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Physiological and histological effects of herbicides in fish

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Abstract: *Physiological and histological effects of herbicides in fish*. Among pesticides used in agriculture, herbicides are used in the largest quantities. These substances enter the aquatic environment using many ways, and monitoring studies show their permanent presence in natural waters. Herbicides cause changes in many physiological parameters in fish, e.g. reduction of acetylcholinesterase activity in internal organs, increased activities of hepatic transaminases or oxidoreductive balance disturbances. Alterations in hematological parameters are also observed, usually indicating anemia and inflammatory process. The most frequently observed histological changes include hyperplasia and hypertrophy of gill epithelium and changes in liver microstructure, such as vacuolation of hepatocytes. Markers of fish exposure to herbicides are generally very sensitive but nonspecific. Analysis of metabolic, hematological and histological parameters of tissues can be helpful in determining fish poisoning with herbicides, but it should always be confirmed by monitoring studies.

Key words: intoxication, pesticides, fish health

INTRODUCTION

Herbicides are chemicals widely used to protect crops by controlling unwanted plants (weeds) in agriculture. They include very diverse group of pesticides – more than 200 active compounds in several thousand commercial formulas. Most herbicides are selective and their selectivity is related mainly to the technique and timing of application adjusted to the stage of development of protected and target plants. Another mechanism of herbicide selectivity derives from the different metabolism of weed and crop plant species. Most of contemporary herbicides are organic compounds (Woznica 2008). Herbicides applied in agriculture may reach aquatic environment using various routes. According to Sadowski et al. (2014), herbicide particles are leached with runoff together with soil particles or are introduced directly to the water due to misapplication (e.g. pouring away spray leftovers, sprayer washing). Monitoring of surface and ground water pollution with herbicides is an important issue in evaluation of the natural environment quality. Contamination of natural waters with herbicides was observed in Poland (Ignatowicz 2006, Sadowski and Kucharski 2007, Sadowski et al. 2014) and in many other countries, e.g. in Italy (Achilli et al. 1995), USA (Clark and Goolsby 2000), Portugal (Cerejeira et al. 2003) or Australia (Tran et al. 2007). Herbicides in water may adversely affect aquatic organisms. Exposure to herbicides causes various pathophysiological effects in fish including metabolic disturbances manifested as changes in enzymatic activity and other biochemical alterations, histopathological lesions and hematological changes. The aim of this work was to review the literature concerning these issues.

PHYSIOLOGICAL EFFECTS

Acetylcholinesterase activity

In fish exposed to herbicides reduction in acetylcholinesterase (AChE) activity is often reported. According to Glusczak et al. (2007), 96 h exposure of *Rhamdia quelen* to glyphosate (0.2 and 0.4 $mg \cdot l^{-1}$) resulted in a decrease of AChE activity in fish brain. *Carassius auratus* exposed for 48 h to 0.5 $mg \cdot l^{-1}$ of carbofuran also showed reduced brain and muscle AChE activity. Similar effect in brain of the same species was observed after 24 and 48 h diuron (0.5 $mg \cdot l^{-1}$, 24 h) and nicosulfuron (0.5 $mg \cdot l^{-1}$) treatments (Bretaud et al. 2000). In *Leporinus obtusidens* exposed for 96 h to 0.5 $mg \cdot l^{-1}$ of clomazone AChE activity was reduced in brain and cardiac muscle, while after 192 h – also in skeletal muscles and eye (dos Santos Miron et al. 2008). Similar results of exposure to the same herbicide (after 12, 24, 48, 96 and 192 h) were observed in brain and muscles of *Rhamdia quelen* (Crestani et al. 2007). Decrease in brain and muscle AChE activities were also reported after 96-hour exposure of *Leporinus obtusidens* to 10 $mg \cdot l^{-1}$ of 2,4-D (da Fonseca et al. 2008). Short-term (96 h) treatment of *Rhamdia quelen* to 600 and 700 $mg \cdot l^{-1}$ of 2,4-D resulted, however, in an increase in brain AChE activity, accompanied by a decrease in muscle (Cattaneo et al. 2008). Exposure of the same fish species to metsulfuron (400, 800 and 1,200 $mg \cdot l^{-1}$) caused reduction of muscle AChE activity and increase in brain (dos Santos Miron et al. 2005). The disturbances of acetylcholinesterase activity considerably affect fish due to a key role of this enzyme in maintaining proper levels of the neurotransmitter acetylcholine in the central and peripheral nervous system. The literature data show that herbicides usually inhibit the activity of this enzyme. Inhibition of AChE activity can impair locomotion and equilibrium in exposed fish (Bretaud et al. 2000). Behavioral effects of waterborne atrazine, diuron and carbofuran observed by Saglio et al. (1996) and Saglio and Trijasse (1998) in *Carassius auratus* also seem to be associated with changes in AChE activity.

