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Effects of an MCPA-based herbicide formulation on the common carp *Cyprinus carpio* Linnaeus, 1758 – haematological, biochemical and histological evaluation

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Original article

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Herbicides (weed control agents) are used in crops on a massive scale. MCPA (2-methyl-4-chlorophenoxyacetic acid) is a herbicide used to control weeds in cereals and other crops. The aim of this study was to investigate the toxic effects of an MCPA-based herbicide formulation (Chwastox Extra® 300 SL) in the common carp (*Cyprinus carpio*). The fish were exposed for 10 days, to a concentration which corresponded to 1 mg/l or 5 mg/l of MCPA. Our analysis showed fluctuations of the haematological parameters during the treatment. Plasma biochemical changes that were statistically significant, i.e. a decrease of the total protein concentration and alanine aminotransferase activity, were observed after 1 day of exposure. No histopathological lesions in the gills, trunk kidney and liver were identified. The results of the present study indicate that Chwastox Extra® 300 SL has a relatively low toxicity for the common carp. It was also observed that the blood indices were more sensitive to the tested herbicide formulation than the microstructure of the selected organs. Further research aimed at studying the effects of Chwastox on water invertebrates and fish of other taxa is recommended.

Key words: pesticide, toxicity, fish, physiological parameter, organ microstructure

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Herbicides are agents used to control unwanted plants (weeds) in crops. They are widely used in agriculture and represent about 50% of all pesticides used throughout the world (De *et al*. 2014). MCPA (2-methyl-4-chlorophenoxyacetic acid) is a herbicide used for the control of annual and perennial weeds in cereals and other crops (PPDB: Pesticide Properties DataBase, 2023). It is moderate toxic for

rats and the northern bobwhite *Colinus virginianus* (Linnaeus, 1758), with low toxicity for honey bees *Apis mellifera* Linnaeus, 1758 (PPDB: Pesticide Properties DataBase, 2023). As was shown in a review by Caux *et al*. (1995), MCPA has been detected in surface and ground water, with a maximum concentration of 0.013 mg/l and 1.0 mg/l, respectively. Despite its presence in the aquatic environment, the data regarding the effects of this compound on aquatic biota is scarce and fragmentary. The acute 96 hour LC_{50} for the common carp *Cyprinus carpio* Linnaeus, 1758 was >100 mg/l, which indicates low toxicity (PPDB: Pesticide Properties DataBase, 2023). However, a study conducted by Lutnicka *et al*. (2018) demonstrated that common carp juveniles treated with MCPA (100 µg/l) exhibited changes in their red and white blood cell parameters, while a weak cytotoxic effect on the hematopoietic system of the fish was also observed. Moreover, the exposure of common carp embryos to MCPA-Na (52, 56 and 60 mg/l) resulted in increased mortality and a greater percentage of malformations, as well as leading to a decreased hatching rate (Sun *et al*. 2021).

To the best of our knowledge, agricultural formulations containing MCPA as active ingredient have not been sufficiently evaluated in the context of their toxicity to aquatic organisms. Nevertheless, according to Caux *et al*. (1996), adjuvants play a predominant role in determining the environmental chemistry, fate and toxicity of the active ingredient. The study performed by Caux *et al*. (1996) showed that an MCPA-based formulation (Amine 500) was much more toxic to *Selenastrum capricornutum* (Chlorophyta) than the active ingredient. We failed to find any scientific data on the influence of MCPA-based herbicide formulations on fish; thus, this issue should be considered to be unknown.

The common carp is a widely cultivated and commercially important freshwater fish species (Rahman 2015). Furthermore, it is commonly used in ecotoxicological studies (e.g. Forouhar Vajargah *et al*. 2018; Banaee *et al*. 2019; Todorova *et al*. 2019; Georgieva *et al*. 2021; Yancheva *et al*. 2022), including in experiments with herbicides (e.g. Wang *et al*. 2018; Ma *et al*. 2019; Socha *et al*. 2021; Yalsuyi *et al*. 2021; Bojarski *et al*. 2022). The size of the common carp allows for the collection of sufficient blood for haematological and plasma/serum biochemical analyses. Our experience has shown that this generally also applies to fry or to sexually mature individuals of a small size.

In the current study, an attempt has been made to evaluate the toxic effects of an MCPA-based herbicide formulation (Chwastox Extra® 300 SL) on the common carp. To detect both functional (pathophysiological) and structural (histopathological) changes, the haematological and blood biochemical parameters as well as the microstructure of selected organs (gills, liver and kidney) were analysed.

Material and Methods

Experiment design

The experiment was approved by the Local Ethics Committee for Animal Testing at the Medical University of Silesia in Katowice (Resolution No. 28/2021 of 19.05.2021). Sexually mature common carp of both sexes (males and females in similar proportions) and the same age, which had always been kept under laboratory conditions, were used in this study. Before the experiment, they were subjected to a parasitological and clinical examination and were deemed to be healthy. The weight of the fish was 66.05 ± 7.16 g (mean ± SD), while the total length was 15.87 ± 0.78 cm (mean ± SD). The fish (99 individuals) were kept in 9 tanks (11 fish per tank). Each tank had a volume of 300 litres and was filled with water to 200 litres. The acclimation lasted for 2 weeks. After acclimation, three equal groups were established (3 tanks with 11 fish in each tank per group). The fish of the control (C) group were kept in water without the addition of toxicants. The fish of Group CH1 were exposed to the commercially available herbicide formulation Chwastox Extra® 300 SL (Ciech Sarzyna, Nowa Sarzyna, Poland) at a lower tested concentration, corresponding to 1 mg/l of MCPA (the active ingredient). The fish belonging to Group CH2 were treated with the same herbicide formulation at a higher tested concentration, which corresponded to 5 mg/l of MCPA. The exposure lasted for 1, 3 or 10 days (after these periods, the fish were euthanised and were not subjected to further exposure). During the treatment, the water (or experimental medium – solution of the applied herbicide formulation) was changed every twelve hours. This procedure was applied to keep the concentration of the herbicide as constant as possible, and to remove nitrogenous metabolites. During the acclimation, the water was exchanged in the same manner. The photoperiod was 14:10 (light:dark). The fish were fed daily with Aller Silver 3 mm feed (0.5% of their body mass). No feed was given on the days of euthanasia. The water quality parameters were regularly controlled (the results of the measurements are presented in Table 1). The temperature and oxygen concentration were deter-

