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Toxicity of 5-Chloro-3-Methyl-Catechol to Rat: Chemical Observations and Light Microscopy of the Tissue

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5-chloro-3-methyl-catechol was first time described by GAUNT and EVANS (1961) as a metabolite of MCPA (4-chloro-2-methylphenoxyacetic acid) which is the most widely used herbicide in the Nordic countries. The toxicity of the metabolite 5-chloro-3-methyl-catechol has not been studied so far. Other catechols like 3,5-dichlorocatechol has been shown to be a metabolite of 2,4-D (BOLLAG et al. 1968a, BOLLAG et al. 1968b), and it has been proven to be toxic to cells by HORVATH (1971). The catechols are metabolized by bacteria finally to muconic semialdehyde (HORVATH 1970, HORVATH and ALEXANDER 1970).

In this work the toxicity of 5-chloro-3-methyl-catechol to male rats was studied in acute and subchronic experiment in order to relate the histopathological changes in the tissues to the residue levels in the same tissues. A TLC method recently developed by SATTAR et al. (1977) was used in the clean-up and the quantitative determination of the catechol residues occurred by glass capillary GLC.

MATERIALS AND METHODS

The rats used were 2 months old male Wistar rats, the average weight of which was 327 g in the acute experiment and 283 g in the subchronic experiment.

5-chloro-3-methyl-catechol was synthesized in the Department of Chemistry, University of Jyväskylä (purity > 99%), and the purity of the compound was controlled by Ri-, NMR- and mass spectrometry. The catechol was dissolved in olive oil and applied by a syringe equipped with a steel canyl (2.5 ml/kg) directly in the stomach.

The LD_{50}-value was determined by the method of WEIL (1962) by oral administration. Following concentrations of catechol were used in the acute experiment: 0, 1300, 1400 and 1508 mg/kg. Each group contained 10 animals. The animals were under control for 24 hours.

The tissue samples for light microscopy were taken from liver, kidney, spleen, oesophagus, small intestine, large intestine and muscle (musculus vastus lateralis) and fixed in 10% buffered formalin and stained with haematoxylin eosin. For the chemical analysis samples were taken from liver, kidney, spleen and muscle, wrapped in aluminium foil and deep frozen in -25°C until analyzed.

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Subchronic experiment: In the subchronic experiment 30 animals were taken in three groups of which Group I obtained catechol 100 mg/kg, Group II 250 mg/kg and Group III served as a control. The catechol was applied daily for four weeks in a solution 2.5 ml/kg. The bigger concentration, however, was impossible to apply by force-feeding, and the animals were killed after two weeks. Before sacrificing the animals were starving 24 hours. A blood sample for analysis was taken from the heart. The samles for chemical analysis were taken from liver, kidney, spleen and muscle. For the histopathological analysis the samples were taken from liver, kidney, stomach, small and large intestine and muscle.

The chemical analysis was carried out only from Group I and Group III but the histopathological analysis from all groups.

Chemical analysis: Extraction. The frozen sample was melted and homogenized in Sorvall Omnimixer with anhydrous Na_2SO_4 and two drops 6-n HCl. The sample was kept for 24 hours at room temperature. The extraction occurred by shaking the sample 2 x 0.5 h with diethylether (redistilled) in a glass stoppered round bottom flask. Ether was used 10 ml for each gram of wet tissue. The extracts were filtered through filter paper Whatman n=1 and the combined extracts were evaporated in Büchi evaporator. The residue was dissolved in 1-5 ml diethylether of which 1 ml was carefully evaporated with nitrogen and applied for TLC.

