e-ISSN 1734-9168
http://www.isez.pan.krakow.pl/en/folia-biologica.html

Folia Biologica (Kraków), vol. 66 (2018), No 3
https://doi.org/10.3409/fb_66-3.15

Effects of Herbicides Pendimethalin and Ethofumesate on Common Carp (*Cyprinus carpio*) Erythrocyte Morphology

Bartosz BOJARSKI✉, Hanna LUTNICKA, Magdalena SWADŹBA-KARBOWY, Joanna MAKULSKA, Mateusz JAKUBIAK, Krzysztof PAWLAK, Barbara TOMBARKIEWICZ, and Małgorzata WITESKA

Accepted November 28, 2018 *Published online November 30, 2018* *Issue online November 30, 2018*

Original article

BOJARSKI B., LUTNICKA H., SWADŹBA-KARBOWY M., MAKULSKA J., JAKUBIAK M., PAWLAK K., TOMBARKIEWICZ B., WITESKA M. 2018. Effects of herbicides pendimethalin and ethofumesate on common carp (*Cyprinus carpio*) erythrocyte morphology. Folia Biologica (Kraków) **66**: 143-149.

The effects of 7-day single and combined exposures to herbicides pendimethalin and ethofumesate at 2 concentrations (2.5 and 25 µg/l for pendimethalin and 0.11 and 1.1 mg/l for ethofumesate) on morphology of common carp erythrocytes was studied. Five types of erythrocyte anomalies were determined: nuclear malformation, cell malformation (changed cell shape), cytoplasm vacuolation, cell swelling and hemolysis. Erythroblasts were also counted. After 3 days of treatment increase of percentage of deformed nucleus in fish exposed to mixture of both tested herbicides at higher concentrations in comparison to the control was observed. In fish exposed to the lower concentration of pendimethalin an increase of percentage of deformed cells and hemolysis in comparison to previous sampling occurred. Decrease of percentage of cell vaculation in fish treated with ethofumesate at the lower concentration and increase of percentage of erythroblasts in fish exposed to mixture of both tested herbicides at higher concentrations were also observed. After 7 days of exposure an increase of percentage of deformed cells in comparison to the control was detected in fish exposed to the lower pendimethalin concentration. The observed minor toxic effects were more time- than concentration-related. The obtained results showed low concentrations of pendimethalin and ethofumesate did not induce serious and persistent alterations in erythrocyte morphology of common carp.

Key words: Red blood cells, herbicides, toxicity, freshwater fish.

Bartosz BOJARSKI✉, Małgorzata WITESKA, Department of Animal Physiology, Institute of Biology, Siedlce University of Natural Sciences and Humanities, Prusa 14, 08-110 Siedlce, Poland. E-mail: bbojarski@o2.pl
Hanna LUTNICKA, Institute of Veterinary Science, University Centre of Veterinary Medicine, University of Agriculture in Krakow, Mickiewicza 24/28, 30-059 Krakow, Poland.
Krzysztof PAWLAK, Barbara TOMBARKIEWICZ, Magdalena SWADŹBA-KARBOWY, Department of Veterinary Science, Animal Reproduction and Welfare, Institute of Veterinary Sciences, University of Agriculture in Krakow, Mickiewicza 24/28, 30-059 Krakow, Poland.
Joanna MAKULSKA, Department of Cattle Breeding, Institute of Animal Sciences, University of Agriculture in Krakow, Mickiewicza 24/28, 30-059 Krakow, Poland.
Mateusz JAKUBIAK, AGH University of Science and Technology, Faculty of Mining Surveying and Environmental Engineering, Department of Environmental Management and Protection, Mickiewicza 30, 30-059 Krakow, Poland.