Table 1

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

Parameter / Group	Control	Group CH1	Group CH2
Temp. [°C]	21.67 ± 0.20	21.66 ± 0.24	21.64 ± 0.22
O_2 [mg/l]	6.38 ± 0.47	6.48 ± 0.51	6.59 ± 0.46
pH	7.51 ± 0.09	7.51 ± 0.10	7.47 ± 0.10
NH_3 [mg/l]	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
NO_2^- [mg/l]	0.04 ± 0.03	0.05 ± 0.04	0.06 ± 0.07
NO_3^- [mg/l]	12.59 ± 5.77	11.90 ± 5.89	12.83 ± 6.07
GH [°GH]	5.93 ± 0.37	6.00 ± 0.00	6.07 ± 0.26
KH [°KH]	3.34 ± 0.55	3.31 ± 0.47	3.21 ± 0.41

mined using an Oxi 3310 oximeter (WTW, Poland), while the pH was measured with a pH meter (Mettler Toledo, Switzerland). Colorimetric kits produced by the Zoolek company (Łódź, Poland) were applied to determine the ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations, as well as the general hardness (GH) and carbonate hardness (KH).

Biological material collection

Sampling of the biological material for the haematological, biochemical and histological analyses was conducted after 1, 3 or 10 days of exposure. Blood from the caudal vein was collected with a needle and syringe into heparinised (Heparinum WZF, Polfa, Warsaw, Poland) polypropylene tubes. The procedure of the blood collection was performed by an experienced ichthyologist without the use of anaesthetics, as it is known that they affect the haematological parameters (e.g. Bishkoul *et al*. 2015; Witeska *et al*. 2017) and plasma biochemical indices (e.g. Velíšek *et al*. 2009; Velisek *et al*. 2011; Lepic *et al*. 2014). This procedure was approved by the Local Ethics Committee and did not raise ethical concerns. Some of the collected blood was used for the haematological analysis, while the rest was centrifuged (3000 g, 20 min) to obtain plasma for a determination of the biochemical parameters. The plasma was stored at -80˚C until the biochemical analysis was performed. After each blood collection, the fish were euthanised with MS 222 (Merck) applied at a concentration of 500 mg/l. The death of the fish was confirmed by decapitation. Then, the gills, liver and trunk kidney were sampled and used for the histological (histopathological) analysis.

Haematological analysis

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). Hayem's solution is one of the diluents routinely used in fish haematological analyses (Witeska *et al*. 2022). The numbers of erythrocytes and leukocytes were counted with a Bürker chamber and a Delta Optical Evolution 300 microscope (100x magnification for the erythrocyte counting and 200x for the leukocyte analysis). The red blood cells were counted in an area of 0.2 mm^2, while the white blood cells were counted in an area of 4 mm^2 (according to Bomski 1983). The counting was completed within 12 hours from the end of the blood collection procedure. The number of erythrocytes/leukocytes per microlitre was estimated using standard formulas (Bomski 1983). For the haematocrit (Ht) determination, glass capillary tubes were filled with the blood to about 3/4 of their volume. Next, they were centrifuged for 5 min using a microhaematocrit centrifuge Type 346 (Unipan, Poland) (according to Bojarski *et al*. 2022). The percentage of the erythrocyte layer was then measured with a standard reader. For the evaluation of the haemoglobin (Hb) concentration, the blood was mixed 1:250 with Drabkin's reagent (Chempur, Poland). The absorbance was read at a 540 nm wavelength using a UV-1601PC UV-visible spectrophotometer (Shimadzu, Japan). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using standard formulas,

according to Bomski (1983). Blood smears were made and stained with May-Grünwald's and Giemsa's solutions (Chempur, Poland) after drying (48-72 hours). Next, the differential leukocyte count (leukogram) was determined at a 1000x magnification using a Nikon Eclipse Ci light microscope. In each smear, 100 white blood cells were inspected (according to Kondera *et al*. 2020; Kondera *et al*. 2021; Bojarski *et al*. 2022). Each haematological parameter was determined in all samples by the same person. The samples were code-labelled and tested 'blind'. For each haematological parameter tested, in the case of each group and at each sampling time, n = 11. In the tables showing the haematological results (Tables 2-4), the statistically significant differences between the experimental groups (CH1 and CH2) and the control group are marked with asterisks. Statistically significant differences between Group CH1 and Group CH2 are denoted with a hash.

