitre was calculated using standard formulas (BOMSKI 1983). For the haematocrit (Ht) evaluation, capillary tubes containing the blood were centrifuged for 5 min using a microhaematocrit centrifuge type 346 (Unipan, Poland), and the percentage of the erythrocyte layer was then measured with a standard Ht reader. For the assessment of the haemoglobin (Hb) concentration, the blood was mixed at a 1:250 ratio with Drabkin's reagent (Chempur, Poland). Next, the absorbance was read at a 540 nm wavelength using a UV-1601PC UV-visible spectrophotometer (Shimadzu, Japan). Standard formulas (BOMSKI 1983) were used to calculate the derived red blood cell parameters: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and the mean corpuscular haemoglobin concentration (MCHC). Blood smears were also made and stained with May-Grünwald's and Giemsa's solutions (Chempur, Poland) for a determination of the differential leukocyte count using a standard optical microscope with a 1000x magnification. Each time, 100 white blood cells were inspected. Each haematological parameter was determined by the same person. The samples subjected to the haematological analysis were code-labelled and tested 'blind'.

Biochemical analysis

All the biochemical determinations were performed for n = 11 (the same as in the case of haematological analysis). Blood plasma was used to measure the concentrations of total protein (TP), glucose (Glu), triglycerides (Tg), cholesterol (Chol) and alanine aminotransferase (ALT) activity. All the biochemical

parameters tested were determined using BioSystems assays, according to the manufacturer's instructions (BioSystems S.A., Barcelona, Spain); the only modification to the procedure was performing ALT activity calculations from two absorbance measurements instead of four absorbance measurements. For the determination of the TP, Glu, Tg and Chol concentrations, an EPOCH microplate reader (BioTek Instruments, USA) was used. The measurement of the ALT activity was conducted using a V-730 UV-visible spectrophotometer (Jasco). The samples subjected to the biochemical analysis were code-labelled and examined 'blind'.

Histological analysis

The organs were taken from 5 randomly selected fish from each group at each sampling time. The dissected gills, livers and trunk kidneys were fixed in 4% buffered formaldehyde (Chempur, Poland) for 10-14 days. The fixative was then rinsed from the tissues by immersing them in 75% ethanol changed multiple times for several days. The tissues were dehydrated in a graded series of ethanol (70%, 96% and 100%), cleared with toluene and embedded in Paraplast Regular (Sigma, St. Louis, MO, USA). Transverse sections of 6 µm were made on a Zeiss Hyrax M55 microtome and affixed to the glass slides. Selected slides were treated with haematoxylin solution according to Delafield (Chempur, Poland; time of staining – 10 minutes), and eosin solution (time of staining – 6 minutes) (H+E) for a general cytology. After treatment, the slides were enclosed in a Thermo Scientific Shandon Consul Mount and observed using a light microscope (Nikon Eclipse E600). The slides subjected to the histopathological analysis were code-labelled and were examined 'blind'. Images were then acquired and processed using a Nikon DS-Fi1c camera and NIS-Elements F software.

To assess histological changes in the fish semi-quantitatively, a protocol proposed by BERNET *et al*. (1999) was used. For each organ that was investigated, the pathological changes were classified into five reaction patterns: circulatory disturbances (C), regressive changes (R), progressive changes (P), inflammation (I) and tumour (T). Next, the following indices were calculated: I_{GC}, I_{GR}, I_{GP}, I_{GI}, I_{GT} (for the gills – G), I_{LC}, I_{LR}, I_{LP}, I_{LI}, I_{LT} (for the liver – L), and I_{KC}, I_{KR}, I_{KP}, I_{KI}, I_{KT} (for the kidney – K). Organ indices (I_G, I_L, I_K) were also determined for the gills, liver and kidney. All the calculations were conducted according to the method described by BERNET *et al*. (1999).

Statistical analysis

For the haematological parameters (except the leukogram) and biochemical parameters obtained after 1 and 3 days of exposure, the assumption of compliance of the data with the normal distribution was verified using the Shapiro-Wilk test. In most cases, the Shapiro-Wilk test did not contradict the compliance of the data with the normal distribution. The assumption of the homogeneity of variances was verified using the Levene test. In most cases (for the haematological parameters, except the leukogram), and in all cases (for the biochemical parameters), the Levene test did not contradict the homogeneity of variances. Thereafter, the one-way ANOVA test, followed (if significant) by the Tukey HSD test as a post-hoc, were performed and the p.adj was given. For the haematological parameters (except the leukogram) and for the biochemical indices obtained after 10 days of exposure, the assumption of compliance of the data with the normal distribution was verified using the Shapiro-Wilk test, which did not contradict the compliance of the data with the normal distribution in any cases. The Welch t-test was also performed. For the leukogram results obtained after 1 and 3 days of exposure, the assumption of compliance of the data with the normal distribution was verified using the Shapiro-Wilk test. In all cases, the Shapiro-Wilk test contradicted the compliance of the data with the normal distribution. The assumption of the homogeneity of variances was verified using the Levene test. In most cases, the Levene test did not contradict the homogeneity of variances. Due to the lack of compliance of the data with the normal distribution, the Kruskal-Wallis test was used. As a post-hoc, the Dunn test with a Bonferroni correction was applied and the p.adj (p-value multiplied by the number of comparisons that was equal to three, or p.adj=1 if the product of the multiplication was greater than 1) was given. For the leukogram results obtained after 10 days of exposure, the assumption of compliance of the data with the normal distribution was verified using the Shapiro-Wilk test. In two cases, the Shapiro-Wilk test did not contradict the compliance of the data with the normal distribution. In one case, the Shapiro-Wilk test contradicted the compliance of the data with the normal distribution. Furthermore, in one case, carrying out the Shapiro-Wilk test was not possible due to the data structure. Thus, the Mann-Whitney U test was performed. For all the tests, the significance level was set at 0.05. The statistical analysis was carried out using the R free software (R Foundation for Statistical Computing, version 4.1.3). No statistical tests were conducted in the case of the histopathological parameters due to the data structure.

Results

In the control group and group R1, no mortality occurred. In group R2, no mortality was noted after 1 and 3 days of exposure, but no fish survived to the last sampling after 10 days. Before death, petechiae were observed on the skin in most of the individuals.