Aquatic environment is constantly loaded with foreign organic chemicals generated as a result of human activity. One of the main threats to surface water is agriculture, which causes pollution with pesticides, including herbicides. Herbicides are considered to be low toxic to mammals (SHANER 2014), however, they can have a negative effect on the fish even at low concentrations (GUILHERME

et al. 2012). Fish organisms represent the final trophic level in the aquatic food web and react to water contamination being sensitive biomonitors of water pollution (DE LEMOS *et al*. 2007). Among different types of herbicides, pendimethalin and ethofumesate have been widely used in Poland to control unwanted weeds. Pendimethalin belongs to the group of the dinitroaniline class. Its primary mode of action is inhibiting the growth of roots and shoots of weeds by preventing plant cell division and elongation. It is used to control annual grasses and broadleaf weeds (GILLIAM *et al*. 1993). Ethofumesate is a representative of benzofuran herbicides. It causes inhibition of lipid synthesis and is used for annual bluegrass control (HAGGAR & KIRKHAM 1981). Herbicides are leached with runoff together with soil particles or are introduced to the water environment due to misapplication (SADOWSKI *et al*. 2014). Pendimethalin enters waters mainly during heavy rainfalls (STRANDBERG & SCOTT-FORDSMAND 2004) being adsorbed to soil or sediment and the amount which reach water ecosystems rapidly decreases (ISENSEE & DUBEY 1983). However, even at low concentrations, this herbicide may cause pathophysiological and histopathological changes in freshwater fish due to hepatotoxic effects (ABD-ALGADIR *et al*. 2011). Pendimethalin is also neurotoxic and genotoxic (AHMAD & AHMAD 2015; TABASSUM *et al.* 2015) and may lead to changes in the hematological profile of fish (BOJARSKI *et al*. 2015). There is very little information concerning the effects of ethofumesate on aquatic organisms and no literature data are available on the mechanisms of toxic action of ethofumesate on fish. Research conducted by BOJARSKI *et al*. (2015) showed that this herbicide caused hematological alterations in *Cyprinus carpio*, similarly as pendimethalin. Hematological analysis is one of the basic tools used to assess toxic effects of aquatic pollution on fish organism. Previous research has shown that hematological indices are reliable and sensitive indicators of pesticide toxicity to fish (LUTNICKA *et al.* 2016). However, detailed hematological studies revealed that morphology of fish erythrocytes is more sensitive to toxic chemicals than basic red blood cell parameters (e.g. WITESKA *et al*. 2010). Thus, various cellular anomalies can be noticed even if the values of such parameters as hematocrit, erythrocyte count or hemoglobin concentration do not change significantly (WITESKA *et al*. 2010). Therefore, investigation of the effects of pendimethalin and ethofumesate action on the common carp erythrocyte morphology may provide more information on the influence of these herbicides on fish organism.

The aim of present study was to evaluate the effects of pendimethalin and ethofumesate in single or combined exposures on morphology of common carp erythrocytes.

Material and Methods

This study was approved by the First Local Ethical Committee on Animal Testing in Krakow, Poland, No. 124/2010 and carried out in accordance with the European Communities Council Directive (86/609/EEC). Common carp (*Cyprinus carpio* L.) juveniles of body mass 80 ± 5.0 g were used. Fish were divided into 7 equal groups: 6 subjected to herbicides and 1 control. The fish harvested from the ponds of the Department of Ichthyobiology and Fisheries, University of Agriculture in Krakow (Poland) were placed in the 800 l tanks, 36 fish in each, and acclimated for two weeks to the laboratory conditions. The values of physical and chemical parameters (temperature 20°C; pH 7.0, dissolved oxygen 7.0 mg O_2/l, and hardness 300 mg $CaCO_3$/l) were appropriate for maintaining the welfare of common carp. Fish of all herbicide-treated groups were subjected constantly to herbicides for 7 days. After 1, 3 and 7 days of exposure blood was sampled from 12 fish from each group by heart puncture using glass capillary tubes.

Two pure herbicide chemicals were used in current research: pendimethalin (certified analytical standard, CAS 40487-42-1) and ethofumesate (certified analytical standard, CAS 26255-79-6). Both herbicides were obtained from the Institute of Industrial Organic Chemistry (Pszczyna, Poland). The lower tested concentration of pendimethalin (2.5 μg/l) was detected in the water environment (SADOWSKI & KUCHARSKI 2007). To our knowledge there is no available data on environmentally realistic concentrations of ethofumesate. Thus, the tested concentrations of this herbicide were chosen on the basis of acute LC_{50} value for *Cyprinus carpio*. The lower tested concentration of ethofumesate (0.11 mg/l) was 1/100 of 96 hour LC_{50}, while the higher one (1.1 mg/l) was 1/10 of 96 hour LC_{50} (PESTICIDE PROPERTIES DATABASE 2014). Fish were subjected to two concentrations of each herbicide: 2.5 μg/l (P1) or 25 μg/l (P2) of pendimethalin and 0.11 mg/l (E1) or 1.1 mg/l (E2) of ethofumesate, and two mixtures: 2.5 μg/l of pendimethalin + 0.11 mg/l of ethofumesate (P1+E1) or 25 μg/l of pendimethalin + 1.1 mg/l of ethofumesate (P2+E2) (Table 1). During the 7 days of exposure water in all tanks was renewed twice to maintain the nominal concentration of the tested herbicides and prevent the accumulation of nitrogen metabolites.