Biochemical analysis

Blood plasma was used to measure the concentrations of total protein (TP), glucose (Glu), triglycerides (Tg), cholesterol (Chol), and to determine the alanine aminotransferase (ALT) activity. All the biochemical parameters tested in the current study (except the Hb concentration, which was considered as a haematological parameter) were determined using BioSystems kits (Barcelona, Spain), according to the instructions provided by the manufacturer; the only modification to the procedure was performing the ALT activity calculations in the bases of two absorbance measurement points instead of four points (according to Bojarski *et al*. 2022). The determination of the non-enzymatic biochemical indices was performed with an EPOCH microplate reader (BioTek Instruments, USA). For the testing of the ALT activity, a V-730 UV-visible spectrophotometer (Jasco) was applied. All the samples were code-labelled and tested 'blind'. For the TP, Glu and Tg concentration as well as the ALT activity, in the case of each group and at each sampling time, n = 11; for the Chol concentration, in the case of each group and at both sampling times of one day and three days of exposure, n = 11; for the Chol concentration and sampling time after 10 days of exposure, n = 8 in the case of both the control group and Group CH2, while n = 9 in the case of Group CH1. In the tables showing the biochemical results (Tables 5-7), the statistically significant differences between the experimental groups (CH1 and CH2) and the control group are marked with asterisks. Statistically significant differences between Group CH1 and Group CH2 are denoted with a hash.

Histological analysis

Tissues for the histological examinations were taken from 5 randomly selected specimens, from the control and experimental groups at each sampling time. The organs (gills, livers and trunk kidneys) were fixed in 4% buffered formaldehyde (Chempur, Poland) for two weeks. To remove the fixative, the tissues were firstly rinsed in distilled water for four days (with numerous changes of the water), then put into 75% ethanol changed multiple times for several days. Next, the tissues were dehydrated by immersing them in a graded series of ethanol (70%, 96% and 100%) and cleared with xylene. The dehydrated tissues were then embedded in Paraplast Regular (Sigma, St. Louis, MO, USA), then transverse sections of 6 µm were made using the Zeiss Hyrax M55 microtome and were affixed to glass slides. The slides were stained in a haematoxylin solution according to Delafield (Chempur, Poland; time of staining – 5 minutes), and an eosin solution (Chempur, Poland; time of staining – 5 minutes) for a general cytology. The slides were then enclosed in a Thermo Scientific Shandon Consul Mount, code-labelled for a 'blind' analysis and observed using a light microscope (Nikon Eclipse E600). Images were acquired and processed using a Nikon DS-Fi1c camera and NIS-Elements F software. The histological procedure described above is similar to the procedure applied in our previous experiment (Bojarski *et al*. 2022).

Statistical analysis

The assumption of the compliance of the data with a normal distribution was verified using the Shapiro-Wilk test. It was assumed that this assumption was not fulfilled (in a given case) if, for a given parameter and a given sampling time, the Shapiro-Wilk test returned $p < 0.05$ for at least one of the groups: CH1, CH2 or the control group.

The assumption of the homogeneity of variances was verified using the Levene test.

For the haematological parameters (except the leukogram) and biochemical parameters obtained after 1, 3 and 10 days of exposure, the assumption of the compliance of the data with a normal distribution was assumed to be fulfilled in most cases, as well as the assumption of the homogeneity of variances. The one-way ANOVA test was followed (if significant) by the Tukey HSD test, as a post-hoc was performed.

For the leukogram results obtained after 1, 3 and 10 days of exposure, the assumption of the compliance of the data with a normal distribution was not assumed to be fulfilled in most cases. The Kruskal-Wallis test was followed (if significant) by the Dunn

test with a Bonferroni correction, as a post-hoc was performed.

Furthermore, in two cases (the percentage of monocytes after 3 and 10 days of exposure), all the values were identical (equal to zero), which implied that no testing was necessary.

The statistical calculations were performed in PQStat software, version 1.8.6.114 (ANOVA, Tukey test, Kruskal-Wallis test and Dunn test) and R free software – R Foundation for Statistical Computing, version 4.1.3 (Shapiro-Wilk test and Levene test).

Results

Haematological parameters

The results of the haematological analyses conducted after 1, 3 and 10 days of exposure are presented in Tables 2-4, respectively.

The only one statistically significant change detected after one day of exposure was a lower percentage of lymphocytes in the fish treated with the tested herbicide at the lower concentration, in comparison to the control group (Table 2). The difference was significant at $p = 0.014645$.

After 3 days of exposure, the value of the MCHC parameter in the fish exposed to the herbicide at the lower tested concentration was statistically significantly higher than the value determined in the case of the control individuals (Table 3). The difference was significant at $p = 0.029722$. The percentage of lymphocytes was statistically significantly higher in Group CH2 than in Group CH1, while the percentage of immature neutrophils was statistically significantly higher in Group CH1 in comparison to Group CH2. The differences were significant at $p = 0.018154$ and $p = 0.042982$, respectively.