Aminotransferases activity

Exposure of fish to herbicides usually causes increase in activities of aminotransferases: alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Treatment of *Cyprinus carpio* to 0.005; 0.01 and 0.02 $mg \cdot l^{-1}$ of trifluralin resulted in elevated gill, liver and serum AST activities, while gill, liver and kidney ALT activities increased only at the highest herbicide concentration. Increased ALT activity was observed in *Rhamdia quelen* after 20 days of glyphosate (3.6 $mg \cdot l^{-1}$) exposure (Soso et al. 2007). Exposure to paraquat (2 h at 1 and 10 $mg \cdot l^{-1}$) caused an increase in ALT and AST activities in *Cyprinus carpio*, *Hypophthalmichthys molitrix* and *Silurus glanis* (Nemcsok et al. 1985). The increase in serum AST and ALT activity might have been related to release of enzymes from the cytosol of damaged cells to the extracellular fluid indicating hepatocyte injury or necrosis (Poleksic and Karan 1999).

Metabolic parameters

Alterations in enzymatic activity observed in fish exposed to various herbicides were often accompanied by changes in other metabolic parameters. Exposure of *Rhamdia quelen* to 0.2 and 0.4 mg·l^{-1} of glyphosate for 96 h caused an increase in hepatic glycogen, lactate, protein and ammonia, while hepatic glucose level decreased. At the same time muscle glycogen and protein levels decreased, while lactate, glucose and ammonia concentrations increased (Glusczak et al. 2007). *Leporinus obtusidens* treated with 10 mg·l^{-1} of 2,4-D showed reduction in muscle glycogen and lactate, increase in protein content, while hepatic protein and lactate were reduced. The fish exposed to 1 and 10 mg·l^{-1}of herbicide showed also a decrease in plasma glucose (da Fonseca et al. 2008). Exposure of *Rhamdia quelen* for 96 h to 400, 600 and 700 mg·l^{-1} of 2,4-D resulted in a drop in hepatic and muscle glycogen, while lactate level decreased in liver and increased in muscle (Cattaneo et al. 2008). Exposure of *Prochilodus lineatus* to 10 mg·l^{-1} of Roundup increased plasma glucose after 24 and 96 h and decreased chloride level after 24 h (do Carmo Langiano and Martinez 2008). The listed metabolic parameters showed a decrease or increase due to exposure to herbicides. The variety of results obtained by various authors makes them difficult to interpret. Therefore, these physiological parameters do not seem to be good indicators of fish exposure to herbicides.