After blood collection blood smears were made and stained with Hemacolor® kit (Merck, USA) according to the manufacturer instructions. The smears were used for evaluation of erythrocyte morphology using light microscope Nikon Eclipse Ci (magnification 1000×). Calculations of

Table 1

Groups of fish used in the experiment and concentrations of tested herbicides

Group	Herbicides	Concentration
Control	none	not applicable
P1	Pendimethalin	2.5 μg/l
P2	Pendimethalin	25 μg/l
E1	Ethofumesate	0.11 mg/l
E2	Ethofumesate	1.1 mg/l
P1+E1	Pendimethalin + Ethofumesate	2.5 μg/l + 0.11 mg/l
P2+E2	Pendimethalin + Ethofumesate	25 μg/l + 1.1 mg/l

the number of immature red blood cells (erythroblasts), normal and abnormal mature erythrocyte frequencies were based on the analyses of 300 cells in each smear, according to WITESKA *et al.* (2010) and WITESKA *et al.* (2011). The obtained data did not show normal distribution and therefore they were analysed using nonparametric Kruskal-Wallis test followed by Bonferroni multiple comparison test using Statistica 13.3 software (Dell Inc., USA). The significance level was set at α=0.05. The data were shown in percent and expressed as mean ±SD.

Results and Discussion

Our study demonstrated that pendimethalin and ethofumesate affected the morphology of common carp erythrocytes, and the observed changes depended on type of herbicide, concentration and time of exposure. The following five types of erythrocyte anomalies were observed: malformations of nucleus, malformation of cell (changed cell shape), vacuolation, swelling and hemolysis. Abnormal nuclei showed distinct cleft (Fig. 1 A), deformed cells had irregular contours (Fig. 1 B), vacuolated erythrocytes showed larger or smaller unstained spots in cytoplasm (Fig. 1 C), swollen cells were distinctly enlarged with pale cytoplasm (Fig. 1 D), while erythrocytes undergoing hemolysis showed disrupted cell membrane and leakage of cytoplasm with hemoglobin outside the cell (Fig. 1 E). Erythroblasts were also identified as round cells smaller than mature erythrocytes, with polychromatophilous cytoplasm and relatively large round nucleus with loose chromatin. All observed erythroblasts had a normal structure. After 1 day of exposure (Table 2) no significant changes were observed. After 3 days (Table 3) significant increase of percentage of deformed nucleus in fish exposed to mixture of both tested herbicides at higher concentrations (P2+E2 group) in comparison to the control was observed (p=0.027109). In fish exposed to lower tested concentration of pendimethalin (P1 group) significant increase of percentage of deformed cells (p=0.004358) as well as hemolysis (p=0.046915) in comparison to the 1st day were noticed. Significant decrease of percentage of cell vaculation (p=0.029351) in animals treated with ethofumesate at lower concentration (E1 group) and increase (p=0.006362) of percentage of erythroblasts in fish of P2+E2 group were detected (Table 3). After 7 days of exposure to tested herbicides (Table 4) significant increase of percentage of deformed cells as compared to the control was observed in P1 group (p=0.000001). There were no statistically significant changes in the percentage of swollen erythrocytes during the experiment.

In our study exposure to herbicides led to occurrence of slight and temporary changes in erythrocyte morphology. The detected toxic effects of the tested chemicals were more time- than concentration-related. The recovery after 7 days of exposure (except for one parameter – deformed cells in fish exposed to pendimethalin at low concentration) indicates the regeneration process taking place in fish despite constant exposure to herbicides.