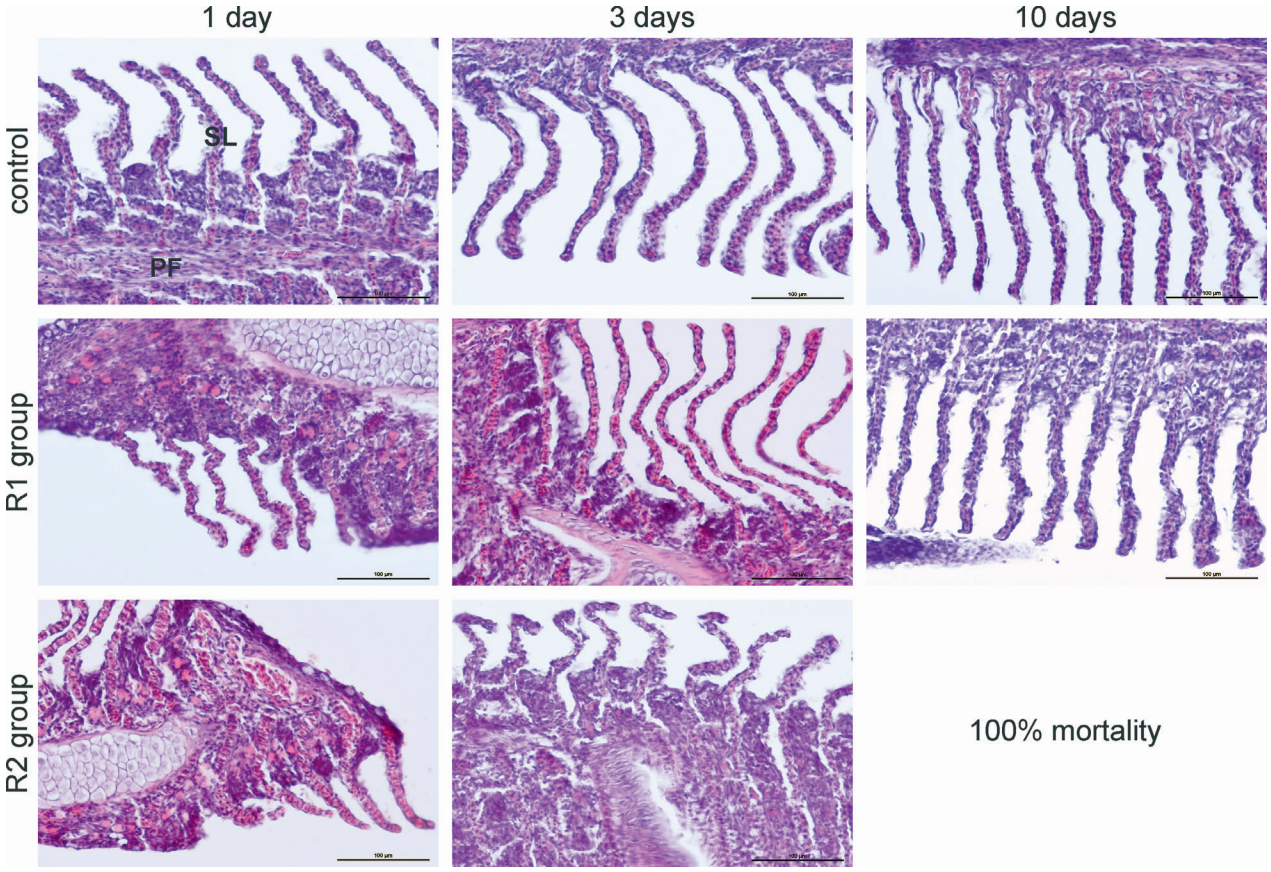

Fig. 1. Photomicrographs of the gills of the common carp. PF – primary filament; SL – secondary lamellae. Haematoxylin-eosin staining. Scale bars: 100 μm.

Histology

Histological images of the examined organs (gills, livers and kidneys) are presented in Figs 1-3. No morphological anomalies belonging to the groups of reaction patterns proposed by BERNET *et al*. (1999) were observed. This applies to both the control and experimental (i.e. the Roundup-exposed) groups. Thus, all the histopathological indices calculated (listed in the Materials and Methods) were equal to zero.

The gills showed a typical architecture with primary and secondary lamellae lined with epithelium (Fig. 1). The experimental fish showed no signs of typical pathological changes resulting from the exposure to pesticides, such as fusion of the secondary lamellae, lamellar lifting, vasodilation and aneurysms of the blood vessels, necrosis, proliferation, oedema and fusion of the epithelial cells.

The histological analysis of the liver revealed a typical structure with polygonal hepatocytes, containing homogenous cytoplasm and a centrally located nucleus, and blood sinusoids as the communication channels between them (Fig. 2). The Roundup-exposed fish showed no signs of pathological changes in the liver parenchyma (e.g. hyperaemia, oedema, necrosis, vacuolar degeneration or inflammation symptoms) and its interstitial tissue (atrophy, hypertrophy, necrosis, architectural or structural alterations).

The trunk kidneys of the fish exposed to Roundup did not show any signs of pathological changes either. The structure of the kidney was the same in the control and experimental groups. It consisted of typical nephrons with glomeruli, renal corpuscles, renal tubules, collecting ducts and interstitial tissue (Fig. 3). No signs of changes categorised as necrosis, proliferation or sclerosis were found.

Haematology

After 1 day of exposure, the ANOVA test showed a significant difference ($p = 0.009$) in the RBC. The Tukey HSD test showed a significant difference between the control group and the fish exposed to Roundup at the higher concentration ($p.adj = 0.0066$). However, it showed no difference between group C and group R1 ($p.adj = 0.367$). The two experimental groups (R1 and R2) did not differ significantly

