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Acute and Short-Term Toxicity of 2,3,4,6-Tetrachlorophenol in Rats

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About 200,000 tons of chlorophenols are manufactured for use as e.g. bactericides, insecticides, fungicides, wood preservatives, and herbicides. In Finland about 1,000 tons of chlorophenolic wood preservatives are used annually (PAASIVIRTA 1978). Pentachlorophenol and the lower chlorinated phenols have been used as fungicides, herbicides, insecticides, and precursors since the early 1930's (AHLBORG & THUNBERG 1980). In Finland analyses have shown chlorophenols even in the most pristine environments (PAASIVIRTA et al. 1976).

Because of the world-wide use of pentachlorophenol, the literature concerning its analysis, toxicology, residues and effects on biological systems is voluminous. The biological action of a series of chlorophenols to the rat has been studied by FARQUHARSON et al. (1958). Short-term toxicity of pentachlorophenol in rats has been studied by KNUDSEN et al. (1974). The effects of pentachlorophenol on the rat liver ultrastructure have been studied by KIMBROUGH & LINDER (1975, 1978). The toxicity and metabolism of tetrachlorophenols in the rat have been studied by AHLBORG (1977).

In this work the toxicity of 2,3,4,6-tetrachlorophenol to the male rat was studied in acute and short-term experiments to relate the histopathological changes in the tissues to the residue levels in the same tissues. The work is a part in a series of studies in which the toxicity, bioaccumulation, and analytical methodology of various chlorinated phenols are studied in our laboratory.

MATERIALS AND METHODS

The chemical. The chemical used was 2,3,4,6-tetrachlorophenol (Fluka, purum) which was distilled several times and finally fractionated by preparative GLC. Purity was checked by IR-, NMR- and mass spectrometry in the Department of Chemistry, University of Jyväskylä (purity > 99 %).

Experimental. In the acute experiment two-month-old Wistar rats, average weight 401 $\pm$ 26 g, were used. The amounts of 2,3,4,6-tetrachlorophenol, dissolved in olive oil and applied intragastrically, was 0, 300, 360, 410, 432, 518, and 632 mg/kg. Each group contained 10 animals. The animals were followed for 24 h and autopsy was performed immediately after death.

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After 24 h all animals were sacrificed and samples for histopathology were taken from liver, kidney, spleen, stomach, small and large intestine, muscle, and brain. Samples for chemical analysis were taken from liver, kidney, spleen, muscle, and brain. Samples for histopathology, were fixed in buffered 10 % formalin, and paraffin sections were stained with haematoxylin-eosin. Tissues for chemical analysis were wrapped in aluminum foil and deep-frozen until analyzed.

In the short-term experiment two-month-old Wistar rats were used, average weight 322 ± 27 g. Tetrachlorophenol which was dissolved in olive oil was applied intragastrically in the following concentrations: 0, 10, 50, and 100 mg/kg seven days a week. The animals were fed ad libitum, and food and water consumption was followed. The animals were weighed every second day. After 55 days the animals were sacrificed and samples were taken as in the acute experiment. In addition to the histopathological studies, blood counts for haemoglobin and differentials were carried out.

Chemical analysis. For the chemical analysis the tissue sample was weighed and homogenized in a Sorvall Omnimixer with anhydrous Na_2SO_4, 4 g for each g of wet tissue and dried at room temperature for 24 h. The tissue was extracted 2 x 0.5 h in Griffin glass shaker with a mixture of chloroform and diethyl ether (1:1 v/v, redistilled) which had been acidified with fuming HCl. The pH of the solvent mixture was < 3 and the amount of solvent was 30 mL for 1-2 g and 50 mL for 2-5 g of the tissue. Extracts were filtered through Whatman no. 1 into a round-bottomed flask. The solvent was then evaporated in a Büchi evaporator and the residue was dissolved in 3 mL ether. Cleanup was conducted by a slightly modified method of RENBERG (1974) in which the buffer contained 1 mL 0.15-N KCl and 2 mL 0.15-N HCl. Derivatization was conducted with diazoethane for 30 min, in sealed tubes after which the ether was carefully evaporated by pure nitrogen and the residue was dissolved in n-hexane. The final analysis was made by GLC by Carlo Erba Fractovap equipped with Ni-EC-detector. The column was 30 m glass capillary SE-30. The phenols were programm from 90° to 160°C, 3°C/min.

RESULTS

Growth and food intake. The weight of the animals increased evenly through the experimental period of 55 days. The average weights at the beginning of the experiment were 290 g (0 mg chlorophenol/kg), 325(10), 345(50), 327(100) and at the end of the experiment 425, 425, 440 and 450 g, respectively.

Histopathology.

Acute experiment. With the exception of a few unspecific changes like hyperaemia, no microscopic changes were observed in the brain. The kidney and muscle were normal in all animals in all groups. In the mucosa of the stomach of three animals, increased infiltration of eosinophilic granulocytes was observed. In the small intestine of some animals, hyperaemia of the mucosa was observed and super-

TABLE I. Histopathological changes of the liver according to the severity of the damage (I the mildest, II medium, III the most severe)

Level I	Level II	Level III
- mild proliferation of the bile canaliculi	- prominent proliferation of the bile canaliculi and inflammatory infiltrates around the bile tracts	- large confluative necroses which covered most of the liver parenchyma
- swelling of the endothelial cells of the bile tract	- hyalinisation of some veins in the portal areas	- veins were extensively dilated and sometimes thrombosed
- groups of leucocytes gathered around the small bile canaliculi	- focal areas with necroses of several hepatocytes	- well preserved hepatocytes remain only around the portal areas
- occasionally necroses of the endothelial cells of canaliculi and some hepatocytes were observed	- around these areas gathering of polymorphonuclear leucocytes, large mononuclear cells, probably histiocytes and active Kupfer cells were observed	
	- areas with large basophilic hepatocytes with large nucleoli and active nuclei	
	- the cells formed more or less distinctive nodules, probably regenerative nodules	

ficial cells were shed out from the epithelium. In the spleen only slight stasis was observed. In the large intestine in the groups which obtained 432 and 518 mg/kg tetrachlorophenol mild necrosis was observed in one animal and in the group which obtained 622 mg/kg chlorophenol approx. 70 % of the animals had real necroses which were, however, situated on the surface cells of the epithelium.

