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Effects of Trifluralin on Carp: Biochemical and Histological Evaluation

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Acute and subacute toxicity of the herbicide trifluralin on fish was investigated in laboratory toxicity tests with carp. Median lethal concentrations were determined in acute tests. The 96-h LC_{50} value was 0.045 (0.036–0.051) mg/L. Fish were exposed to subacute concentrations of the herbicide (0.005, 0.01, and 0.02 mg/L trifluralin) in the 14-day toxicity tests and the effects on the relative growth rate, some biochemical parameters [alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanin aminotransferase (ALT) activities in serum, gills, liver, and kidney], gills, liver, and kidney structure were studied. A decrease in relative growth rate was found. An increase of functional enzyme activities in blood serum and the organs examined, particularly in the highest concentration of trifluralin indicated changes in the vital organs, was confirmed by histological analysis. The most severe changes (although mostly reversible) were found in the gills and kidney of the fish examined.

Key Words: **carp; toxicity; trifluralin; subacute effects.**

INTRODUCTION

Trifluralin is a dinitroaniline compound used as a selective preemergence herbicide. It is licensed and used in Yugoslavia on approx. 30% of agricultural areas (Vitorović and Elezović, 1994).

Trifluralin can get into aquatic ecosystems in runoff and by underground waters, similar to other herbicides used near the water. Its effects on biotic components of a freshwater ecosystem (phyto-, zooplankton, and bottom fauna) in concentrations which could be reached in aquatic environment have been demonstrated (Vidmanić *et al.*, 1994).

The carp (*Cyprinus carpio* L) is a fish species of considerable economic importance in Yugoslavia (Milinković and Mitrović-Tutundžić, 1995). Middle and lower courses of Serbian rivers, flowing by areas with intensive agriculture, are cyprinid regions. Therefore data on the effects of trifluralin on carp could be useful in its risk assessment. This article is, to the authors' knowledge, the first report on trifluralin toxicity on carp.

Biochemical and pathohistological effects on carp were investigated in order to determine possible adverse effects of trifluralin.

MATERIALS AND METHODS

Chemicals

Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) herbicide, 99% analytical standard supplied by Pestanal, was used. Stock solution and all other dilutions were made in water with an ultrasound water bath used to facilitate dilution.

Animals and Maintenance

Carp, obtained from Ečka fish farm, 3.3 ± 0.15 cm in body length and 0.39 ± 0.06 g in body weight (for acute tests) and 15.3 ± 1.1 cm in body length, 154.6 ± 20.9 g body weight (for subacute tests) at the beginning of the experiments, were used.

Fish were acclimated for 21 days to laboratory conditions, fed once a day with aquaria fish mixture.

Dechlorinated tap water (pH 7.8–8.2; dissolved oxygen 7.9–10.5 mg/L; hardness 150–230 mg/L, as $CaCO_3$) was used. The temperature was maintained at $20.0 \pm 1.0°C$, and the light/dark period was 12 h/12 h.

Toxicity Testing

Acute (96 h) toxicity tests on 6-month-old carp fry (*Cyprinus carpio*, L.) and subacute semistatic 14-day toxicity tests (24-h renewal of test solution) on 1.5-year-old carp were performed using the OECD test method (OECD Guideline, Nos. 203 and 204, 1984, 1987, 1990).

In acute tests fish (10 per group) were exposed to the following trifluralin concentrations: 0.025; 0.05; 0.1; 0.2 mg/L for 96 h. Mortality was recorded after 24, 48, and 96 h, and LC_{50} values were calculated by the Litchfield and Wilcoxon method (1949).

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For subacute toxicity tests, fish were randomly divided in four groups (eight fish each): control and three groups exposed to 0.005, 0.01, and 0.02 mg/L trifluralin concentrations. Before the start of the experiments fish were weighed and their length was measured.

During the experiment no mortality was recorded.

After the 14-day exposure period fish were sacrificed by decapitation and blood and organs were collected and prepared for further analysis.

Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanin aminotransferase (ALT) were determined in serum, gills, liver, and kidney of the fish from the control group and fish exposed to different trifluralin concentrations. ALP activity was determined by the method of McComb and Bowers (1972). AST and ALT activities were determined by the method of Bergmeyer *et al.* (1976). For determinations test kits from INEP, Zemun, were used.

For histological investigation, portions of gills, liver, and kidney were sampled from the same fish, fixed in 4% formaldehyde, and processed for histology examination using standard techniques with hematoxylin and eosin staining.