After 10 days of exposure, the RBC count determined in the fish from Group CH1 was statistically significantly higher compared to the value noted in the case of individuals belonging to the control group. The values of MCV and MCH were statistically significantly lower in the individuals from

Table 2

Haematological changes in the common carp (*Cyprinus carpio*) after 1 day of exposure to Chwastox Extra® 300 SL (mean ± SD; significant differences compared to the control values are marked with asterisks; * $0.01 \leq p < 0.05$; 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; $\alpha = 0.05$; $n = 11$)

Parameter	Control group	Group CH1 (1 mg/l of MCPA)	Group CH2 (5 mg/l of MCPA)
RBC [10^6/µl]	1.65 ± 0.17	1.75 ± 0.14	1.63 ± 0.14
Ht [%]	29.02 ± 4.49	27.77 ± 2.26	26.23 ± 2.60
Hb [g/dl]	7.84 ± 0.89	7.77 ± 0.70	7.58 ± 0.71
MCV [fl]	177.86 ± 35.22	159.97 ± 17.99	161.59 ± 20.89
MCH [pg]	47.96 ± 7.34	44.71 ± 5.11	46.83 ± 6.68
MCHC [g/dl]	27.25 ± 2.70	27.95 ± 0.88	29.06 ± 3.09
WBC [10^3/µl]	22.27 ± 6.75	22.82 ± 5.20	21.59 ± 3.52
Lym [%]	90.36 ± 6.74	79.73 ± 9.07 *	85.36 ± 10.30
Seg [%]	2.82 ± 2.27	6.64 ± 4.27	4.55 ± 3.59
ImNeu [%]	6.64 ± 4.97	13.18 ± 6.49	10.09 ± 7.56
Mono [%]	0.18 ± 0.60	0.45 ± 0.69	0.00 ± 0.00

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 (*Cyprinus carpio*) after 3 days of exposure to Chwastox Extra® 300 SL (mean ± SD; significant differences compared to the control values are marked with asterisks; * $0.01 \leq p < 0.05$; significant differences between Group CH1 and Group CH2 are marked with a hash; # $0.01 \leq p < 0.05$; 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; $\alpha = 0.05$; $n = 11$)

Parameter	Control group	Group CH1 (1 mg/l of MCPA)	Group CH2 (5 mg/l of MCPA)
RBC [10^6/µl]	1.61 ± 0.15	1.62 ± 0.38	1.63 ± 0.19
Ht [%]	28.34 ± 2.65	26.02 ± 3.60	27.86 ± 3.92
Hb [g/dl]	7.09 ± 0.49	7.21 ± 0.93	7.59 ± 0.79
MCV [fl]	177.86 ± 23.16	167.47 ± 39.11	171.94 ± 23.88
MCH [pg]	44.58 ± 5.66	46.53 ± 12.05	47.00 ± 6.28
MCHC [g/dl]	25.11 ± 1.49	27.94 ± 3.60 *	27.40 ± 1.70
WBC [10^3/µl]	23.09 ± 3.48	26.89 ± 9.92	28.93 ± 6.47
Lym [%]	96.64 ± 2.69	95.00 ± 2.83 #	98.27 ± 1.85 #
Seg [%]	0.55 ± 1.21	1.00 ± 1.48	0.55 ± 1.51
ImNeu [%]	2.82 ± 2.18	4.00 ± 3.00 #	1.18 ± 1.40 #
Mono [%]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

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

Group CH1 than in the fish belonging to the control group (Table 4). These changes were significant at $p = 0.030862$, $p = 0.026727$ and $p = 0.012539$, respectively. Moreover, the fish from Group CH1 exhibited a significantly lower MCV in comparison to the fish treated with the herbicide at the higher concentration. This difference was significant at $p = 0.044621$.

Blood (plasma) biochemical parameters

The results of the plasma biochemical analyses performed after 1, 3 and 10 days of exposure are given in Tables 5-7, respectively.

After 1 day of treatment, the concentration of total protein determined in the fish exposed to the tested herbicide at the higher concentration was statistically significantly lower in comparison to the concentration noted in the blood plasma of the specimens from the control group. The difference was significant at $p = 0.002888$. Similarly, the ALT activity was statistically significantly lower in the case of Group CH2 compared to the control group (Table 5). The difference was significant at $p = 0.002885$.

After 3 and 10 days of exposure, no statistically significant changes in the plasma biochemical parameters were detected between the control group and the experimental groups (Tables 6 and 7). Nevertheless, the fish from Group CH1 showed a statistically significantly higher concentration of plasma triglycerides in comparison to Group CH2 after 10 days of treatment. The difference was significant at $p = 0.030093$.

Histology

Histological images of the examined organs are shown in Figs 1-3.

The microstructure of the gills was the same in all specimens, regardless the analysed group of fish. The gills presented a typical architecture, with the primary and secondary lamellae lined with epithe-

Table 4

Haematological changes in the common carp (*Cyprinus carpio*) after 10 days of exposure to Chwastox Extra® 300 SL (mean ± SD; significant differences compared to the control values are marked with asterisks; * $0.01 \leq p < 0.05$; significant differences between Group CH1 and Group CH2 are marked with a hash; # $0.01 \leq p < 0.05$; 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; $\alpha = 0.05$; $n = 11$).

Parameter	Control group	Group CH1 (1 mg/l of MCPA)	Group CH2 (5 mg/l of MCPA)
RBC [10^6/µl]	1.46 ± 0.29	1.70 ± 0.14 *	1.50 ± 0.16
Ht [%]	26.77 ± 5.51	25.86 ± 2.06	27.34 ± 3.64
Hb [g/dl]	7.79 ± 1.51	7.41 ± 0.85	7.60 ± 0.82
MCV [fl]	186.13 ± 38.72	153.14 ± 14.42 * #	183.42 ± 26.10 #
MCH [pg]	54.44 ± 11.81	43.86 ± 5.29 *	50.89 ± 5.45
MCHC [g/dl]	29.30 ± 2.71	28.64 ± 2.31	27.96 ± 2.56
WBC [10^3/µl]	20.23 ± 7.45	22.25 ± 5.05	26.48 ± 6.31
Lym [%]	95.45 ± 2.91	96.45 ± 3.67	97.64 ± 2.46
Seg [%]	0.55 ± 0.69	0.45 ± 0.69	0.09 ± 0.30
ImNeu [%]	4.00 ± 2.79	3.09 ± 3.21	2.27 ± 2.33
Mono [%]	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00

