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Biochemical and Histopathological Effects of Glyphosate on Carp, *Cyprinus carpio* L.

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Glyphosate, also known by the trade names Roundup and Rodeo for agricultural use, is a broad-spectrum, translocated herbicide, used primarily in agricultural applications, and for vegetation control in non-crop areas. It is used as non-selective herbicide and for aquatic weed control in fish-ponds, lakes, canals, slow running water, etc. (USDA 1984). Glyphosate is perhaps the most important herbicide ever developed.

Literature of toxicological and ecotoxicological properties of glyphosate is extremely sparse, considering its importance as herbicide. Generally, glyphosate is slightly toxic to mammals and fish, but it may have an impact on the aquatic environment and also on the other aquatic organisms (USDA 1984). Due to this, its toxicity investigation is very important. The study of sublethal effects is of special importance for toxicological evaluation of compound.

The objective of this study was to investigate acute and subacute toxic effects of sublethal glyphosate concentrations in water to carp (*Cyprinus carpio* L.), one of the commercially most imporant fish species populating freshwaters of Yugoslavia.

MATERIALS AND METHODS

Glyphosate (N-/phosphonomethyl/glycine) herbicide, technical grade, purity 62%, supplied by ICI, England, was used. Stock solution, including all other dilutions, were made in water.

Carp (*Cyprinus caprio* L .), 4.0-5.5 g in body weight and 3.6-4.2 cm in body

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length (for acute toxicity tests) and 50.2-54.1 g in body weight and 13.5-15.3 cm in body length (for subacute tests) at the beginning of the experiment, were used. Fish were purchased from Ribokombinat, Belgrade, and they were acclimatized to laboratory conditions for 10 d prior to experiments. During the experiment fish were kept in 10 L glass aquaria (55x25x20 cm), and fed once a day with Rihran a special aquaria fish mixture, purchased from Florine, Ma-ribor, Slovenia. The quantity of food was 2.5% of initial body weight per day.

Chlorine-free tap water with the following physicochemical characteristics was used: pH = 7.0-7.5; total hardness = 141-223 mg/L (as $CaCO_3$); dissolved oxygen = 7.5-11.5 mg/L. The water temperature was kept at 20.0±1.0° C. Water was changed every 24 hr, followed by the addition of fresh glyphosate solution.

Acute toxicity tests were performed according to the OECD procedure (OECD Guideline, No 203, 1984) for semi-static tests. Fish (10 fish per group) were exposed to different glyphosate concentrations (400.0, 500.0, 600.0, 700.0, and 800.0 mg/L) for 96 hr. Mortality was recorded after 48 and 96 hr, and CC-50 was calculated by the Litchfield and Wilcoxon method (1948).

For subacute toxicity test (14 d) fish were randomly divided into four groups of 10 fish each. One group served as control while the remaining three groups were exposed to different herbicide concentrations. At the end of 14 d period, blood samples were taken. After that fish were sacrified by decapitation, and organs (heart, liver and kidneys) were colected, weighed and prepared for biochemical and histopathological analysis.

For biochemical analysis the heart, liver, kidneys and serum of fish were used. Glutamic-oxaloacetic (GOT) and glutamic-pyruvic (GPT) transaminases were determined calorimetrically at 505 nm, by the method of Reitman and Frankel (1957), and Wooton (1964), respectively. Alkaline phosphatase (AP) activity was determined calorimetrically at 510 nm, by the method of McComb and Bowers (I 972). For determinations test kits from INEP, Belgrade-Zemun, Yugoslavia, were used.

For histopathological investigations, portions of gills, livers and kidneys were fixed in 4% neutral formaldehyde, and embedded in paraffin. The paraffin sections were stained with Ehlrich haematoxylin using eosin as counter stain (HE).

To determine the statistical significance of data Student's t-test was used (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Acute toxicity (LC-50) of glyphosate to carp was investigated in semi-static test for 48 and 96 hr. As it can be seen (Table 1), LC-50 value at 48 hr exposure was 645 mg/L, and 620 mg/L (as active ingredient) after 96 hr.