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

RESULTS

Acute Toxicity

Median lethal concentration (LC_{50}) was investigated in semistatic tests for 24, 48, and 96 h (Table 1). LC_{50} value at 24-h exposure was 0.185 mg/L, 0.066 mg/L at 48 h, and 0.045 mg/L after 96 h.

Subacute Toxicity

Effects on Body Weight

Changes in body weight of fish exposed to different concentrations of trifluralin were not statistically significant compared to the control. Relative growth rate (RGR) was calculated according to the method of Crossland (1985), expressed in mg/g/day (Fig. 1).

TABLE 1
Median Lethal Concentrations of Trifluralin to Carp

Duration of exposure (h)	LC_{50} (mg/L)
24	0.185 (0.173–0.189)[a]
48	0.066 (0.054–0.075)
96	0.045 (0.036–0.051)

[a] 95% confidence limits.

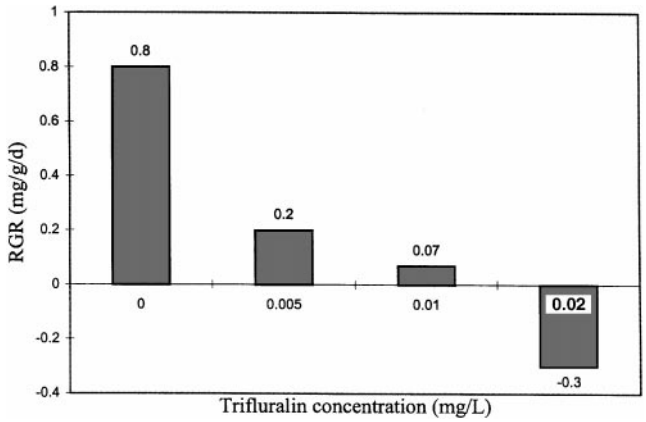

FIG. 1. Relative growth rate (RGR) of carp in different trifluralin concentrations.

Biochemistry

ALP activity. As seen in Fig. 2, a statistically significant ($P < 0.01$) increase in ALP activity (compared to the control) was found in the kidney of carp exposed to the highest trifluralin concentration. In the kidney of fish from two lower concentrations the increase in ALP activity was statistically significant ($P < 0.05$), as well as in the gills of fish exposed to 0.01 and 0.02 mg/L trifluralin and in liver from 0.02 mg/L trifluralin concentration.

AST activity. AST activity markedly increased in the gills of fish exposed to 0.01 and 0.02 mg/L ($P < 0.01$) and to 0.005 mg/L ($P < 0.05$), which correlated with the increase of trifluralin concentration. A uniform but statistically significant ($P < 0.05$) increase of AST activity in all three concentrations tested was found in liver and blood serum (Fig. 3).

ALT activity. A statistically significant ($P < 0.05$) increase in ALT activity was found in all the organs examined from the highest trifluralin concentration (0.02 mg/L) (Fig. 4).

Histology

Gills. The gills of fish from the control group and the group treated with 0.005 mg/L trifluralin had normal histological structure (Fig. 5)

Hyperplasia of epithelium between secondary lamellae which focally led to the fusion of adjacent secondary lamellae were found on the gills of carp treated with 0.01 mg/L herbicide. In addition to this edema of subepithelial space, wrinkled respiratory epithelium, curled secondary lamellae, and some chloride cell hypertrophy were noted (Fig. 6).

On the gills of fish exposed to 0.02 mg/L trifluralin lifting of the secondary epithelium, curled secondary lamellae, and chloride cell hypertrophy were also found. Epithelial hyperplasia led to the fusion of several lamellae (Fig. 7).