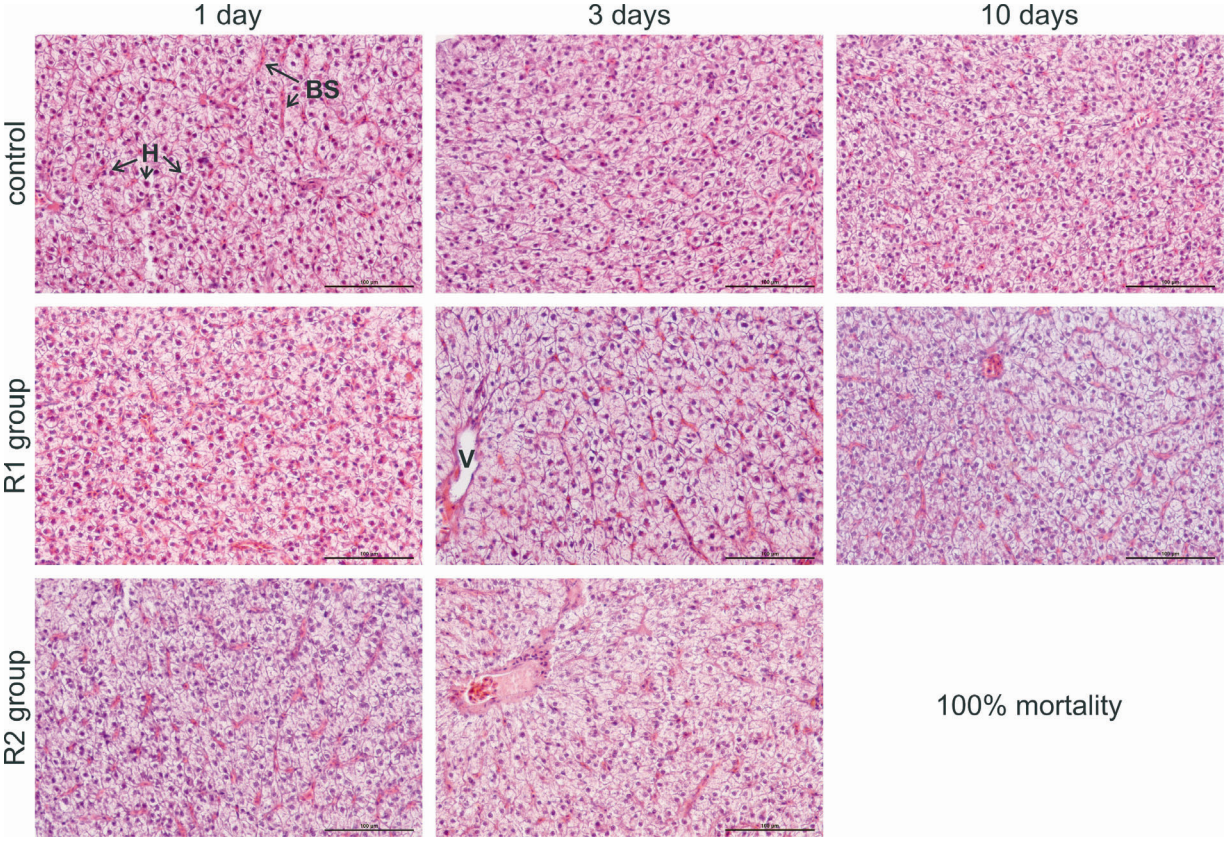

Fig. 2. Photomicrographs of the livers of the common carp in the control and experimental groups. H – hepatocytes; BS – blood sinusoid; V – vein. Haematoxylin-eosin staining. Scale bars: 100 µm.

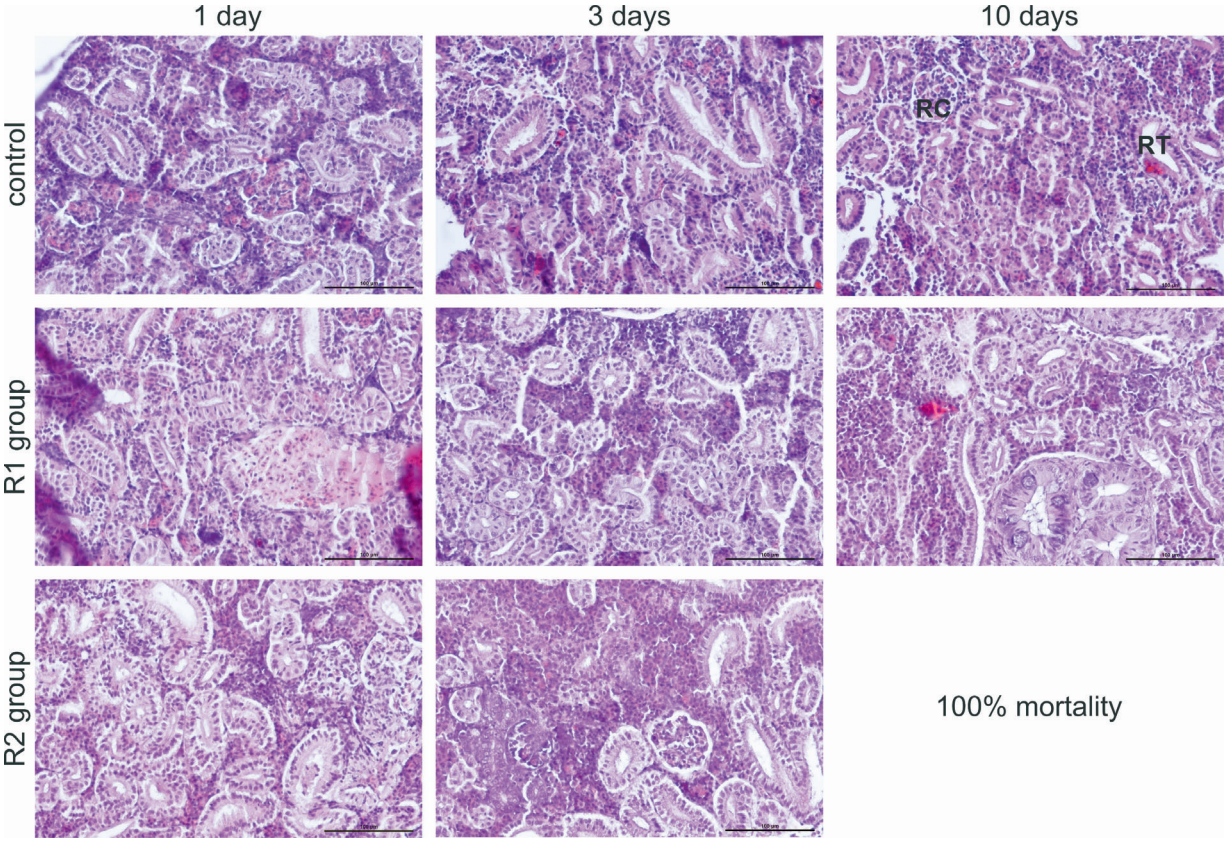

Fig. 3. Photomicrographs of the trunk kidneys of the common carp in the control and experimental groups. RC – renal corpuscle; RT – renal tubule. Haematoxylin-eosin staining. Scale bars: 100 µm.