Most studies on the influence of contaminants present in aquatic environment on fish erythrocyte morphology concern metals, e.g. gallium (YANG & CHEN 2003), zinc (TOMOVA *et al*. 2008) or cadmium (WITESKA *et al*. 2011). Little data about the effects of organic compounds, including pesticides, on the morphology of fish red blood cells has been published. The results obtained by ATEEQ *et al*. (2002) demonstrated that exposure to herbicides: 2,4-D and butachlor changed the morphology of *Clarias batrachus* erythrocytes. Percentage of echinocytes accompanied by nuclear alterations and vacuolation increased in fish exposed to 2,4-D, whereas anisochromasia and anisocytosis of the cells were observed in

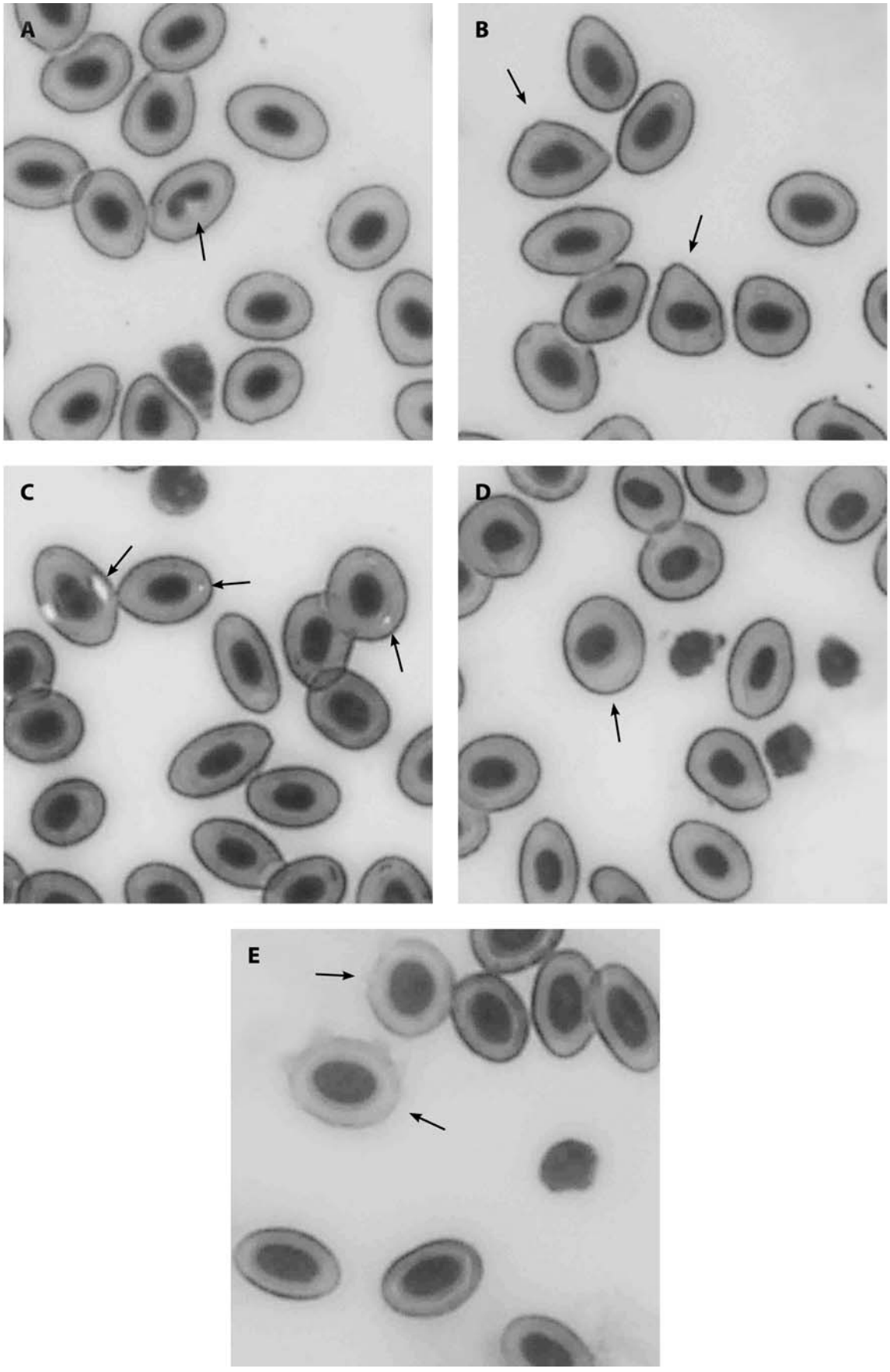

Fig. 1. Examples of erythrocyte anomalies in common carp observed in this study. A – deformed nucleus, B – deformed cells (changed shape of cells), C – vacuolated cells, D – swollen cell, E – hemolysed cells.