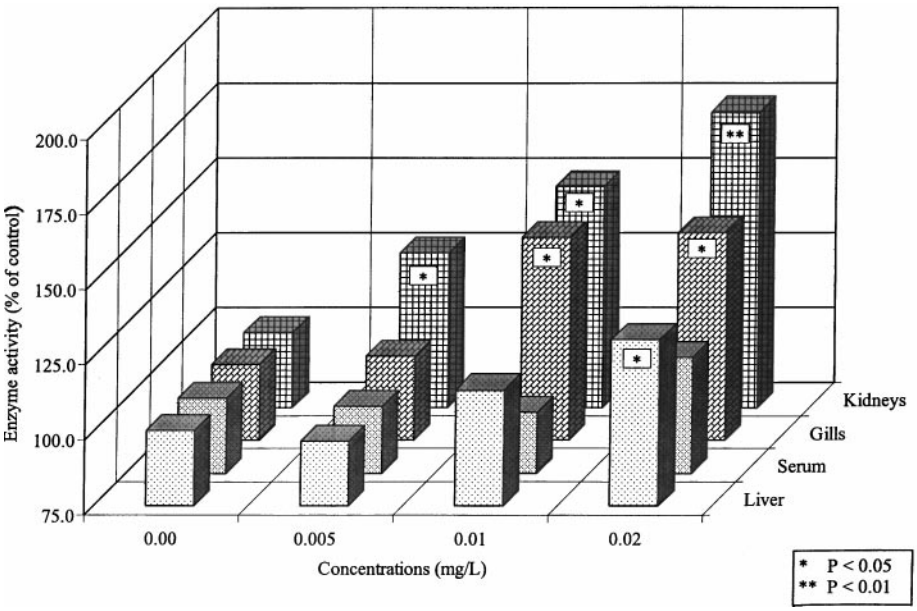

FIG. 2. ALP activity of carp exposed to trifluralin for 14 days.

Changes in supporting cartilage of the gill filament were noted (Fig. 8).

Liver. No difference between the structure of the liver of fish from 0.005 mg/L trifluralin compared to the control group (Fig. 9) was recorded. Only slight vacuolation of hepatocytes in 0.01 mg/L exposed carp was recorded.

In carp exposed to 0.02 mg/L vacuolation and areas of nuclear pycnosis of hepatocytes were noted (Fig. 10).

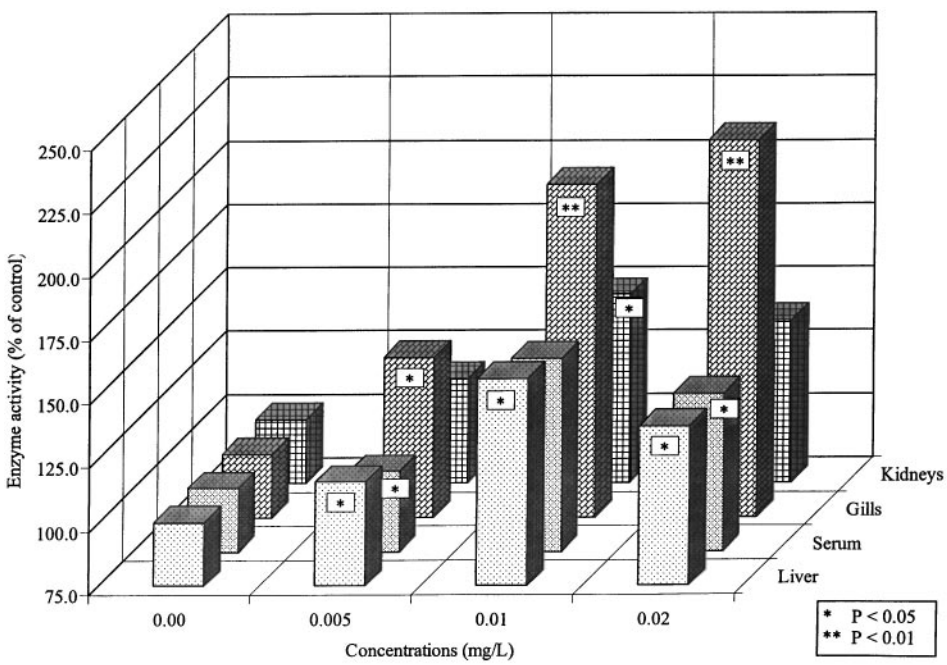

FIG. 3. AST activity of carp exposed to trifluralin for 14 days.