Clean up: TLC plates 24 x 24 cm made of Kieselgel G nach Stahl (layer thickness 1 mm) were used for qualitative and quantitative separation of catechol. The activated plate was devided in 5 sections by upright lines of which 4 were used for samples and one for standard. The evaporation residue was applied in 200 µl ether on the plate, 1 cm from the lower end and 10 µl catechol standard (10 ug/ml) was applied as a standard spot. The plate was developed in the mixture of chloroformdiethylether-acetic acid as described by SATTAR et al. (1977). The plate was dried at room temperature and the standard spot was sprayed with 2% 2,6-dichlorochinonchlorimid in toluene. The rest of the plate was covered with aluminium foil. According to the visible purple spot the catechol fraction was scraped by a spatula in a glass-wool stoppered tube (10 x 1 cm) and the fraction was eluted by 10 ml ether in a 20 ml glass stoppered tube. The solvent was allowed to evaporate at room temperature overnight. The residue was then derivatized for GLC in the following way: 1 drop NaOH solution (10.8 g NaOH in 10 ml water) and 2 drops dimethylsulphate were added carefully on the bottom of the tube which was sealed well and kept in boiling water bath for 40 min. After cooling 5 ml ether was added into the tube and solvent was then evaporated. Ether was then added 1-5 ml on the residue which was determined quantitatively by GLC.

Quantitative analysis: The equipment used was gas chromatograph Carlo Erba Model Fractovap equipped with FID-detector and a grob-type splitless injection system. The column was glass capillary column, a mixture of Carbowax and FFAP, length 15 m and inside diameter 0.35 mm. The injection occurred at 60°C and

a program 30°C/min to 190°C gave a good resolution for the methylderivative. As a standard 5-chloro-3-methylveratrole (4-chloro-6-methyl-1,2-dimethoxybenzene) 30 ng/µl was used. The standard was synthesized in the Department of Chemistry, and the purity was controlled by IR-, NMR- and mass spectrometry. The quantitative determination occurred by comparing the peak height with the corresponding standard. The quantitative analysis was made only from the group which obtained the highest concentration (1508 mg/kg) and from the group which obtained 100 mg/kg catechol for four weeks.

RESULTS

Histopathological study: Acute experiment. The LD_{50}-value for the study was 1412 mg/kg (1371-1453 mg/kg). At the dose level 1300 mg catechol / kg in the acute experiment inflammation was present in all oesophagus samples. The inflammation was phlegmonous through the wall of the oesophagus. Microabscesses were present in the wall of the stomach. In the small intestine the villi were quite normal; in some samples, however, increased inflammatory cells could be observed and vacuolization of the nuclei was found. The changes in the spleen were slight. Germinal centers were few in number and some phagocytosis could be seen. Stasis was not severe. In the liver slight autolytic changes could be observed. In some samples stasis was present and in some places pyknotic eosinophilic cells were found. In the kidney in some areas dilatation of the distal tubules was found and inside the dilated tubules was hyaline round material, probably protein. The muscle and large intestine were normal. At the dose level 1400 mg/kg acute inflammation was present in three stomach samples, and inflammation penetrated through all the sheets of the stomach wall. Also microabscesses could be observed. In two animals the normal mucosa of duodenum was disturbed, inflammatory infiltrates were present and locally the villi were totally absent and only some pyknotic cells of the crypt could be found. Some ghost villi were also observed, some of them, however, because of autolysis.

Kidney: At the dose level 1400 mg/kg the distal tubules showed slight dilatation and often hyaline bodies were present. The spleen was similar to that seen at the dose level 1300 mg/kg. Also the histology of liver, large intestine and muscle were similar to the lower exposure level.

At the dose level 1508 mg/kg the oesophagus was normal. In the duodenum of all animals the mucosa was destroyed 1/2 - 2/3 of its height. In lamina propria oedema and increased number of inflammatory cells could be seen. In some areas all villi were destroyed to the bottom of the crypts. In the kidney focal dilatation of distal tubules and hyaline bodies were seen.

In two animals we saw changes in the white pulp of the spleen: The number of polymorphonuclear leucocytes was increased in the sinusoids. In the marginal zone nuclear dust and dying cells were seen and the germinal centers were partly destroyed.

In the liver sinusoids were filled with erythrocytes indicating stasis. All other tissues were normal.

Subchronic experiment: At the dose level 100 mg/kg all tissues were normal compared to the controls. Although the animals at the dose level 250 mg/kg were killed after two weeks tissue samples were, however, analysed histopathologically in order to find out the lethal effects of the catechol applied daily.

