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Acute Toxicity of Some Chlorinated Phenols, Catechols and Cresols to Trout

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One of the biggest problems in the wood pulp industry are the waste waters of the bleaching process. In Finland the discharge of chlorophenolic wood preservatives is about 1000 tons annually (PAASIVIRTA 1978). The main components in wood protection substances are 2,4,6-tri-, 2,3,4,6-tetra- and pentachlorophenol.

The phenol structure is also very common in nature, e.g. lignin, and the structure when combined with chlorine becomes easily chlorinated. This is the way in which the pulp bleaching and disinfection of waters produce chlorinated phenol residues in the waters of Finland. 30 Tons of these kind of components enter the waters annually (PAASIVIRTA 1978). The occurrence of pentachlorophenol has been analyzed in water, soil and sewage by BUHLER et al. (1973) and STARK (1969), and in fish by STARK (1969) and ZITCO et al. (1974).

Reports on the occurrence of lower chlorinated phenols in the environment are few. In Finland PAASIVIRTA et al. (1978) have reported on the occurrence of some chlorinated phenols in the Lake Päijänne ecosystem and later PAASIVIRTA et al. (1980) on the transportation of chlorinated phenols and guaiacols in aquatic food chains. Beside the chlorophenols, the bioaccumulation of chloroguaiacols and chlorocatechols have been reported in Sweden by LINDSTRÖM & NORDIN (1976).

The toxicity of various chlorinated phenolics to Daphnia magna has been studied by DURKIN (1978), to fish by GOODNIGHT (1942) and KOBAYASHI et al. (1978). A wide review of the toxicity of pentachlorophenol to crustaceans has been published by RANGA RAO et al. (1979).

In the present study the acute toxicity of six chlorinated phenols, four catechols and three cresols to trout was determined and concentration of the majority of the test compounds was analyzed in fish.

MATERIALS AND METHODS

Chemicals. The chemicals used in the experiment were as follows:

2,4-dichlorophenol	+	Fluka AG	97 %
2,6-dichlorophenol	+		
2,3,5-trichlorophenol		Fluka AG	97 %
2,4,5-trichlorophenol	+		
2,3,4,6-tetrachlorophenol	+		
pentachlorophenol		Fluka AG	99 %

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3,5-dichlorocatechol	++	
4,5-dichlorocatechol	++	
3,4,5,6-tetrachlorocatechol	++	
5-chloro-3-methylcatechol	++	
3-chloro-o-cresol	++	
4-chloro-o-cresol	+	Fluka AG 97 %
4-chloro-m-cresol	++	

(+) purified from the commercial product
(++) synthesized in the Dept. of Chemistry, University of Jyväskylä

Purity of the chemicals was controlled by IR-, NMR- and mass-spectrometry. For the experiment the chemicals were dissolved in absolute ethanol which was applied max. 1 mL/10 L H_2O.

Fish. The fish were trout (Salmo trutta); the average weight was 4.5 g. Fish were placed in 10-L aquaria at +5°C, 5 specimens into each and the aquarium was filled with 5-L test solution.

Fish were followed for 24 h and the dead fish were picked up, wrapped in aluminium foil and frozen until analyzed. After 24 h all fish were killed and frozen.

Extraction and cleanup. Chlorinated phenols were extracted from Na_2SO_4-dried material with acidic chloroform-di-ethylether 1:1 (v/v), pH 3). The extraction occurred by shaking the dried fish 2 x 0.5 h in a glass shaker and the solvent was filtered through Whatman no. 1 filter paper. The combined extracts were evaporated in Büchi Evaporator.

The amount of solvent was 10 mL per gram of wet tissue. The cleanup was conducted by the slightly modified method of RENBERG (1974). Chlorinated cresols and catechols were extracted and purified as described by HATTULA et al. (1979).

Analysis. The gas chromatograph used was equipped with a ^{63}Ni-EC-detector and a 30-m SE-glass capillary column. Injection was splitless and compounds were chromatographed from 90 to 180°C, 3°C/min. Quantitative analysis was conducted by comparing peak heights.

