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Toxicity of 4-Chloro-o-Cresol to Fish. Light Microscopy and Chemical Analysis of the Tissue

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The present study is a part of a series in which the toxicity of the metabolites of MCPA (4-chloro-2-phenoxyacetic acid) is studied in rats and fish. 4-chloro-o-cresol was identified as a metabolite of MCPA by GAUNT and EVANS in 1961. It has also been found as an impurity in the technical product of MCPA (RÄSÄNEN et al. 1977). Furthermore, it is found to concentrate in fish tissue (HATTULA et al. 1978a). In the present work the acute and subchronic toxicity was studied in sea trout (Salmo trutta). The trout was chosen because the residues of herbicides get also to water ecosystems and the trout is a commercially important fish which is known to be sensitive to environmental poisons. The fish were studied by light microscopy for histological analyses and the tissue concentrations of the cresol were studied by glass capillary GLC.

MATERIALS AND METHODS

Fish: The trout (age under one year) used were obtained from a fish hatchery and the average weight of the fish in the acute experiment was 2.5 g, and 5.0 g in the subchronic experiments. The fish were kept in 20 l glass aquaria and the aeration occurred by pressure air. The temperature $10 \pm 1^{\circ}C$ was maintained by circulating cold water round the aquaria.

4-chloro-o-cresol: The cresol was purified to >99% product from the commercial product of Fluka and the purity was controlled by IR-, NMR- and mass spectrometry. The compound was dissolved in absolute ethanol and applied in several concentrations. One ml ethanol in 10 ml water was used for the control group.

EXPERIMENTAL

10 fish were placed in 20 l water in the aquaria which were covered by glass plates except for the aeration hole. The solutions were renewed daily. Firstly, the LD_{50}-value was determined by the method of HORN (1956) and the fish were observed for 24 h. The fish were preserved by freezing for chemical analysis in $-25^{\circ}C$.

In the first subchronic experiment (20 fish/aquarium, cresol concentration 0.5 and 1.0 ppm) the fish were killed after four weeks and after three weeks in the second experiment (cresol concentration 0.5, 1.0 and 1.5 ppm). For the pathological analysis the samples were taken from liver, kidney and gills from 10

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specimens. The tissue samples were fixed in buffered formalin and the paraffin sections were stained with haematoxylin eosin for the light microscopy. For chemical analysis 10 fish were wrapped in aluminium foil and frozen in -25°C.

Chemical analysis: The fish were groung in a Sorvall Omni-mixer with two drops 6-n HCl and anhydrous Na_2SO_4 and kept at room temperature for 24 h. The dry sample was shaken 3x0.5 h with 50 ml chloroform-diethylether 1:1 (v/v) (redistilled) in a 100 ml round bottomed flask equipped with a glass stopcock. The combined extracts were evaporated in a Büchi evaporator and the residue was transferred quantitatively by ether in a 10 ml volumetric flask. 1 ml was taken in a 2 ml glass tube with a sharp end and evaporated with 99.999% pure nitrogen.

Clean up: The clean up process occurred by TLC. The plates of 1 mm Kieselgel G nach Stahl (24x24 cm) dried in 120°C for 24 h until used. The plate was devided in five sections of which one served for the standard and four for the samples. 4-chloro-o-cresol 1 mg/l was used 10 µl as a standard. The evaporation residue was applied in 200 µl diethylether at the distance of 1 cm from the lower end and the plate was developed in dichlormethane (redistilled). The plate was covered with aluminium foil except the standard which was made visible by spraying it with 2% 2,6-dichlorchinonchlorimid (Merck) in toluene. The cresol fractions were then scraped by a spatula according to blue standard spot in glass columns (10x1 cm) which were stoppered with glass wool. The cresol was eluted with 2 ml diethylether in 2 ml volumetric tubes and chromatographed by GLC.

The quantitative analysis was carried out by glass capillary GLC. The gas chromatograph used was Carlo Erba Model Fractovap equipped with a FID detector. The column was 15 m glass capillary of FFAP (ø 0.35 mm). The residues were applied at 60°C and chromatographed to 190°C 30/min. The quantitative determination was made by comparing the peak height with the corresponding standard. The standards of different concentrations were treated daily the process above and chromatographed as the samples.