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 5

Biochemical changes in the common carp (*Cyprinus carpio*) after 1 day of exposure to Chwastox Extra® 300 SL (mean ± SD; significant differences compared to the control values are marked with asterisks; ** $0.001 \leq p < 0.01$; the Tukey HSD test; $\alpha = 0.05$; $n = 11$)

Parameter	Control group	Group CH1 (1 mg/l of MCPA)	Group CH2 (5 mg/l of MCPA)
TP [g/l]	20.12 ± 5.96	16.06 ± 2.92	13.55 ± 3.12 **
Glu [mg/dl]	120.62 ± 39.61	113.66 ± 25.29	110.19 ± 25.63
Trigl [mg/dl]	230.60 ± 45.73	204.00 ± 26.80	214.39 ± 26.12
Chol [mg/dl]	218.73 ± 47.43	189.98 ± 65.68	168.37 ± 39.33
ALT [U/l]	60.56 ± 11.73	50.42 ± 8.42	39.23 ± 18.96 **

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

Table 6

Biochemical changes in the common carp (*Cyprinus carpio*) after 3 days of exposure to Chwastox Extra® 300 SL (mean ± SD; the Tukey HSD test; α = 0.05; n = 11)

Parameter	Control group	Group CH1 (1 mg/l of MCPA)	Group CH2 (5 mg/l of MCPA)
TP [g/l]	23.27 ± 1.46	24.25 ± 3.16	25.03 ± 2.43
Glu [mg/dl]	120.49 ± 28.70	132.49 ± 47.09	108.04 ± 19.82
Trigl [mg/dl]	175.03 ± 17.82	181.43 ± 28.70	209.48 ± 81.80
Chol [mg/dl]	155.27 ± 27.77	189.48 ± 82.70	177.11 ± 43.57
ALT [U/l]	50.19 ± 20.56	52.12 ± 17.02	43.03 ± 16.68

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

Table 7

Biochemical changes in the common carp (*Cyprinus carpio*) after 10 days of exposure to Chwastox Extra® 300 SL (mean ± SD; significant differences between Group CH1 and Group CH2 are marked with a hash; $^{\#}$ $0.01 \leq p < 0.05$; the Tukey HSD test; α = 0.05; n = 11 in the case of all the biochemical parameters tested except for cholesterol; for cholesterol, n = 8 in the case of both the control group and Group CH2, n = 9 in the case of Group CH1)

Parameter	Control group	Group CH1 (1 mg/l of MCPA)	Group CH2 (5 mg/l of MCPA)
TP [g/l]	21.67 ± 3.05	24.16 ± 2.63	22.91 ± 2.16
Glu [mg/dl]	106.66 ± 30.48	101.54 ± 25.97	96.63 ± 11.61
Trigl [mg/dl]	212.77 ± 29.91	237.13 ± 34.13 $^{\#}$	203.60 ± 22.41 $^{\#}$
Chol [mg/dl]	153.50 ± 37.72	176.84 ± 26.28	157.89 ± 48.02
ALT [U/l]	72.42 ± 11.88	54.00 ± 14.69	56.94 ± 30.91

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

lium (Fig. 1). None of the pathological changes typical for herbicide exposure (i.e. hypertrophy and hyperplasia of the epithelial cells, proliferation of the mucous cells, lamellar epithelium lifting, fusion of the secondary lamellae, and necrosis of the epithelial cells of the primary and secondary lamellae) were detected.

The microstructure of the liver was also typical – polygonal hepatocytes with homogenous cytoplasm and a centrally located nucleus were observed (Fig 2). Blood sinusoids formed communication channels between the hepatocytes and contained erythrocytes. The experimental groups did not differ from the control group and did not show any symptoms of intoxication in the liver tissue (e.g. cytoplasmic vacuolisation, hypertrophy or deformation of the hepatocytes, nuclear alterations in the hepatocytes, hyperaemia, haemorrhage or necrosis).

The microstructure of the trunk kidney was also typical – nephrons with a renal corpuscle (containing glomerulus), renal tubule, collecting duct and interstitial tissue were observed (Fig. 3). No pathological changes typical for herbicide exposure, such as glomerular degeneration, atrophy of the tubules or tubular necrosis, were detected in the exposed fish.