Oxidoreductive balance

Herbicides were also reported to disturb oxidoreductive balance in fish. In *Prochilodus lineatus* exposed to 10 mg·l^{-1} of Roundup activity of glutathione peroxidase (GPx) was reduced after 6 h, and after 24 h – also activity of superoxide dismutase (SOD) decreased. After 24 and 96 h activity of glutathione transferase (GST) increased. After 6 and 24 h of Roundup exposure hepatic glutathione content also increased (Modesto and Martinez 2010). Exposure of *Carassius auratus* for 96 h to 2.5, 5, 10 and 20 mg·l^{-1} of Roundup resulted in a decrease in brain and kidney SOD activity, while activities of hepatic GST, brain, kidney and liver glutathione reductase (GR) were reduced (Lushchak et al. 2009). A long-term (90 days) exposure to 0.376 mg·l^{-1} of clomazone caused a decrease in hepatic catalase (CAT) activity in *Leporinus obtusidens* (Moraes et al. 2009). Similar effect was observed after 96 and 192 h exposure to the same herbicide at the concentration 0.5 mg·l^{-1} (dos Santos Miron et al. 2008). *Rhamdia quelen* exposed to 0.5 mg·l^{-1} of clomazone showed reduction in hepatic CAT activity after 48, 96 and 192 h, while at 1 mg·l^{-1} of clomazone activity of hepatic CAT decreased already after 12 h of exposure and remained reduced until 192 h post exposure (Crestani et al. 2007). Activity of CAT decreased also in *Leporinus obtusidens* after 90 days treatment with propanil (1.644 mg·l^{-1}) (Moraes et al. 2009). On the other hand, *Carassius auratus* exposed for 96 h to 2.5, 5, 10 and 20 mg·l^{-1} of Roundup showed an increase in hepatic (at 2.5 and 5 mg·l^{-1}) and kidney (at all concentrations) CAT activity (Lushchak et al. 2009). Paraquat (1 mg·l^{-1}, 24 h) induced an increase in ascorbic acid concentration in liver of *Channa punctata*, while the level of uric acid increased in kidney and decreased in gill (Parvez and Raisuddin 2006). These data show that herbicides may disturb the oxido-reductive balance in fish organism. It is not clear whether it is a result of their direct prooxidative activity or oxidative stress is a result of e.g. inflammation process caused by herbicide-induced tissue lesions. Analysis of available literature allows to conclude that one of the often studied oxidative stress indicator, catalase, seems to be a reliable indicator of chemical stress in fish caused by waterborne herbicides. Most authors observed a decrease of the CAT activity after exposure to this group of pesticides.