RESULTS AND DISCUSSION

In Table 1 are presented the LC_{50}-values for different compounds, concentrations as mg/kg tissue and the concentration factors.

The compounds which were common with the study of KOBAYASHI et al (1979) were 2,4-dichloro-, 2,3,4,6-tetrachloro- and pentachlorophenol, the concentration factors of which were 34, 93 and 480. In our study the corresponding values were 10, 450 and 100, respectively.

The remarkable difference in the figures between the two studies was that the amount of phenol found in the dead fish was an order of

TABLE 1. LC_{50}-values (24 h) and accumulation of some chlorinated phenols, catechols and cresols to trout

compound	LC_{50}, ppm	concentration in tissue, mg/kg	concentration factor
2,4-dichlorophenol	1.7	18	10
		SD 7	
2,6-dichlorophenol	4.0	no analyses	
2,3,5-trichlorophenol	0.8	5.7	
		SD 4.6	12
2,4,5-trichlorophenol	0.9	no analyses	
2,4,6-trichlorophenol	1.1	"	
2,3,4,6-tetrachlorophenol	0.5	210	450
		SD 120	
pentachlorophenol	0.2	200	100
		SD 110	
3-chloro-o-cresol	2.0	1.3	
		SD 1.3	
4-chloro-m-cresol	1.3	no analyses	
3,5-dichlorocatechol	2.9	6.4	2.2
		SD 2.5	
4,5-dichlorocatechol	2.3	9.7	4.3
		SD 7.7	
tetrachlorocatechol	1.1	6.3	
		SD 1.6	
5-chlorosalisylic alcohol	4.0	no residues	

magnitude in the study of KOBAYASHI et al. (1979). The concentration factors of 2,4-dichloro- and trichlorophenols in our study were about a half of that observed in the Japanese study and only a fifth as to PCP. On the other hand, the concentration factor of 2,3,4,6-tetrachlorophenol in our study was very high, approx. 450. This might be due to the fact that the tetrachlorophenol was extremely well purified by several fractional distillations and finally by preparative GLC because several batches of tetrachlorophenols contained remarkable amounts of PCP.

The concentration ratio of catechol was small compared with phenols, possibly due to their rapid degradability. The LC_{50}-values for different cresols in this experiment were of the order of the lower chlorine containing phenols.

The smallest lethal concentration of the compounds studied was 0.20 ppm caused by PCP followed by tetrachloro- and trichlorophenols. The cresols were lethal in concentrations 1-2 ppm, and catechols somewhat higher. The lethal catechol dose in tissue was, however, much smaller, 6-10 mg/kg whereas the corresponding value of higher phenols was about 200 mg/kg. As to the differences between this study and that of KOBAYASHI et al. (1979) some differences may be explained by different metabolism between the species because the

trout (S. trutta) which is used for toxicological studies in our laboratory lives in +5°C.

Characterization of acidic phenolic fraction of wood pulp bleachery effluents was first conducted by LINDSTRÖM & NORDIN (1976). In the first chlorination step, chlorophenols and chlorocatechols were identified. The accumulation and toxicity of chlorocatechols has not been studied so far. In the present study the LC_{50}-value for chlorinated catechols was 1-3 ppm depending on the compound, which is of the same order of magnitude as trichlorophenols.

The acute and subchronic toxicity of 4-chloro-o-cresol to trout has been studied by HATTULA et al. (1979). The LC_{50}-value was 2.1 ppm and the tissue concentration 17 mg/kg. In the present study the LC_{50}-value for 3-chloro-o-cresol was 2.0 and for 4-chloro-m-cresol 1.3 ppm, respectively.

The bioaccumulation of chlorinated guaiacols, which are an important group of chemicals in pulp bleachery effluents, has been studied by LANDNER et al. (1977). CHU et al. (1979) have studied the toxic effect of guaiacols in rats to assess the toxic potential of chemicals in pulp mill effluent. The transportation and enrichment of chlorinated phenols, catechols and guaiacols have been studied by PAASIVIRTA et al. (1980).

In order to obtain valuable information of the toxic effects of pulp mill effluents, both acute and chronic experiments with fish and crustaceans should be carried out by testing several compounds together and in relation they appear in natural conditions.

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