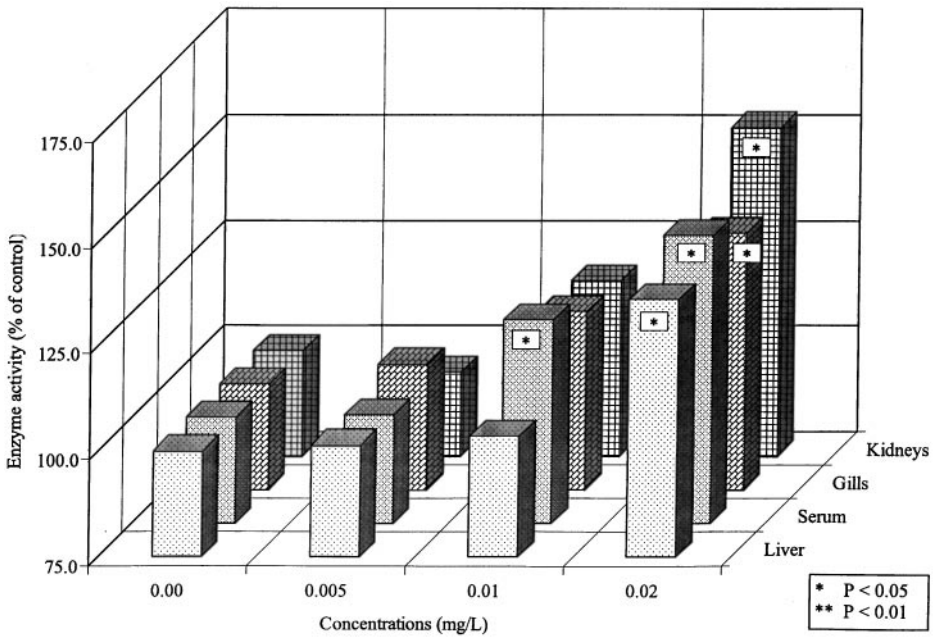

FIG. 4. ALT activity of carp exposed to trifluralin for 14 days.

Kidney. The kidney of fish from the control group, as well as from groups treated with 0.005 and 0.01 mg/L herbicide (Fig. 11), had normal histological structure.

The degeneration of tubular epithelial cells with ectasy of Bowman's capsules and their capillaries was found in the kidney of carp from the 0.02 mg/L exposure concentration (Fig. 12).

DISCUSSION

The data obtained for acute toxicity of trifluralin are in the range of LC_{50} concentrations established for young rainbow trout and bluegill sunfish (0.01–0.04 and 0.02–0.09 mg/L, respectively (Tomlin, 1994).

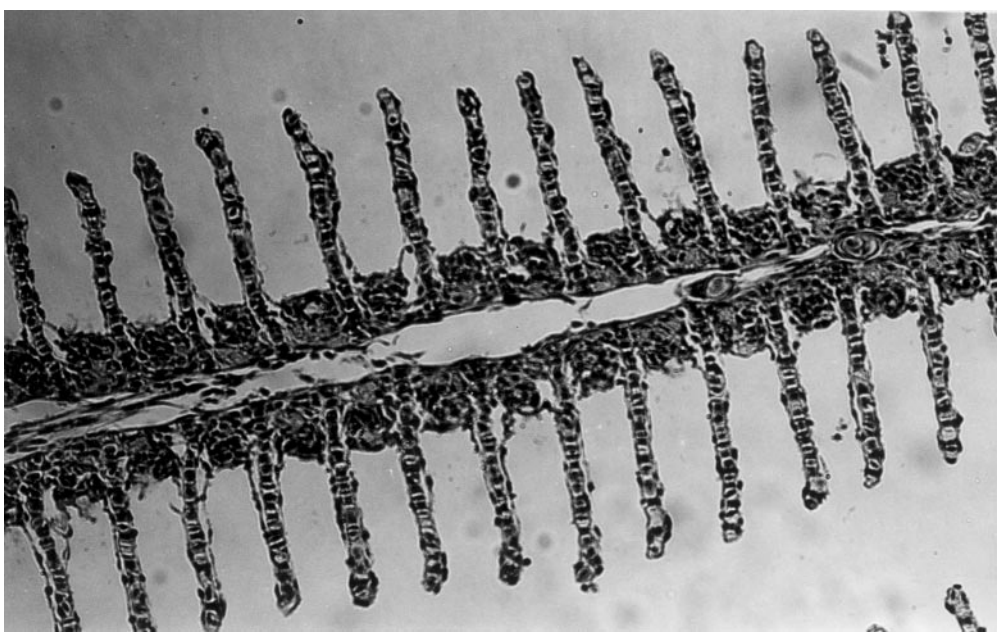

FIG. 5. Gills of carp from the control group. HE, $\times 64$.