Table 1. Acute toxicity (LC-50) of the herbicide glyphosate to carp (*Cyprinus carpio* L.)[a]

Herbicide	Duration of Exposure (hr)	LC-50 (mg/L)
Glyphosate	48	645 (632-655)[b]
	96	620 (607-638)

"There were 10 fish in each group; [b]95% Confidence limits

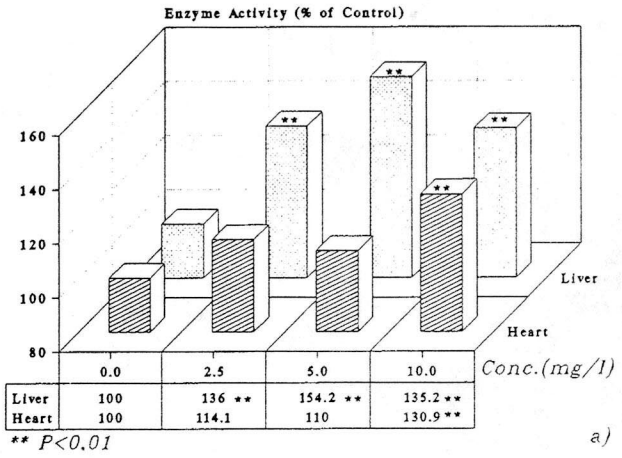
The acute toxicity of glyphosate (Roundup and Rodeo) has been investigated on the different fish species, and in different environmental conditions (physico-chemical water characteristics, pH, temperature, etc.). Mitchell et al. (1987) investigated the acute toxicity of glyphosate (Roundup and Rodeo) to rainbow trout (*Salmo gairdneri*), chinook salmon (*Oncorynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) , using varying dilution water types (dechlorinated, dechlorinated and reconstituted, and natural lake water), pH (6.1-7.7), hardness (4.5-85.0 mg/L as $CaCO_3$), and conductivity (12-132 umhos/cm). The results showed that 96 hr LC-50 value varies from 15-26 mg/L (Roundup) and 600-1440 mg/L (Rodeo), depending on fish species, water characteristics, pH and hardness (total formulations were used). At the same time, Servizi et al. (1987) reported that 96 hr LC-50 values for Roundup, composed of glyphosate (30.5%) and surfactant MONO818 (15%), for rainbow trout are 28.0, and 42.0 mg/L for coho salmon, respectively. Some other investigations (USDA 1984) showed that LC-50 values of technical glyphosate to fish range from 16-168 mg/L and 2.3-48.0 mg/L Roundup. and over 1000 mg/L Rodeo, depending on fish species used in the experiments and exposure time.

As it is obvious, our results on glyphosate acute toxicity are mostly in accordance with the results of the authors mentioned. Based on its acute toxicity of 620 mg/L (96hr LC-50) it may be concluded that glyphosate is slightly toxic (practically non-toxic) to carp (Clarke et al. 1970).

In subacute toxicity test, the effects of sublethal glyphosate concentrations (2.5, 5.0 and 10.0 mg/L) on the enzyme activity in the serum and organs, as well as on morphological changes of fish organs were performed. Concerning biochemical effects, statistically significant ($P<0.01$) increase in AP activity in liver (all three concentrations tested) was registered (Figure 1). Also, statistically significant ($P<0.01$) increase in the AP activity in heart of fish exposed to 10.0 mg/L glyphosate concentration was registered. Slightly lower effect in the GOT and GPT activity was noted (Figure 1). In the fish exposed to 2.5 and 5.0 mg/L glyphosate concentrations, statistically significant ($P<0.05$) increase in the GOT activity in liver and kidneys was registered. It was also registered the statistically significant ($P<0.05$) increase in the GPT activity in kidneys (glyphosate concentration: 2.5 mg/L), and in serum (glyphosate concentrations: 5.0 and 10.0 mg/L). In all other cases no statistically significant changes in the GOT and GPT activity were noted, in relation to the control.

Our results of subacute toxic effects of glyphosate to carp (Figure 1) are, in high degree, in accordance with results of other authors (Nemcsok et al. 1987; Sing and Reddy 1990; Ferando and Andreu-Moliner 1991; Neškovic et al. 1993). Their results, also, proved that pesticides cause changes in some fish enzyme activity (in the first place transaminases) as well as biochemical alterations of some organ and tissue constituents (total lipids, glucose, glycogen, etc.). The results show that the effects depend on fish species, type of compound, its concentrations in water, and exposure time.

