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4-Chloro-3-Methylphenol: Salmonella/Mammalian-Microsome Mutagenicity Test and Subacute Toxicity Test in Rats

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4-chloro-3-methylphenol, CMP, (4-chloro-m-cresol) is a potent bactericide. It is used as a preservative in pharmaceutical products and cutting oils.

A previous investigation in the Salmonella/mammalian-microsome test (Rapson et al. 1980) has revealed no mutagenic potential of CMP. Available information on oral toxicity is limited to Dieke et al. (1949), stating a LD_{50}-value of 500 mg/kg bw or higher based upon 2 rats.

The present investigation was carried out in order to obtain more detailed information concerning the mutagenic potential and oral toxicity of the substance, as CMP has been detected in drinking water in Denmark, probably as a result of contamination of waterpipes with cutting oil.

MATERIALS AND METHODS

4-chloro-3-methylphenol, CAS-number 59-50-7, Fluka no. 24940 purum.

Salmonella typhimurium, strains TA 100, TA 1535, TA 1537 and TA 98 were provided from Dr. B. N. Ames, Berkeley, U.S.A. The Salmonella/mammalian microsome test was carried out according to Ames et al. (1975). The S 9 microsome fraction from Aroclor 1254 induced male Wistar rat liver was used for metabolic activation, in a concentration of 2 mg protein per plate. CMP was dissolved in dimethylsulphoxide (DMSO). DMSO also served as a negative control. In tests without S 9, sodium azide served as a positive control for the tester strains TA 1535 and TA 100, while 2-nitroflourene served as positive control for the tester strains TA 1537 and TA 98. In the tests performed with S 9, 2-antramine served as a positive control for all strains. The following concentrations were selected for testing as doses higher than 800 µg CMP per plate exerted strong toxic effects on the tester strains. 800, 160, 32, 6.4, and 1.28 µg per plate. Each dose level was tested in five parallel plates, and all tests were performed in two independent experiments.

Statistical evaluation was performed according to Andersen and Jensen (1984).

4 groups of 10 male and 10 female rats were given 0, 50, 200 or 400 mg CMP/kg bw/day for 28 days. CMP was dissolved in soy bean oil (food grade), as the solubility in water is low and dosed by gavage, 5 ml/kg bw/day. Animal strain, diet, caging, environmental conditions, and clinical observations were as described by Thorup et al. (1983). Blood samples for hematological and clinical chemical analyses were taken from the periorbital plexus from 8 males and 8 females in each group after 21 days dosing. Hemoglobin, PCV, total erytrocyte count, and glucose were examined in whole blood. Concentrations of creatinine, urea, and activity of aspartate aminotransferase was determined in plasma. Methods were as described by Thorup et al (1983). Alkaline phosphatase was determined in plasma (Empfelungen der Deutsches Gesellschaft für Klinishe Chemie 1972). The rats were killed by exanguination in CO_2-anaesthesia after 4 weeks of dosing. The following organs were excised and weighed at necropsy: kidneys, spleen, brain, adrenals, liver and testes. Samples from these organs and from stomach, small intestine, pancreas, lung, aorta, heart, thymus, thyroid, and parathyroid were fixed in 10% buffered formalin. The samples were prepared for light microscopy and stained with hematoxylin-eosin (all) PAS (liver) and Pearl (liver and spleen). Freeze sektions of liver and heart were stained with Oil red O.

Students t-test was performed on body weight and weight gain. Analysis of variance was performed on relative organ weights, hematological, and clinical chemical parameters. The statistical analyses were calculated for males and females separately.

RESULTS AND DISCUSSION

The results of the salmonella mammalian-microsome test are given in tabel 1. 4-chloro-3-methylphenol at doses 1.28, 6.4, 32, 160 an 800 µg per plate did not show mutagenic potential in Salmonella tester strain TA 1535, TA 100, TA 1537, and TA 98 with and without S 9.

The number of His+ revertants per plate were in the same range as the negative control at the three lowest dose levels, i.e. 1.28, 6.4 and 32 µg per plate in experiments without S 9. Concentrations of 160 and 800 µg per plate generally showed an increasing toxic effect on the tester strains. Addition of S 9 mix seemed to make CMP less toxic to the bacteria.

In the subacute toxicity test the animals in the highest dosed group exhibited a significant decrease in weight gain during the last week of dosing (table 2). No other clinical signs of adverse effects were observed.

The hematological and clinical chemical parameters were found to be within the normal range in all groups. No significant alterations were noted in the relative organ weights. There were no pathological changes attributable to the dosing with 4-chloro-3-methylphenol.

Table 1. Number of revertants after exposure to 4-chloro-3-methylphenol in the salmonella/mammalian microsome test. Presented as mean of 10 plates ± S.E.M.

Dosage	TA 1535		TA 100		TA 1537		TA 98	
µg per plate	-S 9	+S 9	-S 9	+S 9	-S 9	+S 9	-S 9	+S 9
DMSO	8±1	13±1	107±5	112±5	14±1	11±1	12±1	29±2
1.28	9±1	14±1	105±4	113±5	13±1	12±1	15±2	27±2
6.4	9±1	12±1	107±4	117±6	12±1	12±1	10±1	34±2
32	8±1	11±1	105±4	105±5	9±1	12±1	10±1	31±2
160	3±1	10±1	83±3	116±6	6±1	12±1	7±1	33±2
800	3±1	11±1	0[a]	69±6	0[a]	6±1	0[a]	22±2
Sodium azide	404±15***		394±16***					
2-nitrofluorene					134±5***		800±18***	
2-anthramine		251±4***		734±30***		156±7***		892±78***

a = toxic response
*** = $p < 0.001$

Table 2. 4-chloro-3-methylphenyl subacute toxicity test: Weight gain week 4.

mg CMP/kg bw/day	0	50	200	400
	weight gain (g)			
male rats	20,7±5,0	21,9±7,5	21,1±7,0	14,1± 5,7*
female rats	9,3±3,0	11,9±7,5	8,4±3,9	5,5±2,9**

* $p < 0,05$ ** $p < 0,01$

Although not associated with pathological or biochemical changes the growth retardation of both males and females in the highest dosed group is considered a toxic effect of the test substance. On this basis the no effect level in this 28-days oral toxicity study with CMP in rats was 200 mg/kg bw/day.

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