Hematological parameters

The basic indicators of chemical stress in fish include hematological parameters. Velisek et al. (2009) revealed that short-term (96 h) metribuzin exposure of *Cyprinus carpio* (175.1 mg·l^{-1}) caused a decrease in Hct, Hb, MCV, and WBC values. Sub-chronic exposure of the same fish species to the terbutryn (2, 20, and 40 mg·l^{-1}) led to an increase in RBC, while MCV and MCH values were significantly reduced (Velisek et al. 2010). Short-time (96 h) Roundup exposure (3, 6, 10, and 20 mg·l^{-1}) of *Leporinus obtusidens* decreased RBC and Hct values and Hb level (Glusczak et al. 2006). Ramesh et al. (2009) showed that acute atrazine treatment (18.5 mg·l^{-1}, 24 h) caused reduction of RBC count and Hb content compared to control group, whereas WBC increased. Hussein et al. (1996) revealed that the exposure of *Oreochromis niloticus* and *Chrysichthyes auratus* to 3 and 6 mg·l^{-1} of atrazine resulted in a decrease of RBC, Hb and Hct as compared the control group in both species. Also, there were significant changes of MCV, MCH and MCHC for both species. The investigators noticed that *Chrysichthyes auratus* were much more affected by atrazine exposure than *Oreochromis niloticus*. Dobsikova et al. (2011) exposed *Cyprinus carpio* to 13 mg·l^{-1} of Gardoprim Plus Gold 500 SC (corresponding to 2.25 mg·l^{-1} and 3.75 mg·l^{-1} of terbuthylazine and S-metolachlor, respectively) for 96 h. Exposure to the preparation caused a decrease of Hct and WBC values, as well as lymphocyte count. Studies conducted by Modesto and Martinez (2010) demonstrated an increase of Hct, RBC, and WBC in *Prochilodus lineatus* exposed to Roundup Transorb® (5 mg·l^{-1}) after 24 and 96 h of exposure. Increase in the percentage of lymphocytes and decreased percentage of neutrophils after 96 h exposure were also observed. Kreutz et al. (2011) revealed that RBC and WBC were decreased in the blood of *Rhamdia quelen* from the glyphosate-exposed fish (96 h; 0.73 mg·l^{-1}) as compared to the non-exposed fish. However, Hct level did not change. Crestani et al. (2006) revealed that clomazone caused a decrease of Ht value after 96 h of exposure at a concentration of 0.05 and after 192 h at 0.05 and 1.0 mg·l^{-1} in *Rhamdia quelen*. After 192 h of purification the Hct level in treated fish (0.5 and 1.0 mg·l^{-1}) were similar to control values. Butachlor administration led to various hematological alterations in *Labeo rohita*. RBC decreased in fish exposed to tested herbicide at concentration of 0.5 to 1.0 mg·l^{-1} for 72 and 96 h of exposure, as well as in fish exposed to concentration of 0.75 to 1.0 mg·l^{-1}for 48 h. The Hb concentration decreased significantly in fish after 96 h at 0.5 to 1.0 mg·l^{-1} and after 72 h at 1.0 mg·l^{-1}. Hct was reduced in fish treated with herbicide at concentration of 0.75 and 1.0 after 48 h of exposure and in groups of 0.5–1.0 mg·l^{-1} after 72 and 96 h of exposure. The WBC was increased in all tested concentrations of butachlor and times of fish exposure (Ghaffar et al. 2015). Experiments conducted by Bojarski et al. (2015) revealed that exposure of *Cyprinus carpio* to pendimethalin at concentration of 2.5 µg·l^{-1} resulted in significant increase of Hb content and MCHC value after 7 days of treatment. Higher concentration (25 µg·l^{-1}) led to decrease of Hb level after 1 day, decrease of Hct and increase of MCH after 3 days and increase of RBC and Hct values and Hb content after 7 days of herbicide exposure. Ethofumesate (0.11 µg·l^{-1}) caused increase of WBC after 3 days of exposure, while longer treatment (7 days) led to increase of RBC and Hct values and Hb content. The same herbicide at higher concentration (1.1 µg·l^{-1}) caused increase of Hb level and MCHC value after 3 days of exposure. In fish exposed to mixture of both herbicides (2.5 µg·l^{-1} of pendimethalin + 0.11 µg ·l^{-1} of ethofumesate) increased RBC and Hct values after 1 as well as 3 days of exposure were observed. Fish exposed simultaneously to pendimethalin and ethofumesate at higher concentrations (25 and 11 µg·l^{-1}, respectively) exhibited similar hematological alterations: RBC and Hct were increased after 1 and 3 days of exposure, while MCHC was decreased (1 day), and Hb content was increased (3 days). In contrast to above, longer exposure (7 days) resulted in reduction of RBC and Hct values and increase of MCV and MCHC. Also, exposure to molinate of *Anguilla anguilla* at sublethal concentration of 11.15 mg·l^{-1} for 96 h showed the effects in hematological indices. Hct, Hb, RBC and WBC decreased only during recovery period (72 and 96 h) after exposure (Sancho et al. 2000). Based on the literature data it can be concluded that the fish organism usually shows anemic response as a result of herbicide pollution which probably results from direct action of herbicides on circulating erythrocytes and shortening of their life span. Sometimes observed increase in the values of red blood parameters may be related to stress reaction. The increase in WBC observed by some authors may be a result of inflammation process caused by the action of waterborne herbicides on fish organs.