Table 2

Erythrocyte morphology (%) after 1 day of common carp exposure to herbicides. No significant differences between control and experimental groups were observed (n=12; α=0.05; Bonferroni multiple comparison test)

Group	Deformed nucleus	Deformed cell	Cytoplasm vacuolation	Cell swelling	Hemolysis	Erythroblasts
Control	0.25±0.45	4.17±1.98	0.92±1.16	0.00±0.00	0.75±2.05	4.42±2.06
P1	0.42±0.67	5.33±1.24	1.00±1.13	0.50±0.54	0.00±0.00	4.25±3.01
P2	1.00±1.75	7.50±2.32	2.92±1.51	0.58±0.68	2.58±3.55	4.83±3.47
E1	0.75±1.22	7.17±1.74	1.75±1.13	0.58±0.79	1.50±2.01	5.25±2.53
E2	2.08±2.35	5.83±1.18	1.08±1.57	0.33±0.51	1.58±2.06	5.33±1.66
P1+E1	0.75±1.06	6.08±2.06	0.92±0.91	0.42±0.53	1.25±1.84	4.67±2.09
P2+E2	0.33±0.79	5.83±1.95	0.92±1.37	0.08±0.29	2.67±2.80	3.58±1.38

Table 3

Erythrocyte morphology (%) after 3 days of common carp exposure to herbicides. Statistically significant differences between control and experimental groups are marked with an asterisk. Significant differences between the 3rd day and the previous sampling (1st day) are marked with ↑ (increase) or ↓ (decrease) (n=12; α=0.05; Bonferroni multiple comparison test)

Group	Deformed nucleus	Deformed cell	Cytoplasm vacuolation	Cell swelling	Hemolysis	Erythroblasts
Control	0.17±0.58	7.42±2.11	0.50±0.80	0.25±0.62	2.92±2.58	4.33±2.34
P1	0.33±0.49	10.42±1.44↑	0.42±0.51	0.08±0.29	2.92±2.03↑	6.42±2.49
P2	1.08±0.78	5.42±1.62	0.83±0.83	0.00±0.00	1.33±1.49	4.75±2.80
E1	1.42±1.56	5.25±1.19	0.08±0.29↓	0.50±0.80	0.75±1.62	4.08±0.99
E2	1.67±1.60	5.25±1.27	0.42±0.68	0.00±0.00	0.83±1.47	5.17±1.47
P1+E1	1.25±1.27	4.92±1.52	0.67±0.91	0.17±0.39	3.25±1.69	4.83±1.94
P2+E2	2.17±1.47*	4.83±1.49	0.42±0.68	0.50±0.52	2.08±1.60	6.92±1.59↑

Table 4

Erythrocyte morphology (%) after 7 days of common carp exposure to herbicides. Statistically significant differences between control and experimental groups are marked with an asterisk. No significant differences between the 7th day and the previous sampling (3rd day) were observed (n=12; α=0.05; Bonferroni multiple comparison test)

Group	Deformed nucleus	Deformed cell	Cytoplasm vacuolation	Cell swelling	Hemolysis	Erythroblasts
Control	0.75±0.77	2.83±1.11	0.17±0.39	0.08±0.29	2.00±1.36	5.17±0.69
P1	0.58±0.79	8.75±2.63*	0.17±0.39	0.08±0.29	0.08±0.29	3.42±1.64
P2	0.75±0.74	5.42±0.92	0.08±0.29	0.00±0.00	1.17±1.67	4.25±1.00
E1	1.33±1.85	5.58±2.00	0.42±0.79	0.08±0.29	2.50±2.44	5.00±1.60
E2	0.25±0.87	6.08±2.69	0.08±0.29	0.00±0.00	1.08±1.57	4.17±1.70
P1+E1	0.50±0.66	5.50±0.99	0.08±0.29	0.00±0.00	2.50±1.92	6.08±1.30
P2+E2	0.58±0.65	5.83±1.44	0.58±0.78	0.00±0.00	0.83±1.77	5.08±1.29

butachlor-treated fish. The percentage of malformations increased in a time- and concentration-dependent way. The research conducted by RUIZ DE ARCAUTE *et al*. (2016) showed that 2,4-D treatment of *Cnesterodon decemmaculatus* led to increase in percentage of nuclear abnormalities. SAWHNEY & JOHAL (2000) revealed that insecticide malathion led to anisocytosis and swelling of erythrocytes in *Channa punctata*. Number of swollen cells was increasing during the insecticide exposure. Abnormal (shrunken) erythrocytes were observed in *Anabas testudineus* exposed to insecticide cypermethrin (BABU *et al.* 2014).