Table 2

Haematological changes in the common carp after 1 day of exposure to Roundup (mean ± SD; significant differences compared to the control values are marked with asterisks; * 0.01 ≤ p.adj < 0.05; ** 0.001 ≤ p.adj < 0.01; *** 0 < p.adj < 0.001; the Tukey HSD test was used in the case of RBC, Ht, Hb, MCV, MCH, MCHC and WBC; the Dunn test with a Bonferroni correction was used in the case of Lym, Seg, ImNeu and Mono; α = 0.05; n = 11)

Parameter	Control group	Group R1	Group R2
RBC [10^6/µl]	1.40 ± 0.25	1.26 ± 0.13	1.06 ± 0.30 **
Ht [%]	30.05 ± 2.52	29.11 ± 3.68	31.66 ± 1.94
Hb [g/dl]	9.68 ± 1.38	9.76 ± 2.02	12.04 ± 1.60 **
MCV [fl]	218.86 ± 27.04	233.86 ± 39.01	318.84 ± 84.83 ***
MCH [pg]	70.00 ± 7.84	77.87 ± 14.41	120.91 ± 35.47 ***
MCHC [g/dl]	32.14 ± 2.80	33.68 ± 6.84	37.98 ± 4.05 *
WBC [10^3/µl]	24.32 ± 5.09	41.07 ± 4.64 ***	35.41 ± 7.95 ***
Lym [%]	85.09 ± 7.87	89.09 ± 7.25	73.18 ± 10.60
Seg [%]	1.64 ± 2.20	2.09 ± 2.51	3.09 ± 3.24
ImNeu [%]	13.18 ± 7.68	8.73 ± 6.08	23.64 ± 11.27
Mono [%]	0.09 ± 0.30	0.09 ± 0.30	0.09 ± 0.30

RBC – red blood cell count; Ht – haematocrit value; Hb – haemoglobin concentration; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; WBC – white blood cell count; Lym – lymphocytes; Seg – segmented neutrophils; ImNeu – immature neutrophils; Mono – monocytes.

Table 3

Haematological changes in the common carp after 3 days of exposure to Roundup (mean ± SD; significant differences compared to the control values are marked with asterisks; * 0.01 ≤ p.adj < 0.05; ** 0.001 ≤ p.adj < 0.01; *** 0 < p.adj < 0.001; the Tukey HSD test was used in the case of RBC, Ht, Hb, MCV, MCH, MCHC and WBC; the Dunn test with a Bonferroni correction was used in the case of Lym, Seg, ImNeu and Mono; α = 0.05; n = 11)

Parameter	Control group	Group R1	Group R2
RBC [10^6/µl]	1.79 ± 0.28	1.61 ± 0.32	1.48 ± 0.20 *
Ht [%]	30.98 ± 4.05	31.70 ± 4.24	31.27 ± 3.73
Hb [g/dl]	9.12 ± 1.28	9.39 ± 1.32	10.68 ± 1.64 *
MCV [fl]	177.96 ± 38.47	202.12 ± 40.49	214.02 ± 34.39
MCH [pg]	52.34 ± 10.86	59.96 ± 13.29	73.57 ± 16.43 **
MCHC [g/dl]	29.46 ± 1.74	29.59 ± 1.16	34.12 ± 3.07 ***
WBC [10^3/µl]	23.61 ± 6.55	28.27 ± 6.23	25.39 ± 7.08
Lym [%]	87.45 ± 11.24	92.45 ± 4.80	56.09 ± 18.00 **
Seg [%]	1.09 ± 0.83	0.45 ± 0.69	2.00 ± 2.41
ImNeu [%]	11.36 ± 11.23	6.91 ± 4.68	41.73 ± 17.40 **
Mono [%]	0.09 ± 0.30	0.18 ± 0.60	0.18 ± 0.40

RBC – red blood cell count; Ht – haematocrit value; Hb – haemoglobin concentration; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; WBC – white blood cell count; Lym – lymphocytes; Seg – segmented neutrophils; ImNeu – immature neutrophils; Mono – monocytes.

(p.adj = 0.145) (Table 2). After 3 days of exposure, the ANOVA test showed a significant difference (p = 0.044) in the RBC. The Tukey HSD test showed a significant difference between the control group and the group exposed to the herbicide at the higher concentration (p.adj = 0.035). It again showed no difference between the control group and group R1 (p.adj = 0.306). The two experimental groups (R1 and R2) did not differ significantly (p.adj = 0.506) (Table 3). After 10 days of exposure, the Welch t-test showed no statistically significant difference in the RBC between the control value and the value recorded for group R1 (p = 0.97) (Table 4).

Table 4

Haematological changes in the common carp after 10 days of exposure to Roundup (mean ± SD; significant differences compared to the control values are marked with asterisks; * $0.01 \leq p < 0.05$; ** $0.001 \leq p < 0.01$; the Welch t-test was used in the case of RBC, Ht, Hb, MCV, MCH, MCHC and WBC; the Mann-Whitney U test was used in the case of Lym, Seg, ImNeu and Mono; $\alpha = 0.05$; $n = 11$)

Parameter	Control group	Group R1
RBC [10^6/µl]	1.32 ± 0.18	1.32 ± 0.21
Ht [%]	31.25 ± 6.30	34.02 ± 4.25
Hb [g/dl]	8.13 ± 1.62	10.02 ± 1.25 **
MCV [fl]	239.32 ± 50.65	262.04 ± 47.63
MCH [pg]	62.39 ± 14.14	77.31 ± 15.09 *
MCHC [g/dl]	26.32 ± 3.47	29.51 ± 1.89 *
WBC [10^3/µl]	23.61 ± 7.04	23.02 ± 3.11
Lym [%]	94.55 ± 3.27	87.00 ± 8.72 *
Seg [%]	0.36 ± 0.67	1.09 ± 1.38
ImNeu [%]	5.09 ± 3.02	11.82 ± 8.13 *
Mono [%]	0.00 ± 0.00	0.09 ± 0.30

RBC – red blood cell count; Ht – haematocrit value; Hb – haemoglobin concentration; MCV – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – mean corpuscular haemoglobin concentration; WBC – white blood cell count; Lym – lymphocytes; Seg – segmented neutrophils; ImNeu – immature neutrophils; Mono – monocytes.