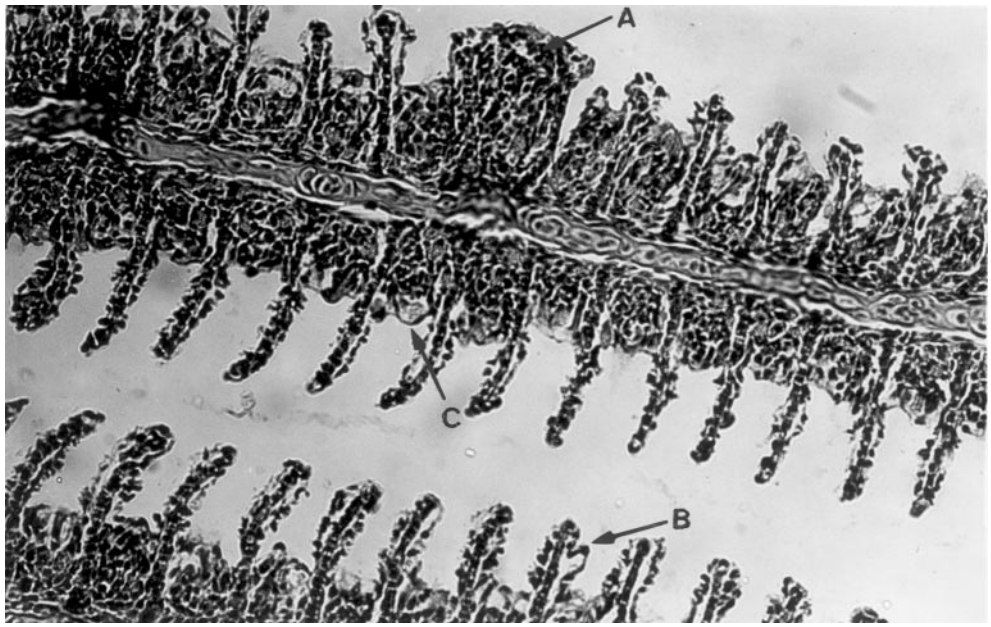

FIG. 6. Gills of carp exposed to 0.01 mg/L trifluralin. HE, magnification × 64. A, epithelial hyperplasia; B edema in subepithelial space and wrinkled respiratory epithelium; C, hypertrophy of chloride cells.

In subacute experiments a decrease in relative growth rate with the increase of trifluralin concentration indicated an effect of trifluralin on body weight.

Rapid uptake from water and rapid metabolism of trifluralin (Schulz and Hayton, 1993) could explain the increase in ALP activity in the carp's kidney exposed to subacute concentrations of the herbicide found in the current study. It was confirmed by pathohistology, since degenerative and vascular changes were found in the kidney of carp from the highest trifluralin concentration.

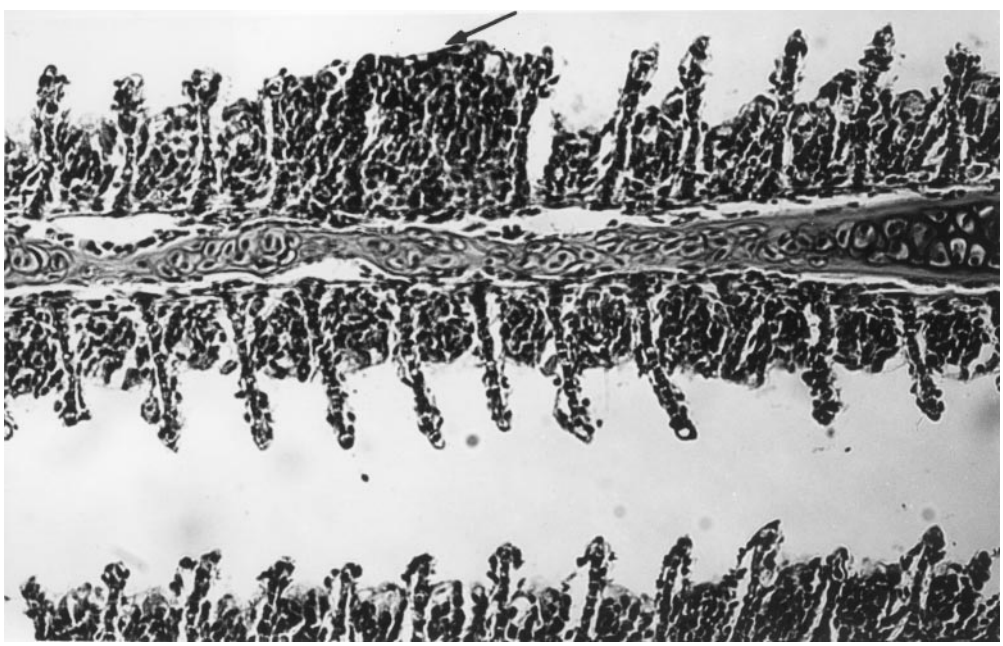

FIG. 7. Gills of carp exposed to 0.02 mg/L trifluralin. HE, × 6. Fusion of secondary lamellae (arrow).