Our research and the referred studies demonstrated that the exposure of fish to various types of pesticides may result in disturbances in their erythrocyte morphology. The results of our study showed that low concentrations of pendimethalin and ethofumesate induced only minor and temporary alterations in carp erythrocyte morphology.

Acknowledgments

The study was carried out in the University of Agriculture in Krakow, Faculty of Animal Science, Institute of Veterinary Sciences, Department of Veterinary Science, Animal Reproduction and Welfare; Al. Mickiewicza 24/28, 30-059 Krakow, Poland.

This study has been supported by grant 387/14/S granted by the Ministry of Science and Higher Education (Department of Animal Physiology, Siedlce University of Natural Sciences and Humanities, Siedlce, Poland), grant N N304 279440 (National Science Center, Poland) and DS 3263/ZWRiDZ (Department of Veterinary Science, Animal Reproduction and Welfare, University of Agriculture in Krakow, Poland).

Author Contributions

Research concept and design: B.B.; Collection and/or assembly of data: B.B., H.L., M.S., M.J.; Data analysis and interpretation: B.B., J.M., K.P., M.W.; Writing the article: B.B., M.S., M.J., B.T.; Critical revision of the article: B.B., H.L., K.P., B.T.; M.W.; Final approval of article: B.B., K.P., B.T., M.W.

Conflict of Interest

The authors declare no conflict of interest.

References

ABD-ALGADIR M.I., IDRIS O.F., ELKHIER M.K.S. 2011. Effect of pendimethalin herbicide on fish (*Tilapia nilotica*) skeletal muscles, gills and its influence on human. World J. Life Sci. Med. Res. **1**: 5-10.

AHMAD I., AHMAD M. 2015. Fresh water fish, *Channa punctatus*, as a model for pendimethalin genotoxicity testing: a new approach toward aquatic environmental contaminants. Environ. Toxicol. **31**: 1520-1529. https://doi.org/10.1002/tox.22156

ATEEQ B., FARAH M.A., ALI M.N., AHMAD W. 2002. Induction of micronuclei and erythrocyte alterations in the catfish *Clarias batrachus* by 2,4-dichlorophenoxyacetic acid and butachlor. Mut. Res. **518**: 135-144. https://doi.org/10.1016/S1383-5718(02)00075-X

BABU V., MARIADOSS S., IPEK C.E., SERBEST B., ALID S. 2014. Surface structures of gill, scale and erythrocyte of *Anabas testudineus* exposed to sublethal concentration of cypermethrin. Environ. Toxicol. Pharmacol. **37**: 1109-1115. https://doi.org/10.1016/j.etap.2014.04.009

BOJARSKI B., LUDWIKOWSKA A., KUREK A., PAWLAK K., TOMBARKIEWICZ B., LUTNICKA H. 2015. Hematological alterations in common carp (*Cyprinus carpio* L.) exposed to herbicides: pendimethalin and ethofumesate tested separately and in mixture. Folia Biol. (Kraków) **63**: 167-174. https://doi.org/10.3409/fb63_3.167

DE LEMOS C.T., RODEL P.M., TERRA N.R., DE OLIVEIRA N.C.D., ERDTMANN D. 2007. River water genotoxicity evaluation using micronucleus assay in fish erythrocytes. Ecotoxicol. Environ. Saf. **66**: 391-401. https://doi.org/10.1016/j.ecoenv.2006.01.004

GILLIAM C.H., EAKES D.J., OLIVE J.W. 1993. Herbicide use during propagation affects root initiation and development. J. Environ. Horticult. **11**: 157-159.