After 1 and 3 days of exposure, the ANOVA test showed no statistically significant difference in the Ht value ($p = 0.117$ and $p = 0.913$, respectively) (Tables 2 and 3). After 10 days of exposure, the Welch t-test showed no statistically significant difference in the Ht level between the control group value and the value recorded for the fish in group R1 ($p = 0.24$) (Table 4).

After 1 day of exposure, the ANOVA test showed a significant difference ($p = 0.003$) in the Hb concentration. The Tukey HSD test showed a significant difference between the control group and group R2 (p.adj = 0.00713). Group R1 also differed significantly from group R2 (p.adj = 0.00973). The Tukey HSD test showed no difference between group C and group R1 (p.adj = 0.992) (Table 2). After 3 days of exposure, the ANOVA test showed a significant difference ($p = 0.035$) in the Hb content. The Tukey HSD test showed a significant difference between the control group and group R2 (p.adj = 0.0407); however, it showed no difference in the Hb concentration between groups C and R1 (p.adj = 0.901) and between R1 and R2 (p.adj = 0.102) (Table 3). After 10 days of exposure, the Welch t-test showed that the Hb level in the control fish and the exposed (R1) fish differed significantly ($p = 0.00617$) (Table 4).

After 1 day of exposure, the ANOVA test showed a significant difference ($p = 0.000429$) in terms of the MCV. The Tukey HSD test showed a significant difference between the control fish and those exposed to the herbicide at the higher concentration (p.adj = 0.000666). The Tukey HSD test also showed a significant difference between the fish from groups R1 and R2 (p.adj = 0.00358). There was no significant difference in the MCV in groups C and R1 (p.adj = 0.807) (Table 2). After 3 days of exposure, the ANOVA test also showed no statistically significant difference ($p = 0.092$) in terms of the MCV (Table 3). Similarly, after 10 days, the Welch t-test showed no difference in the MCV between the control group and group R1 ($p = 0.291$) (Table 4).

After 1 day of exposure, the ANOVA test showed a significant difference ($p = 0.0000168$) in the MCH. The Tukey HSD test showed a significant difference between the control fish and the fish treated with Roundup at the higher concentration (p.adj = 0.0000299). The Tukey HSD test also showed a significant difference between the fish from groups R1 and R2 (p.adj = 0.000295). There was no significant difference in the MCH between groups C and R1 (p.adj = 0.695) (Table 2). After 3 days of exposure, the ANOVA test showed a significant difference ($p = 0.004$) in the MCH. The Tukey HSD test showed a significant difference between the control group and group R2 (p.adj = 0.00292). However, there was no significant difference in the MCH between groups C and R1 (p.adj = 0.405). There was also no significant difference in the MCH between groups R1 and R2 (p.adj = 0.0673) (Table 3). The Welch t-test performed after 10 days of exposure showed that the MCH value of the fish in the control group differed significantly ($p = 0.0267$) from that of the fish in group R1 (Table 4).

After 1 day of exposure, the ANOVA test showed a significant difference (p = 0.024) in the MCHC. The Tukey HSD test showed a significant difference between the control group and group R2 (p.adj = 0.0225). There was no difference in the MCHC between groups C and R1 (p.adj = 0.739), and there was also no difference in the MCHC parameter between groups R1 and R2 (p.adj = 0.113) (Table 2). After 3 days of exposure, the ANOVA test showed a significant difference (p = 0.0000123) in the MCHC. The Tukey HSD test showed a significant difference between the control fish and the fish exposed to Roundup at the higher concentration (p.adj = 0.0000511). The Tukey HSD test also showed a significant difference between the fish from groups R1 and R2 (p.adj = 0.0000761). However, the values of the MCHC parameter in groups C and R1 were similar (p.adj = 0.989) (Table 3). The Welch t-test performed after 10 days of exposure showed that the value of the MCHC parameter for the fish in the control group differed significantly (p = 0.0142) from the value determined for the fish from group R1 (Table 4).

After 1 day of exposure, the ANOVA test showed a significant difference (p = 0.0000015) in the WBC. The Tukey HSD test showed a significant difference between the control group and group R1 (p.adj = 0.00000111), as well as between group C and group R2 (p.adj = 0.000497). The two groups exposed to the herbicide (R1 and R2) did not differ significantly (p.adj = 0.09) (Table 2). After 3 days of exposure, the ANOVA test showed no statistically significant difference (p = 0.266) in terms of the WBC (Table 3). After 10 days of exposure, the Welch t-test showed no statistically significant difference in the WBC between the control group and the group of fish exposed to the herbicide at a lower concentration (p = 0.802) (Table 4).

After 1 day of exposure, the Kruskal-Wallis test showed a significant difference (p = 0.00165) in the percentage of lymphocytes. The Dunn test showed a difference between groups R1 and R2 (p.adj = 0.00125). However, there were no differences between the control group and group R1 (p.adj = 0.633) and between the control group and group R2 (p.adj = 0.0679) (Table 2). After 3 days, the Kruskal-Wallis test showed a significant difference (p = 0.000171) in the percentage of lymphocytes. The Dunn test showed a significant difference between groups C and R2 (p.adj = 0.00381), as well as between groups R1 and R2 (p.adj = 0.000294). However, this test showed no significant difference between the percentage of lymphocytes found in the fish from groups C and R1 (p.adj = 1) (Table 3). The Mann-Whitney U test performed after 10 days of exposure showed that the percentage of lymphocytes in the blood of the control fish differed significantly (p = 0.0148) from the value found in the fish from group R1 (Table 4).

After 1 and 3 days of exposure, the Kruskal-Wallis test found no statistically significant difference in the percentage of segmented neutrophils (p = 0.313 and p = 0.0936, respectively) (Tables 2 and 3). The Mann-Whitney U test performed after 10 days of exposure also showed no significant difference (p = 0.17) in the percentage of segmented neutrophils between the fish from the control group and the fish in group R1 (Table 4).