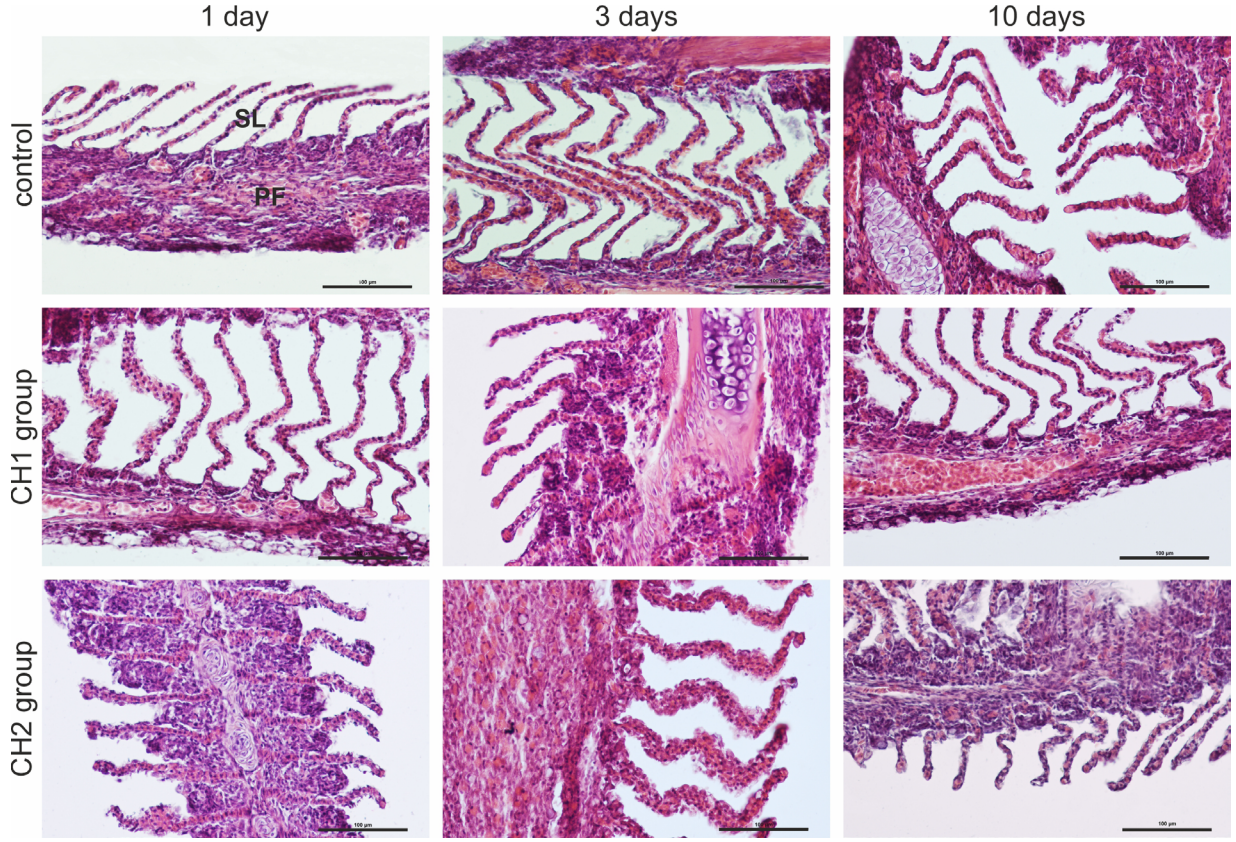

Fig. 1. Photomicrographs of the gills of the common carp in the control and experimental groups. PF – primary filament; SL – secondary lamellae. Haematoxylin-eosin staining. Scale bars: 100 µm. No histopathological changes were found.