GUILHERME S., GAIVAOB I., SANTOSA M.A., PACHECOA M. 2012. DNA damage in fish (*Anguilla anguilla*) exposed to a glyphosate-based herbicide – Elucidation of organ-specificity and the role of oxidative stress. Mut. Res. Genet. Toxicol. Environ. Mutagen. **743**: 1-9. https://doi.org/10.1016/j.mrgentox.2011.10.017

HAGGAR R.J., KIRKHAM F.W. 1981. Selective herbicides for establishing weed-free grass. I. Evaluation of ethofumesate and methabenzthiazuron. Weed Res. **21**: 141-151. https://doi.org/10.1111/j.1365-3180.1981.tb00109.x

ISENSEE A.R., DUBEY P.S. 1983. Distribution of pendimethalin in an aquatic microecosystem. Bull. Environ. Contam. Toxicol. **30**: 239-244. https://doi.org/10.1007/BF01610128

LUTNICKA H., BOJARSKI B., LUDWIKOWSKA A., WROŃSKA D., KAMIŃSKA T., SZCZYGIEŁ J., TROSZOK A., SZAMBELAN K., FORMICKI G. 2016. Hematological alterations as a response to exposure to selected fungicides in common carp (*Cyprinus carpio* L.). Folia Biol. (Kraków) **64**: 235-244. https://doi.org/10.3409/fb64_4.235

PESTICIDE PROPERTIES DATABASE – PPDB, University of Hertfordshire, https://sitem.herts.ac.uk/aeru/ppdb, 2014.

RUIZ DE ARCAUTE C., SOLONESKI S., LARRAMENDY M.L. 2016. Toxic and genotoxic effects of the 2,4-dichlorophenoxyaceticacid (2,4-D)-based herbicide on the Neotropical fish *Cnesterodon decemmaculatus*. Ecotoxicol. Environ. Saf. **128**: 222-229. https://doi.org/10.1016/j.ecoenv.2016.02.027

SADOWSKI J., KUCHARSKI M. 2007. Monitorowanie stanu zanieczyszczeń herbicydowych w wodach powierzchniowych i gruntowych na terenach rolniczych. [Monitoring of the status of herbicide contamination in surface water and groundwater in agricultural areas.] Studia i Raporty IUNG-PIB **8**: 87-97. (In Polish).

SADOWSKI J., KUCHARSKI M., DZIĄGWA M. 2014. Influence of changes in the scope of registered plant protection products on the level of herbicide contamination of waters in agricultural areas. Progr. Plant Prot. **54**: 191-197. (In Polish).

SAWHNEY A.K., JOHAL M.S. 2000. Erythrocyte alterations induced by malathion in *Channa punctatus* (Bloch). Bull. Environ. Contam. Toxicol. **64**: 398-405.

SHANER D.L. 2014. Herbicide safety relative to common targets in plants and mammals. Pest. Manag. Sci. **60**: 17-24. https://doi.org/10.1002/ps.782

STRANDBERG M., SCOTT-FORDSMAND J.J. 2004. Effects of pendimethalin at lower trophic levels – a review. Ecotoxicol. Environ. Saf. **57**: 190-201. https://doi.org/10.1016/j.ecoenv.2003.07.010

TABASSUM H., AFJAL M.A., KHAN J., RAISUDDIN S., PARVEZ S. 2015. Neurotoxicological assessment of pendimethalin in freshwater fish *Channa punctata* Bloch. Ecol. Indic. **58**: 411-417. https://doi.org/10.1016/j.ecolind.2015.06.008

TOMOVA E., ARNAUDOV A., VELCHEVA I. 2008. Effects of zinc on morphology of erythrocytes and spleen in *Carassius gibelio*. J. Environ. Biol. **29**: 897-902.

WITESKA M., KONDERA E., SZCZYGIELSKA K. 2011. The effects of cadmium on common carp erythrocyte morphology. Polish J. Environ. Stud. **20**: 783-788.

WITESKA M., KONDERA E., SZYMAŃSKA M., OSTRYSZ M. 2010. Hematological changes in common carp (*Cyprinus carpio* L.) after a short-term lead (Pb) exposure. Polish J. Environ. Stud. **19**: 825-831.

YANG J.L., CHEN H.C. 2003. Effects of gallium on common carp (*Cyprinus carpio*): acute test, serum biochemistry, and erythrocyte morphology. Chemosphere **53**: 877-882. https://doi.org/10.1016/S0045-6535(03)00657-X