After 1 day of exposure, the Kruskal-Wallis test showed a significant difference (p = 0.00224) in the percentage of immature neutrophils. The Dunn test showed a difference between groups R1 and R2 (p.adj = 0.00162). However, there were no differences between the control group and group R1 (p.adj = 0.565) and between the control group and group R2 (p.adj = 0.0959) (Table 2). After 3 days, the Kruskal-Wallis test showed a significant difference (p = 0.000156) in the percentage of immature neutrophils. The Dunn test showed a significant difference between groups C and R2 (p.adj = 0.00278), as well as between groups R1 and R2 (p.adj = 0.000321). However, it did not show a significant difference between groups C and R1 (p.adj = 1) (Table 3). The Mann-Whitney U test performed after 10 days of exposure showed that the percentage of immature neutrophils in the blood of the control fish differed significantly (p = 0.0193) from the value detected in the fish belonging to group R1 (Table 4).

After 1 and 3 days of exposure, the Kruskal-Wallis test showed no statistically significant difference in the percentage of monocytes (p = 1 and p = 0.794, respectively) (Tables 2 and 3). The Mann-Whitney U test performed after 10 days of exposure showed no significant difference (p = 0.363) in the percentage of monocytes in the fish from the control group and the fish from group R1 (Table 4).

Blood biochemistry

After 1 and 3 days of exposure, the ANOVA test showed no statistically significant difference in the TP concentration (p = 0.518 and p = 0.131, respectively) (Tables 5 and 6). After 10 days of exposure, the Welch t-test also showed no significant difference in the TP concentration between groups C and R1 (p = 1) (Table 7).

After 1 day of exposure, the ANOVA test showed a significant difference (p = 0.003) in the glucose concentration. The Tukey HSD test showed a significant difference between the control group and group R2 (p.adj = 0.00237). However, there was no difference between the control group and group R1 (p.adj = 0.354), and there was also no difference between groups R1 and R2 (p.adj = 0.07) (Table 5). After 3 days of exposure, the ANOVA test showed no statistically significant difference in the glucose concentration (p = 0.71) (Table 6). After 10 days of exposure,

Table 5

Biochemical changes in the common carp after 1 day of exposure to Roundup (mean ± SD; significant differences compared to the control values are marked with asterisks; ** $0.001 \leq p.adj < 0.01$; Tukey HSD test; $\alpha = 0.05$; n = 11)

Parameter	Control group	Group R1	Group R2
TP [g/l]	29.14 ± 9.15	29.60 ± 6.54	26.55 ± 2.46
Glu [mg/dl]	89.20 ± 37.34	110.80 ± 25.14	146.43 ± 43.65 **
Trigl [mg/dl]	176.60 ± 28.05	165.39 ± 41.44	157.11 ± 30.98
Chol [mg/dl]	202.28 ± 54.93	202.23 ± 27.28	206.99 ± 68.74
ALT [U/l]	80.17 ± 35.57	117.02 ± 54.63	76.17 ± 32.60

TP – total protein; Glu – glucose; Trigl – triglycerides; Chol – cholesterol; ALT – alanine aminotransferase.

Table 6

Biochemical changes in the common carp after 3 days of exposure to Roundup (mean ± SD; significant differences compared to the control values are marked with asterisks; * $0.01 \leq p.adj < 0.05$; Tukey HSD test; $\alpha = 0.05$; n = 11)

Parameter	Control group	Group R1	Group R2
TP [g/l]	25.38 ± 3.35	27.24 ± 3.51	24.32 ± 3.11
Glu [mg/dl]	82.44 ± 26.59	84.78 ± 18.67	89.95 ± 18.79
Trigl [mg/dl]	187.47 ± 25.13	193.92 ± 37.43	186.97 ± 28.09
Chol [mg/dl]	164.42 ± 20.49	207.16 ± 52.47 *	138.69 ± 31.10
ALT [U/l]	35.63 ± 17.20	20.13 ± 11.86	30.75 ± 19.25

TP – total protein; Glu – glucose; Trigl – triglycerides; Chol – cholesterol; ALT – alanine aminotransferase.

Table 7

Biochemical changes in the common carp after 10 days of exposure to Roundup (mean ± SD; Welch t-test; $\alpha = 0.05$; n = 11)

Parameter	Control group	Group R1
TP [g/l]	25.80 ± 3.50	25.80 ± 3.89
Glu [mg/dl]	119.68 ± 33.39	100.46 ± 16.25
Trigl [mg/dl]	180.07 ± 13.13	180.35 ± 16.69
Chol [mg/dl]	166.38 ± 46.84	159.68 ± 38.62
ALT [U/l]	16.90 ± 4.39	15.86 ± 4.92

TP – total protein; Glu – glucose; Trigl – triglycerides; Chol – cholesterol; ALT – alanine aminotransferase.

the Welch t-test showed no significant difference in the glucose concentration between groups C and R1 ($p = 0.102$) (Table 7).

After 1 and 3 days of exposure, the ANOVA test showed no statistically significant difference in the concentration of triglycerides ($p = 0.413$ and $p = 0.84$, respectively) (Tables 5 and 6). After 10 days of exposure, the Welch t-test showed no significant difference in the concentration of triglycerides between groups C and R1 ($p = 0.965$) (Table 7).

After 1 day of exposure, the ANOVA test showed no statistically significant difference in the cholesterol concentration ($p = 0.971$) (Table 5). However, after 3 days, the ANOVA test showed a significant difference in the cholesterol concentration ($p = 0.000624$). The Tukey HSD test showed a significant difference between the control group and group R1 ($p.adj = 0.0296$), as well as between groups R1 and R2 ($p.adj = 0.000447$). There was no difference in this biochemical parameter in the blood plasma of the fish

from groups C and R2 (p.adj = 0.251) (Table 6). After 10 days of exposure, the Welch t-test showed no significant difference in the cholesterol concentration between groups C and R1 (p = 0.718) (Table 7).

After 1 and 3 days of exposure, the ANOVA test showed no statistically significant difference in the ALT activity (p = 0.057 and p = 0.094, respectively) (Tables 5 and 6). In addition, after 10 days of exposure, the Welch t-test showed no significant difference in the ALT activity between groups C and R1 (p = 0.604) (Table 7).