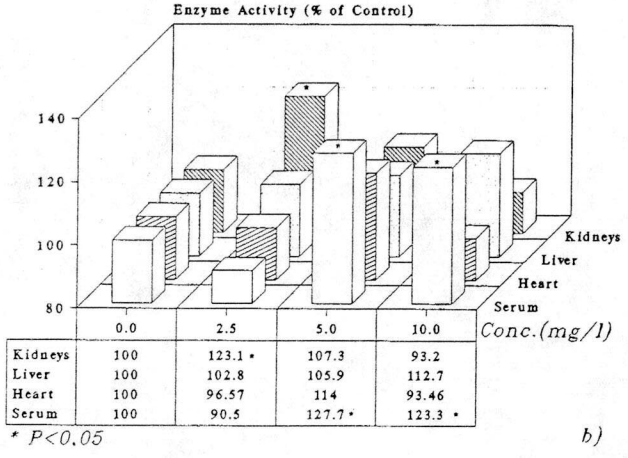
For histopathological changes gills, liver and kidneys were examined. Gills of the control fish had normal morphological structure (Figure 2). On the gills of fish exposed to 5.0 mg/L glyphosate concentration (Figure 3), epithelial hyperplasia and subepithelial edema were found. Similar changes remained at 10.0 mg/L glyphosate, but were more pronounced and followed with leukocyte infiltration, slight hypertrophy of chloride cells, as well as lifting and rupture of the respiratory epithelium on some secondary lamellae (Figure 4). Liver in the control fish had normal structure (Figure 5). Changes in liver structure appeared only at 10.0 mg/L glyphosate concentration. Congestion of few sinusoid and, on some places, signs of early fibrosis (Figure 6) were recorded. Kidney structure was not affected by the glyphosate, since no histopathological changes were found in the kidney of fish exposed to different sublethal glyphosate concentrations.



	0.0	2.5	5.0	10.0
Liver	100	136 **	154.2 **	135.2 **
Heart	100	114.1	110	130.9 **

** P<0.01

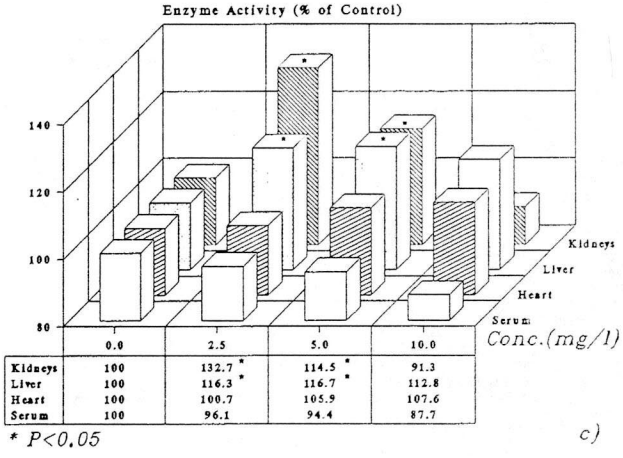
a)



	0.0	2.5	5.0	10.0
Kidneys	100	123.1 *	107.3	93.2
Liver	100	102.8	105.9	112.7
Heart	100	96.57	114	93.46
Serum	100	90.5	127.7 *	123.3 *

* P<0.05

b)



	0.0	2.5	5.0	10.0
Kidneys	100	132.7 *	114.5 *	91.3
Liver	100	116.3 *	116.7 *	112.8
Heart	100	100.7	105.9	107.6
Serum	100	96.1	94.4	87.7

* P<0.05

c)

Figure 1. Enzyme activites in serum and some organs of controls and carp (*Cyprinus carpio* L.) exposed to various glyphosate concentrations during the experiment (14 days).

(a) Alkaline phosphatase (AP);
(b) Glutamic-oxaloacetic transaminase (GOT);
(c) Glutamic-pyruvic transaminase (GPT).