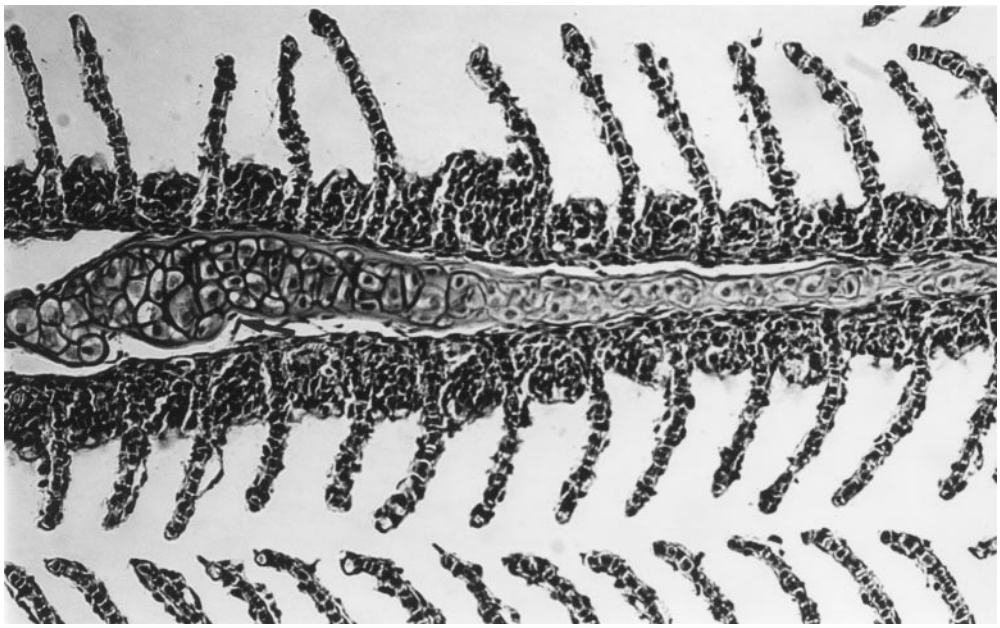

FIG. 8. Gills of carp exposed to 0.02 mg/L trifluralin. HE, ×64. Changes in cartilage tissue of the gill filament (arrow).

Significant increase in AST activity ($P < 0.01$) in the gills of fish exposed to 0.01 and 0.02 mg/L herbicide concentrations probably led to the appearance of hyperplasia and edema of primary and secondary epithelium, a known defense mechanisms of the gills. Gill chloride cell hypertrophy found in both 0.01 and 0.02 mg/L exposure concentrations could point to the role of those cells in branchial elimination of the herbicide which exceeds urinary elimination of hydrophobic chemicals such as trifluralin (Schulz and Hayton, 1994). Another structural alteration found on the gills of carp from the highest concentration was a degeneration of chondrocytes in the gill filament cartilage. Changes in