HISTOLOGICAL EFFECTS

Gills histopathology

Fish exposure to herbicides may also lead to pathological changes in various internal organs, including kidney (Jiraungkoorskul et al. 2002) and brain (Deivasigamani 2015). However, majority of studies concerning the influence of these substances on fish organism focus on the structure of gills and liver. Very often histological lesions are observed in gills being the site of first contact with environmental pollutants. They relatively quickly react to changes in environmental conditions and the presence of toxic substances in water. In the gills of *Cyprinus carpio* exposed to 0.01 mg·$\cdot l^{-1}$ of trifluralin for 14 days Poleksic and Karan observed hyperplasia of secondary lamellae and their partial fusion accompanied by folding of respiratory epithelium and hypertrophy of some chloride cells. At the concentration of 0.02 mg·l^{-1} trifluralin caused local lifting of secondary lamellae epithelium and its hyperplasia which resulted in lamellar deformation and fusion, while chloride cells were hypertrophic (Poleksic and Karan 1999). *Labeo rohita* exposed for 5 days to 0.18 mg·l^{-1} of atrazine showed increased gill mucosecretion, hyperplasia of primary lamellae and their merging, while in secondary lamellae separation of epithelium and lamellar fusion were observed. Destruction of pillar cells was also noted accompanied by vacuolation and necrosis of secondary lamellae epithelium (Jayachandran and Pugazhendy 2009). The effects of seven-day exposure of *Ctenopharyngodon idella* to 0.5 and 1 mg·l^{-1} of atrazine included local epithelial cell proliferation, epithelium lifting and fusion of secondary lamellae (Botelho et al. 2012). *Channa punctatus* exposed for 10 days to 1.21 mg·l^{-1} of alachlor showed deformed primary and secondary gill lamellae. Secondary lamellae were shortened and often fused, while their epithelial cells often showed vacuolation. Karyorhexis of pillar cells was also observed (Butchiram et al. 2009). According to Gosiewski et al. (2012), exposure to 15 mg·l^{-1} of Roundup caused edema, hypertrophy and hyperplasia of secondary lamellae epithelium. These changes were mode pronounced after 10 days of exposure compared to 6 months. Hued et al. (2012) reported that short-term (96 h) treatment of *Jenynsia multidentata* with 5 mg·l^{-1} of Roundup resulted in edema, hypertrophy and separation of secondary epithelium. Higher concentration (10 mg·l^{-1}) caused focal separation of secondary lamellae epithelium. In the lamellae dilated capillaries and chloride cell hypertrophy were also observed. At 20 mg·$l^{-1}$of Roundup slight thickening of secondary lamellae occurred due to epithelial hyperplasia. At the highest concentration – 35 mg·l^{-1} increased mucosecretion and considerable epithelial hyperplasia were observed which resulted in lamellar fusion. Subchronic exposure (28 days) to the same herbicide at the concentration 0.5 mg·l^{-1} caused strong mucosecretion and shortening of secondary lamellae (Hued et al. 2012). After 48 h of exposure to 36 mg·l^{-1} of Roundup *Oreochromis niloticus* showed different thickness of primary lamellae epithelium, edema and lifting of secondary lamellae epithelium and leukocyte infiltration among the epithelial cells (Jiraungkoorskul et al. 2002); after 96 h – apical enlargement of secondary lamellae and considerable epithelium thickening in primary lamellae. The results obtained by Ayoola (2008a) revealed cellular infiltration between the secondary lamellae in *Oreochromis niloticus* exposed for 96 h to 2, 9 and 97 mg·l^{-1} of glyphosate, uneven epithelium thickness at 30 mg·l^{-1} and gill edema and necrosis at 310 mg·l^{-1}. The gills of *Clarias gariepinus* treated with glyphosate for 96 h Ayoola observed cellular infiltration (at 19, 42, 94, 207 and 455 mg·l^{-1}), congestion (at 42, 94, 207 and 455 mg·l^{-1}), hemorrhages (at 94, 207 and 455 mg·l^{-1}) and necrotic lesions (at 207 and

455 mg·l^{-1}) (Ayola 2008b). These data indicate that herbicide poisoning leads primarily to hyperplasia and hypertrophy of the gill epithelium, and thus probably may impair the functions of this vital organ: respiration, osmoregulation and secretion. Therefore, it can be assumed that the exposure of fish to herbicides may indirectly cause further physiological disorders such as hypoxemia, hypercapnia and osmotic disturbances.