Discussion

In this study, a reduction of the red blood cell count was observed, indicating an anaemic response to the Roundup exposure. The increase in the value of the other erythrocyte parameters (Hb concentration, MCV, MCH and MCHC) that was observed may indicate the activation of compensatory mechanisms. KONDERA *et al*. (2018) demonstrated that Roundup-treated common carp showed a higher frequency of head kidney erythroblasts, which indicated an acceleration of erythropoiesis. The authors hypothesised that this accelerated erythropoiesis was a response to the shortened life span of the red blood cells. It can therefore be assumed that the reduction in the RBC count observed in the current research resulted from a reduction of the life span of the erythrocytes. An increase of the Hb concentration, MCH and MCHC was also observed in tambaqui *Colossoma macropomum* (Cuvier, 1816) exposed to Roundup Original® (BRAZ-MOTA *et al*. 2015). In addition, a decrease of the RBC count was noted in the case of glyphosate-exposed rohu (GHAFFAR *et al*. 2021). The similarity of the results of the current study with the results obtained by the abovementioned authors suggests that the physiological response to Roundup/glyphosate is not specific to common carp, but may also occur in fish of other species.

In the current study, most of the changes in the analysed erythrocyte indices occurred faster in the fish exposed to Roundup at the higher tested concentration, while the exposure to the lower concentration resulted in similar changes that appeared after a longer exposure time, thus indicating the concentration-dependent and time-dependent nature of these changes. KONDERA *et al*. (2018) observed an increase of Ht and a decrease of the Hb content and the MCHC value in Roundup-exposed common carp. In contrast to the results of the current study, the haematological changes revealed by these authors were not directly related to the herbicide concentration. However, the changes in the haematological parameters that occurred in glyphosate-exposed rohu appeared in a dose and time-dependent manner (GHAFFAR *et al*. 2021). The discrepancies in the abovementioned observations may be the result of various factors, such as the age of the fish, sex distribution in the experimental population, physiological status of the fish or/and the experimental conditions. It is well known that both endogenous and exogenous factors can lead to changes in the haematological parameters in fish (FAZIO 2019; AHMED *et al*. 2020).

The increase in the white blood cell count observed in the present study indicates the presence of inflammation. It was confirmed by an increase in the percentage of immature neutrophils. According to PEILLEX & PELLETIER (2020), it seems that glyphosate affects the immune system and alters the complement cascade, phagocytic function and lymphocyte responses, as well as increases the synthesis of pro-inflammatory cytokines in fish. The reduction in the percentage of lymphocytes observed in the present study may indicate immunosuppression. A decrease of the lymphocyte percentage and an increase of the neutrophil ratio were observed in rohu treated with glyphosate (GHAFFAR *et al*. 2021). Immunosuppression was also detected in Roundup-exposed Nile tilapia, which showed a decreased WBC count (ABDELMAGID *et al*. 2022). In contrast to the current results, KONDERA *et al*. (2018) did not observe significant changes in the percentages of lymphocytes, neutrophils and monocytes in Roundup-exposed common carp, while the WBC count was decreased. It can be assumed that the disorders of the immune system caused by herbicides may lead to an increased incidence of infectious diseases in fish. This was partially confirmed by the research conducted by LE DU-CARREE *et al*. (2022), which demonstrated that glyphosate-based herbicide formulations (i.e. Viaglif Jardin® and Roundup Innovert®) modulated the susceptibility of rainbow trout to the infectious haematopoietic necrosis virus (*IHNv*). The authors revealed that the exposure of parental fish to Viaglif Jardin® led to an increased mortality in the experimentally infected juveniles. On the other hand, the exposure of parental individuals to Roundup Innovert® resulted in a decreased mortality in the infected juveniles (LE DU-CARREE *et al*. 2022).

The present study indicates that the haematology parameters (both red blood cell and white blood cell indices) are sensitive and rapidly-responding markers of the exposure to Roundup in fish. The aforementioned literature (KONDERA *et al*. 2018; GHAFFAR *et al*. 2021; ABDELMAGID *et al*. 2022) confirms this statement. However, it should be noted that in the experiment conducted by GHAFFAR *et al*. (2021), glyphosate alone (not as a herbicide formulation) was used. Moreover, it should be added that the interpretation of the haematological results in fish is difficult, due to the lack of commonly accepted reference values for most species. According to WITESKA *et al*. (2016), the determination of haematological reference values in fish is much more difficult than in

higher vertebrates due to their poikilothermy and high spatial, temporal and individual variability.

In the present study, only minor and temporary changes (increased glucose and cholesterol concentrations) in the blood biochemical parameters were observed. The increase in the glucose concentration may have been the result of a stress reaction, while the increase of the cholesterol level is difficult to explain. Nevertheless, an increase of the cholesterol concentration in Roundup-exposed common carp was also observed in the research conducted by KONDERA *et al*. (2018). However, in that case the glucose concentration was not significantly affected by the herbicide treatment (KONDERA *et al*. 2018). Major serum biochemical alterations were observed in Roundup-exposed Nile tilapia in the study performed by ABDELMAGID *et al*. (2022). These authors noted a decrease of the total protein, albumin, globulin and total immunoglobulin concentrations, as well as a decrease of the lysozyme activity indicating immunosuppression. The glucose, cortisol, urea and creatinine concentrations, as well as the activity of the hepatic aminotransferases (ALT and AST) were increased, which indicate a stress response and disturbances in the functioning of the liver and kidneys. An increased concentration of serum creatinine and the concentration of serum urea, as well as increased ALT and AST activity have also been observed in glyphosate-treated rohu (GHAFFAR *et al*. 2021). In the present study, the biochemical parameters tested turned out to be significantly less-sensitive markers of Roundup exposure than the analysed haematological indices. However, as has been shown in the abovementioned literature, the situation may be different in the case of other fish species. According to BOJARSKI & WITESKA (2020), the haematological and blood biochemical parameters appear to be useful biomarkers for an evaluation of the physiological state of fish exposed to pesticides; however, they are not specific markers of intoxication.