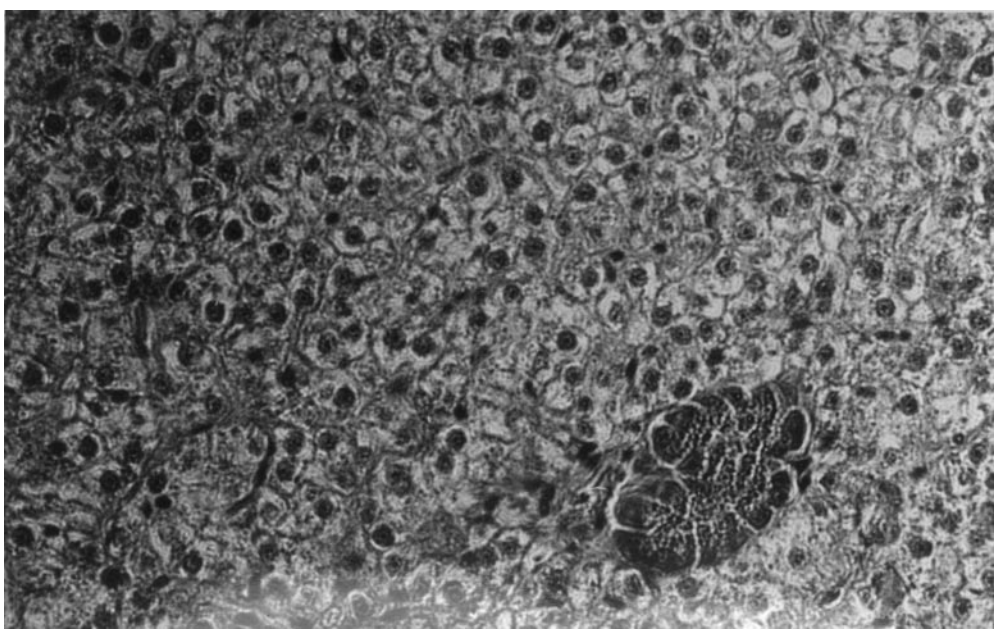

FIG. 9. Liver of carp from the control group. HE, ×128.

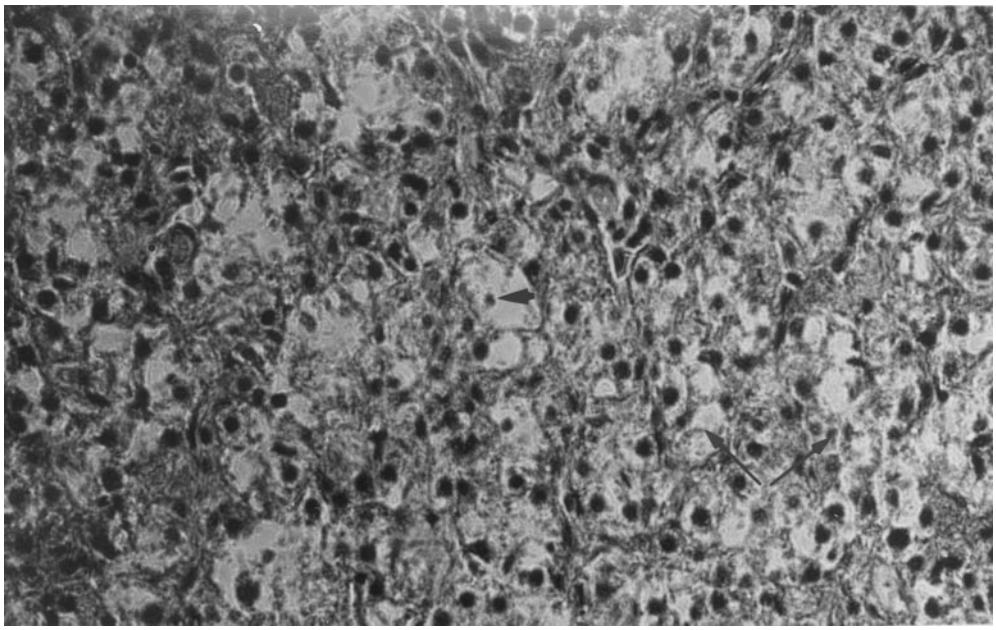

FIG. 10. Liver of carp exposed to 0.02 mg/L trifluralin. HE, ×128. Vacuolation of hepatocytes (arrows); pycnotic nucleus of hepatocyte (arrow head).

supporting tissues, more precisely vertebral dysplasia, were reported by Couch *et al.* (1979) in sheepshead minnow exposed to trifluralin, although in the mentioned study cartilage changes were not found.

The increase in serum and gill transaminase activity in fish exposed to trifluralin could indicate possible leakage of enzymes across damaged plasma membranes and/or the increased synthesis of the enzyme in the tissues (Gingerich, 1982). A rather uniform increase in AST activity in all concentrations in liver and serum could be the cause of hepatocyte necrosis in 0.02 mg/L exposed fish.