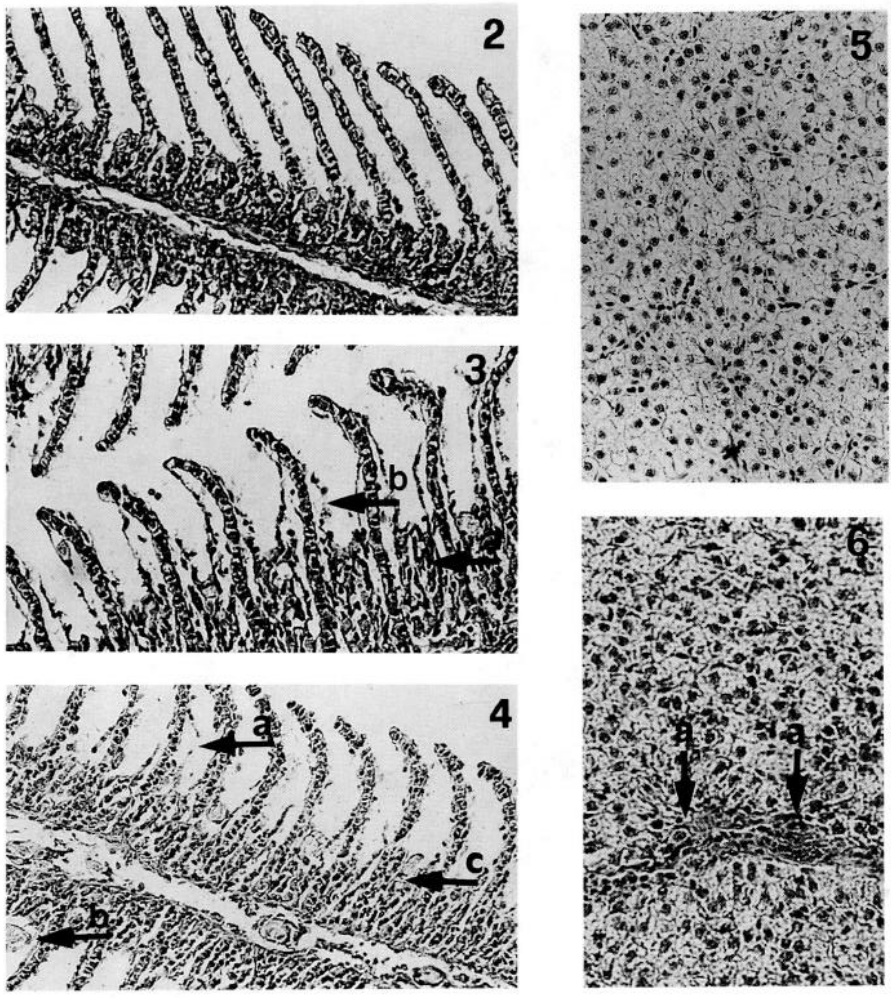


Figure 2. Gills of control fish (H+E x 160)

Figure 3. Gills of fish exposed to 5.0 mg/L glyphosate concentration (H+E x 160). (a) Epithelial hyperplasia; (b) Subepithelial edema.

Figure 4. Gills of fish exposed to 10.0 mg/L glyphosate concentration (H+E x 160). (a) Lifting of epithelium with leucocyte infiltration; (b) Chloride cell hypertrophy; (c) Epithelial hyperplasia.

Figure 5. Liver of control fish (H+E x 237.5)

Figure 6. Liver of fish exposed to 10.0 mg/L glyphosate concentration (H+E x 237.5). (a) Focal fibrosis.

Histopathological changes observed in the carp organs following exposure to different sublethal glyphosate concentrations appeared in the gills (glyphosate concentrations: 5.0 and 10.0 mg/L). Changes in liver were recorded only in fish exposed to the highest glyphosate concentration (10.0 mg/L). Histological structure of the kidneys of fish exposed to different glyphosate concentrations was almost normal.

The appearance of leucocyte infiltration in the gills (highest concentration tested) supports the inflamatory reaction indicated by hyperplasia and lifting of the respiratory epithelium .

Chloride cells hypertrophy and proliferation (hyperplasia of those cells was also found) recorded on the gills of fish exposed to 10.0 mg/L glyphosate concentration could be explained by the role of these cells in the regulation of the acid-base balance of the gill apparatus and their possible detoxification function (Rojik et al. 1983; Mallatt 1985). Gill changes observed in this study are not particularly severe, i.e., these changes could be reparable if the fish is transferred into the clean water.

Liver lesions found in this study were of limited value, because they were found only focally and not in all livers of fish examined. Congestion of sinusoids was the only pathological effect of organochlorine herbicides reported by Couch (1975), and was also recorded in our glyphosate study. On the other hand, focal fibrosis is a progressive type of change and could indicate possible serious damage of liver structure.

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