In the present study, histopathological changes were not observed in the Roundup-treated common carp. However, it cannot be ruled out that a longer exposure or an exposure to higher concentrations would cause histopathological changes, especially since many authors have shown that Roundup/glyphosate caused changes in the microstructure of fish vital organs, such as the gills. BRAZ-MOTA *et al*. (2015) observed that a short-term exposure of tambaqui to Roundup Original® resulted in filament and lamellar hyperplasia and hypertrophy, lamellar epithelium lifting, as well as the proliferation of mitochondria rich cells, proliferation of mucous cells, lamellar fusion and aneurism. Moreover, Nile tilapia exposed to Roundup showed a proliferation of the respiratory epithelium, oedema and lifting of the secondary lamellar epithelium and clubbing at the tips of the secondary lamellae. Leukocyte infiltration was also observed (JIRAUNGKOORSKUL *et al*. 2002). Similarly, *J. multidentata* treated with Roundup exhibited a lifting of the secondary lamellar epithelium, oedema formation, hypertrophy of the epithelial cells and chloride cells, lamellar aneurysm, proliferation of the mucous cells, and hyperplasia of the epithelial cells, which led to a fusion of the secondary lamellae (HUED *et al*. 2012). GHAFFAR *et al*. (2021) revealed that rohu exposed to glyphosate exhibited a disruption of the cartilaginous core, sloughing of the secondary lamellar epithelium and disruption of the primary lamellae. Disorganisation in the arrangement of the primary and secondary lamellae, curling of the gills and fusion of the lamellae were also noted. Moreover, severe pathological lesions occurred, such as necrosis of the epithelial cells of the primary and secondary lamellae, congestion, atrophied lamellae, lamellar stunting and fusion of the lamellae.

Previous studies have shown that Roundup/glyphosate affected the microstructure of fish liver and resulted in various histopathological alterations in this organ. Pathological lesions, e.g. degeneration of the cell membrane, cytoplasm vacuolation and pyknotic nuclei in the hepatocytes, as well as the infiltration of leukocytes in the liver were detected in Nile tilapia exposed to Roundup (JIRAUNGKOORSKUL *et al*. 2002). Similarly, HUED *et al*. (2012) demonstrated that the liver of *J. multidentata* treated with Roundup showed hydropic degeneration, blood sinusoid dilation, infiltration of leukocytes, vascular congestion and necrosis. Moreover, GHAFFAR *et al*. (2021) noted the occurrence of vacuolar degeneration, congestion, pyknosis and karyorrhexis of the hepatocytes in glyphosate-exposed rohu. The liver of Roundup-intoxicated African catfish showed severe congestion in the hepatoportal blood vessels. Multifocal areas of coagulative necrosis invaded with numerous leukocytes and erythrocytes were observed. Severe hydropic degeneration and macrovesicular steatosis were also detected (MOUSTAFA *et al*. 2016). In the liver of Roundup-treated pirapitinga *Piaractus brachypomus* (Cuvier, 1818), congestion, lipidic vacuolisation and the presence of hyaline droplets in the hepatocytes, as well as multifocal necrotic processes were detected (RAMIREZ-DUARTE *et al*. 2008). The study performed by DO CARMO LANGIANO & MARTINEZ (2008) showed that streaked prochilod exposed to Roundup exhibited cytoplasmic and nuclear degeneration, bile stagnation, hyperaemia, and the occurrence of vacuoles in the cytoplasm and nucleus of the hepatocytes.

Histopathological changes have also been observed by other authors in the kidneys of fish exposed to Roundup/glyphosate. Some of the glomeruli of Nile tilapia exposed to Roundup were collapsed or distorted; while the epithelium of many of the tubules had become exfoliated (JIRAUNGKOORSKUL *et al*. 2002). Other alterations were also noted

(JIRAUNGKOORSKUL *et al*. 2002). The kidneys of glyphosate-treated rohu exhibited, among other changes, oedema, haemorrhages, atrophy of the tubules, glomerular degeneration and tubular necrosis (GHAFFAR *et al*. 2021). The results of a histopathological examination conducted by MA *et al.* (2015) revealed vacuolisation of the renal parenchyma and intumescence of the renal tubule in the kidney collected from glyphosate-exposed common carp. Moreover, multifocal haemorrhages in the periphery of the organ and multifocal necrosis of the tubular epithelium were observed in the kidney sampled from Roundup-treated pirapitinga (RAMIREZ-DUARTE *et al*. 2008).

The histopathological changes observed by the abovementioned investigators indicate the structural damage of organs resulting from exposure to Roundup/glyphosate. The results obtained by LI *et al.* (2019) suggest that glyphosate-based herbicides induce damage in fish tissues via a heat shock proteins-related immune response and oxidative stress. The previously discussed haematological and biochemical changes confirm the dysfunction of various organs and indicate homeostasis disorders in intoxicated fish. Differences in the results of the histological analysis of the gills, liver and kidneys obtained between the present study and the experiments carried out by other authors may be the result of various experimental conditions or species differences. Thus, the sensitivity of the model organism should be taken into account when designing toxicological studies. Due to the lack of histopathological lesions occurring in the current research, we recommend the use of more sophisticated research methods, such as a cytological analysis with scanning and/or transmission electron microscopy, for studies aimed at determining whether a Roundup treatment causes structural changes in fish. Ultrastructural changes (appearance of myelin-like structures, swelling of the mitochondria and disappearance of the internal membrane of the mitochondria) in the hepatocytes of Roundup-treated common carp was previously detected by SZAREK *et al*. (2000). Moreover, Glifosato II® (another glyphosate-based herbicide formulation) caused severe alterations in the gill ultrastructure in pejerrey *Odontesthes bonariensis* (Valenciennes, 1835) (MENENDEZ-HELMAN *et al.* 2020).

Conclusions

The current study showed that the haematological parameters were the most sensitive and reliable markers of the exposure of the common carp to Roundup. Far fewer changes were observed in the blood biochemical indices, while the histological structure of the analysed organs was unchanged. Thus, a haematological analysis seems to be a basic and necessary tool in the evaluation of the effects of the exposure of common carp to the tested herbicide; however, for an overall toxicological assessment, both functional (pathophysiological) and structural (histopathological) changes should be studied.

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Author Contributions

Research concept and design: B.B.; Collection and/or assembly of the data: B.B., A.O., S.H., J.S., A.R.-B.; Data analysis and interpretation: B.B., A.O., S.H., L.S.; Writing the article: B.B., L.S.; Critical revision of the article: B.B.; Final approval of the article: B.B., A.O., S.H., L.S.

Conflict of Interest

The authors declare no conflict of interest.

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