Liver histopathology

Liver in which metabolism of xenobiotics takes place is another organ that often shows pathological lesions in intoxicated fish. Unfortunately, the data concerning hepatic histpathologies in fish due to herbicide exposures are scarce. *Cyprinus carpio* exposed for 14 days to 0.01 or 0.02 mg·l^{-1} of trifluralin showed a concentration-related vacuolation of hepatocytes, accompanied at higher concentration by karyopyknosis (Poleksic and Karan 1999). Treatment of *Rhamdia quelen* to 1 mg·l^{-1} of clomazone for 192 h also resulted in hepatocyte cytoplasm vacuolation (Crestani et al. 2007). In *Channa punctatus* subjected to 1.21 mg·l^{-1} of alachlor for 10 days hepatocyte vacuolation and cytoplasm damage were observed accompanied by blood vessel breakdown and alterations in hepatocyte organization (Butchiram et al. 2009). *Prochilodus lineatus* exposed to 7.5 mg·l^{-1} of Roundup for 24 h showed hepatic vessel congestion, cytoplasm degeneration and hypertrophic hepatocyte nuclei; after 96 h at the same concentration of Roundup – hepatocyte vacuolation, progressive degeneration and pyknosis accompanied by cholestasis. Vacuolation and cholestasis accompanied by karyorrhexis and hepatocyte hypertrophy were observed in fish exposed for 24 h to 10 mg·l^{-1} of herbicide (do Carmo Langiano and Martinez 2008). Histopathological alterations accompanied by increase of liver transaminase activities indicate that liver is a sensitive to herbicide toxicity. Lesions in this organ may result in disruption of xenobiotic metabolism and increased susceptibility to chemical stress, as well as disturbances in other metabolic functions. Therefore, microstructure of fish liver, as well as gills, seems to be a sensitive indicator of water pollution by herbicides.

CONCLUSIONS

Pollution of aquatic environment with herbicides becomes an increasing problem. The data shown in present review indicate that reactions of fish organism to intoxication with various herbicides are nonspecific which makes identification of contaminant very difficult or impossible and implies the necessity of environmental monitoring. These data indicate that toxic effects of herbicides depend on various factor such as type and concentration of herbicide, time of exposure and fish species. Comprehensive evaluation of the harmfulness of herbicides requires performing biochemical, hematological and histopathological tests probably supplemented with other indicators as immune parameters and reproductive indices.

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Streszczenie: *Fizjologiczne i histologiczne skutki działania herbicydów na ryby*. Pośród pestycydów stosowanych w rolnictwie herbicydy są wykorzystywane w największych ilościach. Substancje te przedostają się do środowiska wodnego na wiele sposobów, a badania monitoringowe wykazują stałą ich obecność w wodach naturalnych. Herbicydy powodują zmiany wielu parametrów fizjologicznych u ryb, np. zmniejszenie aktywności acetylocholinoesterazy w narządach wewnętrznych, zwiększenie aktywności aminotransferaz wątrobowych czy zaburzenie równowagi oksydoreduktancyjnej. Obserwuje się również zmiany parametrów hematologicznych, zwykle wskazujące na anemię i proces zapalny. Najczęściej notowanymi zmianami histologicznymi są hiperplazja i hipertrofia nabłonka skrzeli oraz zmiany w mikrostrukturze wątroby, takie jak wakuolizacja hepatocytów. Markery ekspozycji ryb na herbicydy są na ogół bardzo wrażliwe, ale niespecyficzne. Analiza parametrów metabolicznych, hematologicznych i histologicznych może być pomocna w diagnostyce zatrucia ryb herbicydami, ale zawsze powinna być potwierdzona badaniami monitoringowymi.

Słowa kluczowe: intoksykacja, pestycydy, zdrowie ryb

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