RESULTS

Histopathological study: All fish from water containing 0.5 ppm 4-chloro-o-cresol were histologically normal but some specimens from water containing 1.0 ppm cresol had histopathological changes. In one specimen slight cytoplasmic vacuolar changes were observed in the liver cells. In one specimen there were changes in the kidney. The epithelium of the tubules was degenerated, the cytoplasm of the epithelial cells was eosinophilic, nuclei pycnotic and the cells had lost their contact to the basement membrane. Inside the tubules there were necrotic cells and probably protein globes.

The fish from water containing 1.5 ppm cresol some changes were observed in kidneys and gills. In the kidneys the changes were very slight; some vacuolization, pycnotic nuclei and eosinophilic coloration in the cytoplasm of the epithelial cells in the tubules were observed. In the gills a remarkable amount of

lamellar teleangiectasic changes in the secondary filaments were observed. The changes were observed in all but one fish and the globes in the filaments were of different sizes. The globes were bigger and more numerous in the fish from the higher concentrations.

Chemical study: The concentrations of 4-chloro-o-cresol in the wet tissue of the fish in the subchronic experiments are presented in Table 1. The LD_{50}-value for the fish was 2.12 ppm (1.88-2.39). The chemical concentration of cresol of the LD_{50}-experiment (17.37 mg/kg) is an average of all specimens which were kept in 1.82, 2.00, 2.20 and 2.42 ppm. In two fish from the highest concentrations 39 and 41 ppm cresol in the wet tissue was found. The average cresol concentration in the fish from LD_{50} experiment was 17.37 (s_d 13.37).

TABLE 1

Concentration of 4-chloro-o-cresol mg/kg in the wet weight of the fish. 10 specimens were analysed.

	Subchronic experiment Concentration in water ppm		
	0.5	1.0	1.5
Experiment I			
	3.45	5.18	
s_d	2.55	2.86	
Experiment II			
	3.15	4.19	6.39
s_d	1.37	1.60	4.58

DISCUSSION

MCPA is the most widely used herbicide in the Nordic countries. When the toxicity of MCPA is considered it must be remembered that the chemical is not sold as a pure compound. One of the main impurities of MCPA is 4-chloro-o-cresol which has been studied in our work. This cresol is also the first soil metabolite of MCPA. In our preliminary experiments we found that 4-chloro-o-cresol is accumulated both in rodents and fish from the technical grade product. The figures concerning the concentrations of cresol in MCPA are not available but the analysis of one batch of technical grade MCPA in the Department of Chemistry, University of Jyväskylä, showed that 4% 4-chloro-o-cresol was as an impurity in the product.

In the earlier study (HATTULA et al. 1978a) when the enrichment of MCPA in the fish was studied, it was found that when the concentration of technical grade MCPA in the water was 100 ppm the concentration of cresol in the tissue was 4 ppm which was approximately the same as the concentration of MCPA. This is probably due to the fact that 4-chloro-o-cresol is fat soluble and is absorbed more easily through the gills.

Our results show that the histopathological changes due to

cresol are biggest in the gills and in the kidney. Especially in the gills the changes were remarkable due to lamellar telangiectasis in some filaments. Corresponding changes were not found in the fish which were kept in 10 and 30 ppm MCPA technical grade solution for some unknown reason.

On the basis of the results obtained in the toxicological experiments by MCPA and 4-chloro-o-cresol in rodents and fish in our laboratory a statement can be made that the accumulation of cresol is bigger than the enrichment of MCPA. It is also evident that the histopathological changes are bigger than in the MCPA-experiment (HATTULA et al. 1978a). Our studies concerning the toxicity of MCPA and 4-chloro-o-cresol (HATTULA et al. 1977, HATTULA et al. 1978a, 1978b) show that the main impurity, 4-chloro-o-cresol may increase its toxicity which depends on the amount of the impurity.

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