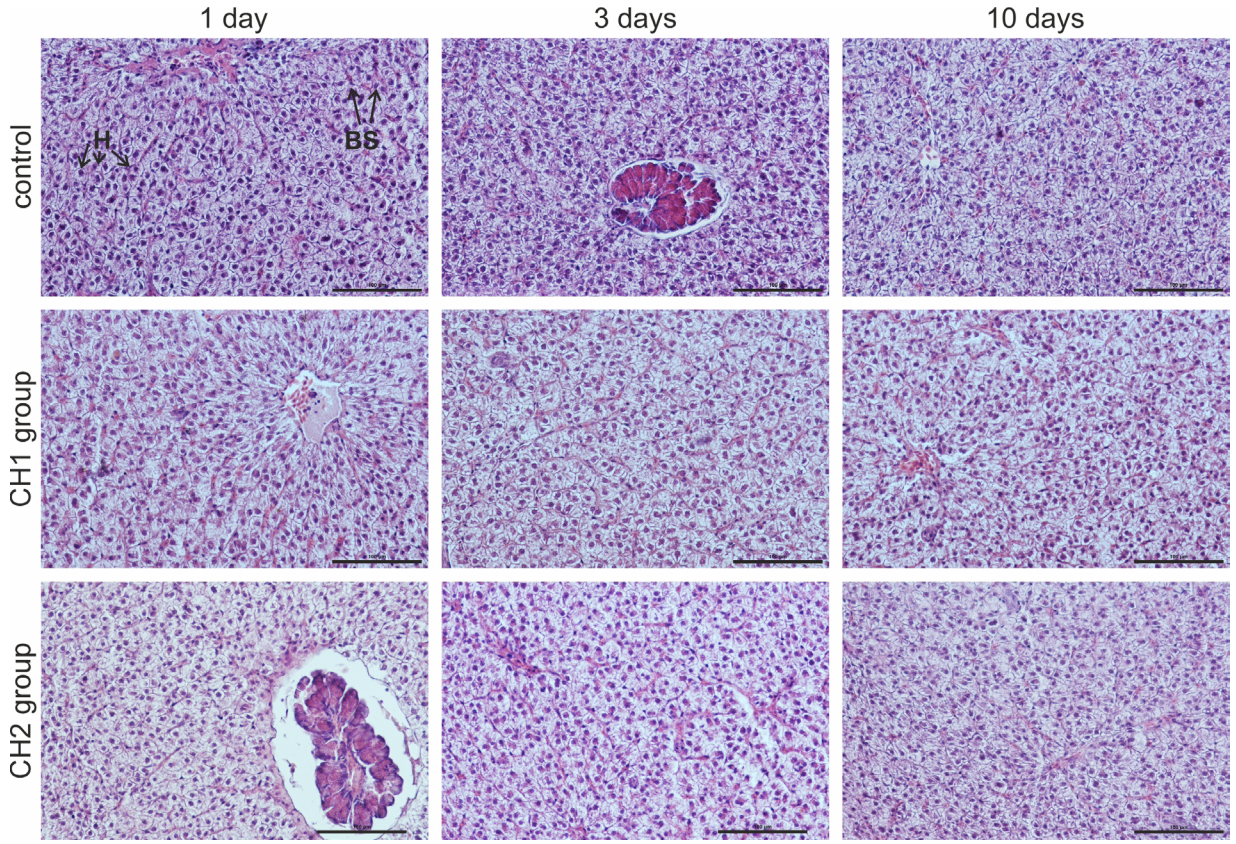

Fig. 2. Photomicrographs of the liver of the common carp in the control and experimental groups. H – hepatocytes; BS – blood sinusoid. Haematoxylin-eosin staining. Scale bars: 100 µm. No histopathological changes were found.

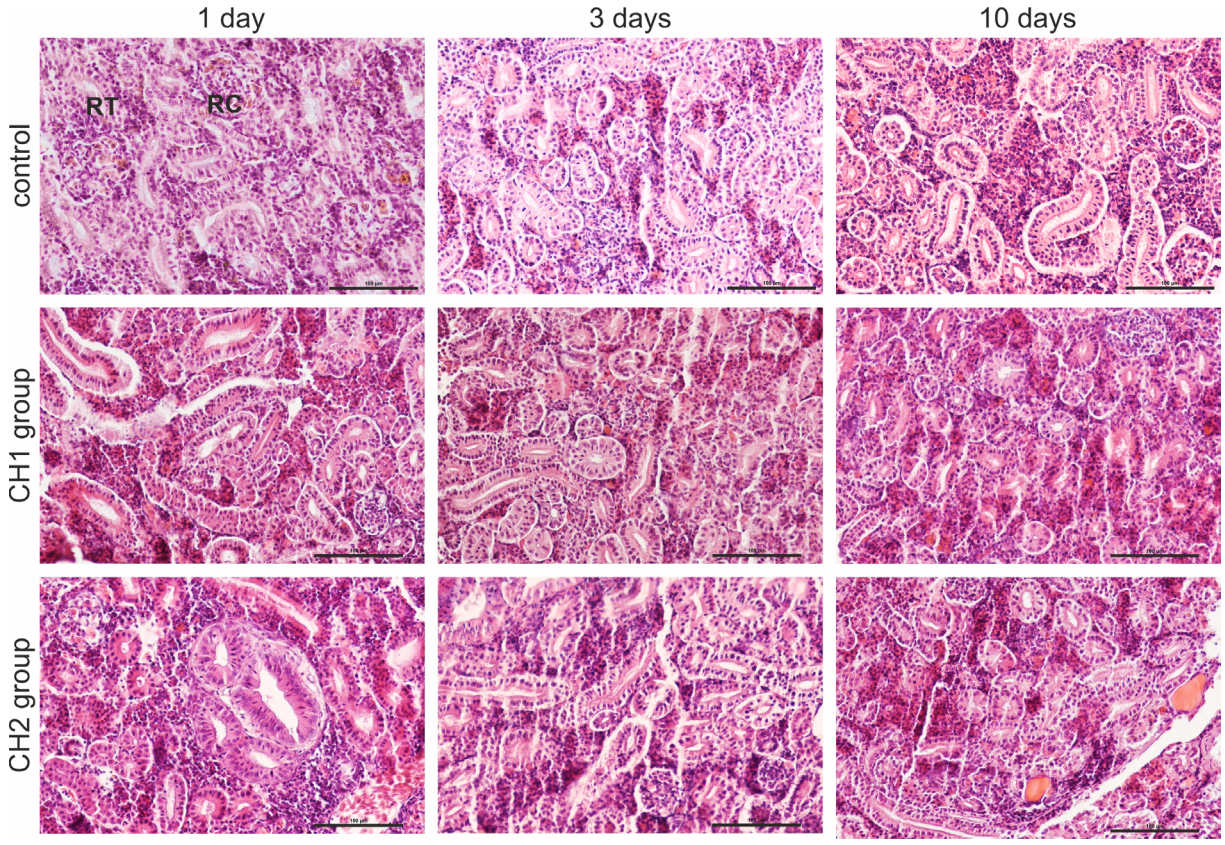

Fig. 3. Photomicrographs of the trunk kidney of the common carp in the control and experimental groups. RC – renal corpuscle; RT – renal tubule. Haematoxylin-eosin staining. Scale bars: 100 µm. No histopathological changes were found.

Discussion

Only minor haematological changes were observed in the present study. The decrease in the percentage of lymphocytes in Group CH1 may indicate a negative effect of the tested herbicide on the immune system of the common carp, while the increased value of the MCHC may suggest a compensatory mechanism; on the other hand the MCV and MCH were found to have decreased. The increase in the RBC count could have been a result of increased erythropoiesis.

The biochemical changes detected in the present study were minor and transient. A decreased concentration of total protein in the blood plasma could be a result of disorders of the liver or the digestive system functioning. A decrease in ALT activity is a rare and difficult to interpret phenomenon. For example, it may potentially be the result of a disorder in the synthesis of the enzyme molecules. Undoubtedly, the haematological and biochemical changes observed in the current study indicate that only minor disorders of homeostasis resulted from the exposure to Chwastox. These changes indicate a low toxicity of the tested herbicide formulation to the common carp. This conclusion generally confirms the data mentioned in the Introduction of the present paper; however, those example refer to MCPA, not MCPA-based formulations.