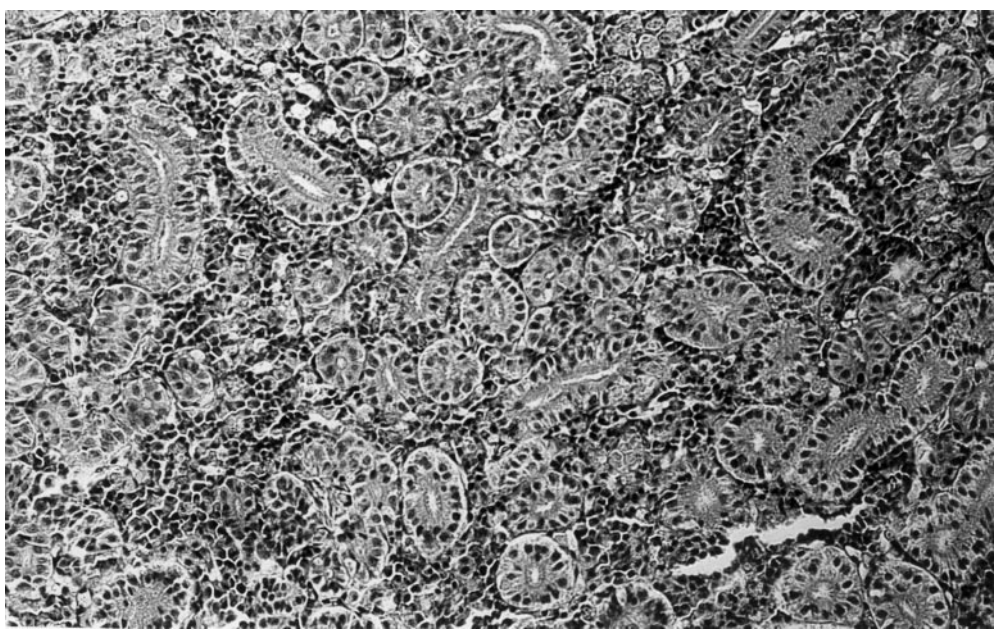

FIG. 11. Kidney of carp from the control group. HE, ×64.

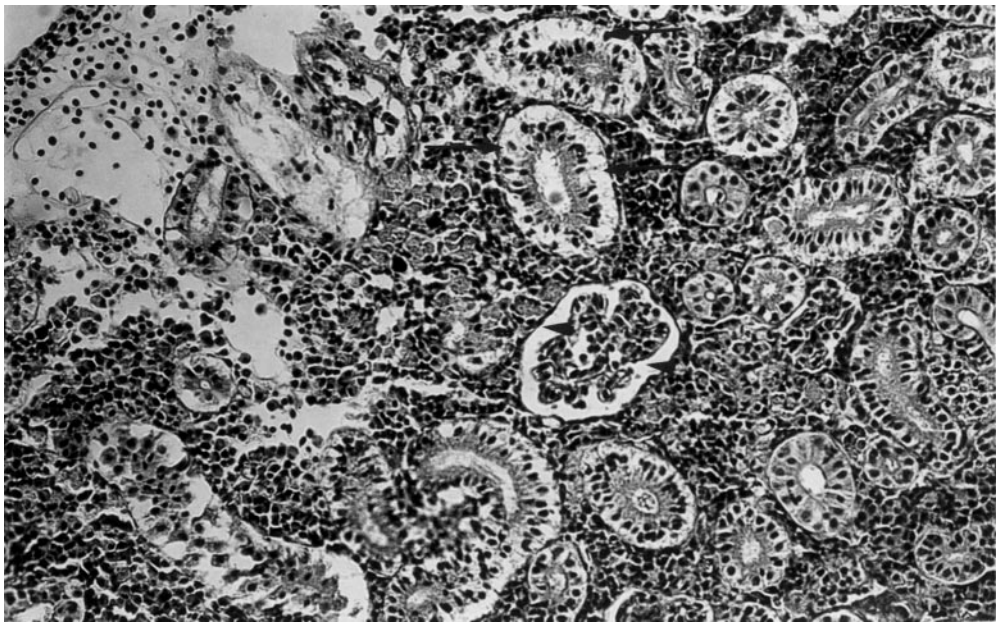

FIG. 12. Kidney of carp exposed to 0.02 mg/L trifluralin. HE, × 64. Degeneration of tubular epithelial cells (arrows); Ectasy of Bowman's capsules (arrow heads).

CONCLUSION

An increase in some enzyme activities in serum and organs recorded in this study is pointing out changes in metabolic state of the fish. It was confirmed by pathohistology of the organs examined. The most severe changes were found in the gills and kidneys, probably due to the role of these organs in rapid elimination of the compound.

Although the herbicide trifluralin should be classified in the group of highly toxic substances, according to its LC_{50} values to fish (OECD, Chemical group and management committee, 1992), changes recorded in this study were of moderate intensity and probably reversible. However, it should be emphasized that although reversible, the changes found could affect fish health, making them more sensitive to environmental changes and less resistant to diseases.

In addition to this, the results of the present study have determined the importance of both biochemical and pathohistological approaches in the evaluation of nonlethal effects of pesticides and other chemicals on fish.

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