The most conspicuous changes were observed in the liver. The changes could be divided according to light microscopical changes into three levels denoted by (I), (II) and (III) in the text and presented in Table 2. The same changes could also be observed in the short-term experiment.

Short-term experiment. Haematology. The results of the blood analysis are shown in Table 2.

Histopathology. With the exception of occasional unspecific changes such as hyperaemia in a few animals no pathological changes were observed in the brain. The muscles were normal but in the stomach mild dilatation of the veins of the mucosa was observed. The small intestine was normal at the two lowest dosage levels but necroses we found in three animals in the group which obtained tetrachlorophenol 100 mg/kg. The large intestine was normal in all animals and in all groups. The most serious changes were observed in the liver. At the lowest level (10 mg/kg) no changes were observed in the liver pa renchyma. Only one animal, which had obtained tetrachlorophenol at 50 mg/kg, necroses typical to level (III) of liver damages were observed. In the rates which had obtained tetrachlorophenol at 100 mg two animals had changes in the liver corresponding to the levels (II and (III).

Results of the chemical analysis of the short-term experiment are shown in Table 2.

TABLE 2. Concentration (ppm) of 2,3,4,6-tetrachlorophenol in tissue

Tissue	DOSE mg/kg		
	10	50	100
muscle	n.d.	0.22	0.46
sd	n.d.	0.26	0.48
brain	n.d.	0.98	1.2
sd	n.d.	0.77	0.68
spleen	0.04	1.4	3.2
sd	0.02	1.8	2.6
kidney	0.03	1.0	5.1
sd	0.03	0.72	3.5
liver	0.01	0.65	2.2
sd	0.01	0.51	1.9

n.d. = not detected

DISCUSSION

This experiment was carried out to study the histopathological effects of 2,3,4,6-tetrachlorophenol and relate the results to the residue levels in the same tissues.

In the acute experiment the highest residue concentrations were found in spleen which contained tetrachlorophenol at 146 mg/kg followed by kidney and liver which contained residues of 47 and 34 mg/kg, respectively. In the short-term experiment at the lowest application level (10 mg/kg) no residues were found in muscle and brain and the concentrations in other tissues were very low. The highest concentration at the application level of 50 mg/kg was found in spleen and kidney, 1.4 and 1.0 ppm and the corresponding figures at the application level 100 mg/kg were 3.2 and 5.0 ppm, respectively. The concnetrations were smallest in muscle and brain.

Acute toxicity of tetrachlorophenols has been studied by AHLBORG (1977). The acute toxicity of 2,3,4,6-tetrachlorophenol was 131 mg/kg administered intraperitoneally. In our study the corresponding figure was 360 mg/kg administered intragastrically. The biochemical behavior of chlorophenols has been studied by ARRHENIUS et al. (1977a, 1977b), CARLSON (1978) and KNUDSEN et al. (1974). According to KNUDSEN et al. (1974) the liver weight was increased by pentachlorophenol and the activity of microsomal liver enzymes was increased, also. Pentachlorophenol has also been known as an uncoupler of microsomal detoxification by inhibition of the terminal oxygenation enzyme P-450 (ARRHENIUS et al. 1977b). Trichlorophenols did not induce xenobiotic metabolism in the rat: 2,3,5-, 2,3,6- 2,4,5- and 2,4,6-trichlorophenol at the doses of 400 mg/kg p.o. daily for 14 days did not alter EPN detoxification by CARLSON (1978).

The acute experiment showed that histopathological changes were almost completely focused on the liver which according to the chemical analysis contained approx. 34 ppm tetrachlorophenol. In regard to histopathological changes also in the short-term experiment the tissue damage was mostly confined to the liver. Similar results were obtained by KIMBROUGH & LINDER (1975, 1978) when pentachlorophenol was used. In our study the liver seems to be affected between 50 and 100 mg/kg, because in only one animal were there changes in the group which received 50 mg/kg 2,3,4,6-tetrachlorophenol.

The severe necrosis of the liver parenchymal tissue seen in this experiment might be due to the metabolic poisoning of the liver parenchymal cells, especially mitochondria. From the experiments of ARRHENIUS et al. (1977b), it is known that at least pentachlorophenol acts as a potent uncoupler of oxidative phosphorylation. On the other hand, the specific damage in the liver may be due to the fact that chlorophenols are metabolized in the liver by a mixed function oxidase system. Furthermore, it is known that at least pentachlorophenol selectively inhibits the terminal oxygenation enzyme P-450 of this system. This inhibition may play an important role in the induction of the liver damage by initiation of free

radicals and hence lipid peroxidation.

The inflammatory changes seen in the bile ducts are a secondary process which is preceded by the specific parenchymal cell damage due to the damage of mitochondria by uncoupling and endoplasmic reticulum by lipid peroxidation.

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