In all but one tissue sample of the liver, necrotic cells could be seen and there was a prominent mononuclear infiltration around these areas. Stasis was a prominent finding in all liver samples. Microvesicular changes were found in the cytoplasm of the liver cells. The finding indicated a slight toxic hepatitis and a pronounced fatty degeneration of the liver. In the spleen distinct stasis was observed. Kidney, stomach, small and large intestine and muscle were normal.

Blood analysis: The results of the blood analysis are shown in Table 1. With the exception of leucocytes no obvious differences were observed between the two groups.

TABLE 1

Composition of blood in the subchronic experiment (dose 100 mg/kg).

	Hb g/l	Hkr %	L_2 mm	E 10^6mm	Polymorphonuclear leucocytes	L-monocytes	L-lymfocytes
	154	49	10900	8.19	8.3	2.6	88.7
Control	163	48	6100	7.76	8.8	2.8	88.4

Chemical analysis: The concentrations of 5-chloro-3-methyl-catechol expressed as ppm in wet tissue are shown in Table 2.

TABLE 2

Concentrations of 5-chloro-3-methyl-catechol (ppm in wet tissue) after acute (15 animals) and subchronic (5 animals) exposure. The figures of the acute experiment concern all three groups.

	Acute mg/kg	Chronic 100 mg/kg
Liver	571.5	-
s_d	15.4	
Kidney	177.9	-
s_d	13.2	
Spleen	194.2	-
	34.5	
Muscle	44.7	-
s_d	18.8	

The results show that the highest concentration was found in the liver and the lowest in the muscle. In the subchronic

experiment not even traces of the compound were found in the tissues due probably to a rapid decomposition of the compound in the tissues.

DISCUSSION

5-chloro-3-methyl-catechol is the third known soil metabolite of MCPA. It has also been found that the chlorinated catechols are metabolites of chlorinated phenols. Therefore their appearance in the nature may be remarkably higher than has been assumed.

Compared to the toxicity of MCPA and 4-chloro-o-cresol (HATTULA et al. 1977, HATTULA et al. 1978) it is evident on the basis of our work that 5-chloro-3-methyl-catechol is far more toxic. The biggest changes in histopathology were seen in liver, oesophagus and small intestine in the acute experiment. At the highest dose level 1508 mg/kg the mucosa of the small intestine was totally destroyed. In the subchronic experiment the changes were biggest in liver samples and the findings indicate slight toxic hepatitis. In the small intestine the changes were smaller than in the acute experiment. In the blood analysis an obvious inflammation reaction was seen as an elevated level of leucocytes.

The chemical analysis of the tissue showed that in the acute experiment the compound was most found in the liver and spleen. The histopathological changes in these organs agree well with the chemical findings. Our study shows that the chronic toxicity of 5-chloro-3-methyl-catechol is bigger than the toxicity of other metabolites studied (HATTULA et al. 1977). The mutagenicity test of 5-chloro-3-methyl-catechol was negative (RÄSÄNEN et al. 1977).

At the present time there is no conception of the fact how rapidly the compound is metabolized in the nature and what is the half-life of it. In our study it was not found in the subchronic experiment and it gives support to the assumption that it is rapidly metabolized further.

REFERENCES

BOLLAG, J.M., C.S. HELLING and M. ALEXANDER: J. Agr. Food Chem. 16, 826 (1968a).

BOLLAG, J.M., E.G. BRIGGS and J.E. DAWSON: J. Agr. Food Chem. 16, 829 (1968b).

HATTULA, M.L., H. ELO, H. REUNANEN, T.E. SORVARI and A.U. ARSTILA: Bull. Environ. Contam. Toxicol. 18, 2 (1977).

HATTULA, M.L., H. REUNANEN and A.U. ARSTILA: Accepted to Bull. Environ. Contam. Toxicol.

HORVATH, R.S.: Biochem. J. 119, 871 (1970).

HORVATH, R.S.: J. Agr. Food Chem. 19, 291 (1971).

HORVATH, R.S. and M. ALEXANDER: Can. J. Microbiol. 16, 1131 (1970).

GAUNT, J.K. and V.C. EVANS: Biochem. J. 79, 25 (1961).

SATTAR, M.A., J. PAASIVIRTA, R. VESTERINEN and J. KNUUTINEN: J. Chromatog. 135, 395 (1977).