Lutnicka *et al*. (2018) revealed that common carp treated with MCPA (100 µg/l) showed a higher Hb concentration, MCH and MCHC value in comparison to a control group after 1 day of exposure, while the RBC count was increased after 7 days of exposure. On the other hand, after 14 days of treatment the Hb level and MCHC value were found to have decreased. The authors identified the changes in the red blood cell parameters as minor and transient. After 1 day of exposure a decreased percentage of mature neutrophils was observed, while after 3 days of treatment the lymphocyte ratio had decreased and monocyte percentage had increased. An exposure for 7 days resulted in an increased WBC count, percentage of mature neutrophils and monocyte ratio. However, a longer exposure (14 days) did not result in significant changes in the white blood cell indices. According to the authors, the changes in the white blood cell parameters indicate a possible inflammatory process and immunosuppression caused by

MCPA. *Galaxias maculatus* (Jenyns, 1842) exposed to MCPA-Na exhibited a lower haematocrit level and leukocrit value in comparison to non-treated individuals (Davies *et al*. 1994). On the other hand, the administration of MCPA did not affect the red or white blood cell counts in rats nor rabbits; in this case, the haemoglobin concentration and haematocrit value were also unchanged (Kobal & Budihna 1999).

In the current study, no histological lesions in the gills, liver and trunk kidney were detected. To the best of our knowledge, there is no scientific data on the effects of MCPA-based herbicide formulations on the microstructure of the vital organs of fish. Thus, it was not possible to compare our results with other articles related to these animals. Lutnicka *et al*. (2018) demonstrated that common carp exposed to MCPA exhibited minor ultrastructural anomalies in the hematopoietic tissue. Hematopoietic precursor cells with a blurred ultrastructure, some vacuoles in the cytoplasm, melanomacrophage structures and myelin-like structures were observed. It should also be mentioned that histopathological changes were observed in other vertebrates exposed to MCPA or MCPA-based formulations. The experiment conducted by Takagi (1990) showed that the chronic exposure of mice to MCPA resulted in leukaemia and neoplastic infiltration in the liver. In the study conducted by Bara (2008), it was demonstrated that chick embryos treated with Erbitox E30 (a formulation containing MCPA sodium-potassium salt) exhibited vacuolisation of the hepatocytes. Occasional bile thrombi were also observed. The author revealed that the gallbladder was frequently empty, which could indicate disturbances in the efflux of bile from the liver (Bara 2008). Moreover, Zaffaroni *et al*. (1986) revealed that newts (*Triturus cristatus carnifex*) exposed to Agroxone 3 (a herbicide formulation containing sodium salt of MCPA as the active ingredient) showed vacuolar degeneration of the liver parenchyma.

The differences between the results of the histopathological analysis obtained in the current study and the results obtained by other authors may result from various factors. The above-mentioned studies often refer to pure MCPA, not to herbicide formulations. Moreover, except for one study (i.e. Lutnicka *et al*. 2018), they concern vertebrates other than fish, so the observed differences in the results may be related to taxonomic differences. Furthermore, it can be assumed that the concentration/dose, time and manner of exposure, environmental conditions, physiological state of the animals and individual variability also play an important role.

In the study conducted by Bojarski *et al*. (2022), an attempt was made to determine the toxicity of another herbicide formulation (Roundup® 360 Plus) on the common carp, using the same endpoints as in the current study. Similarly to the present research, the concentrations used corresponded to 1 and 5 mg/l of the active ingredient, which in the case of Roundup® 360 Plus is glyphosate in the form of potassium salt. In the above-mentioned study, a reduction of the RBC count was observed, indicating an anaemic response to the Roundup exposure. The authors hypothesised that the increase in the value of the other erythrocyte parameters (Hb concentration, MCV, MCH and MCHC) that they observed may have indicated the activation of compensatory mechanisms. An increase in the WBC count indicating the presence of inflammation was also observed. The authors added that the presence of an inflammatory state was confirmed by an increase in the percentage of immature neutrophils. The reduction in the percentage of lymphocytes, which was interpreted as a result of a potential immunosuppression, was also detected (Bojarski *et al*. 2022). In the cited study, only minor and temporary changes in the blood biochemical parameters were observed. An increase in the glucose concentration was interpreted as the result of a stress reaction. No histopathological changes in the gills, liver and trunk kidney were detected (Bojarski *et al*. 2022). Overall, the differences in the results obtained in the above-mentioned experiment and the current research suggest a lower toxicity of Chwastox Extra® 300 SL in comparison to Roundup® 360 Plus.

As was demonstrated in the Introduction of the current article, the toxicity of MCPA is rather low. It seems that the other ingredients in Chwastox Extra® 300 SL do not have a significant effect on the toxicity of this formulation. However, this conclusion should be confirmed by further research including other aquatic organisms; in particular, fish belonging to other species and various water invertebrates. We would also like to point out the need to investigate the effects of the simultaneous exposure of fish (and other aquatic organisms) to MCPA (or MCPA-based formulations) and other pesticides. In addition, there is a need to study the concentrations of MCPA in the surface water of particular countries, as the available data may be considered to be out of date and fragmentary.

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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., T.K.-G., A.R.-B.; Data analysis and interpretation: B.B., L.S., A.O., S.H., K.U.; Writing the article: B.B., A.O., L.S; Critical revision of the article: B.B.; Final approval of the article: B.B., L.S., A.O., S.H., K.U., T.K.-G., A.R.-B.

Conflict of Interest

The authors declare no conflict of interest.

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