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Toxicity Studies on Chlorinated Guaiacols in the Rat

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Trichloroguaiacol (3CG) and tetrachloroguaiacol (4CG) are formed by the reaction of chlorination agents with phenolic lignins during the bleaching process in the manufacture of pulp and paper. These compounds have been identified in river water receiving pulp mill effluents and have been shown to be toxic to fish (LEACH and THAKORE 1975). Since chloroguaiacols present in river water can occur in drinking water, it is important to have information on the toxicity of these compounds. The present study was carried out as part of a general program to evaluate the toxicity of pulp mill effluent (VILLENEUVE 1977) and was designed specifically to investigate the acute and subacute toxicities of 3CG and 4CG.

MATERIALS AND METHODS

Trichloroguaiacol was prepared in the following manner: Chlorine gas was bubbled into a stirred solution of guaiacol (12.4 g) in glacial acetic acid for a period of 50 min while the reaction mixture was kept at 12°C by external cooling. The mixture was then poured onto crushed ice (500 g) and the solids that formed were filtered, washed and dried. One recrystallization from hexane produced 3CG (22 g) as white needles, mp 112-114°, lit. mp. 107-108° (HEILBRON 1965).

Tetrachloroguaiacol was synthesized in quantitative yield by a similar method described for 3CG except that chlorine gas was bubbled into the reaction mixture for 2 h. Mp. 122-124°, lit. mp 119-120° (HEILBRON 1965). NMR and GC-MS of the synthesized products were found to be consistent with those of 3CG and 4CG. Purities were established to be greater than 99% by GC.

Acute Toxicity Study. Male Sprague-Dawley rats (Biobreeding Laboratories, Ottawa, Canada) weighing 250-300 g were intubated with single oral doses of 3CG and 4CG dissolved in corn oil (Mazola Corn oil, 0.5 mL/100 g body weight). There were five dose groups (888, 1333, 2000, 3000 and 4500 mg/kg body weight) each with ten animals. The animals were fed ad libitum with standard laboratory feed (Master Feed). LD_{50} values were calculated according to the method of LITCHFIELD and WILCOXON (1949) based on an observation period of 14 days.

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Subacute Toxicity Study. Seven groups of male weanling rats six animals each group, were given 3CG and 4CG at 0, 50, 500 or 5000 ppm incorporated in the diet for 28 days. Body weight gains and food consumption were measured daily for the entire period. After 28 days all animals were anesthetized with ether and exsanguinated. The serum collected was assayed for sorbitol dehydrogenase activity (Calbiochem, La Jolla, Cal.). The liver, spleen, kidney, heart and brain were excised and weighed. Tissues were fixed with 10% buffered neutral formalin, and paraffin sections made and stained with hematoxyline, phloxine and saffaron. Part of the liver was homogenized for the determination of aniline hydroxylase activity using the method of BECKING (1973).

For tissue residue analysis, tissues (ca. 1 g) were homogenized with nine volumes of water, and the homogenate (1 mL) was extracted with hexane (10 mL). An aliquot of the hexane extract (1 mL) was methylated using diazomethane. The reaction mixture was allowed to stand 30 min at room temperature. The solvent was evaporated to dryness under a stream of N_2 gas and the residue was taken up in hexane (2 mL). The hexane solution was washed with sulfuric acid (1 mL), and dried over sodium sulfate for the GC analysis using Tracor MT-220 gas chromatograph equipped with a 6 ft X 1/4 in. glass column packed with 4% SE-30 and 6% QF-1 on Supelcoport, 80-100 mesh. Temperature of the electron capture detector was 285°, column temperature 170° and injector port 170°. Tissue homogenates (1 mL) of control rats after being fortified with 3CG or 4CG (24 μ g), were analyzed in a similar manner as described above. Recoveries were greater than 90%, and the values reported in this study were uncorrected for recovery.

RESULTS AND DISCUSSION

Acute Toxicity Study. Trichloroguaiacol was found to have an oral LD_{50} of 3000 mg/kg (2400 - 3700 mg/kg), whereas 4CG showed and LD_{50} of 1690 mg/kg (1400 - 2000 mg/kg). These LD_{50} values placed 3CG and 4CG in the moderate toxic group rated by the toxic scale of GLEASON et al (1976).

Subacute Toxicity Study. Decreased body weight gains occurred only in the group of rats fed with the diet containing 50 ppm 3CG (control rats 210 $\pm$ 9 g, test rats 190 $\pm$ 15 g, statistically significant at $P \leq 0.05$), but not in the rats on 500 or 5000 ppm diet. Reduction in body weight gains was not noted in rats receiving 4CG. Food consumption was not affected by 3CG or 4CG at any dietary level.

Trichloroguaiacol caused a slight increase in liver weight at 50 ppm concentration (control: 5.17 $\pm$ 0.26%, expressed as % of body weight, test animal : 5.96 $\pm$ 0.29%). However, no change was noted in organ weights of animals on other 3CG dietary levels. Tetrachloroguaiacol had no effect on organ weights at any dose level.

Aniline hydroxylase activity was elevated by 3CG at 50 and 5000 ppm level and by 4CG at all dose levels (Table 1). A dose-dependent effect was not found.

TABLE 1

Aniline Hydroxylase Activity of Rats[a]

(μ mole PAP/g liver/hour)

	3CG	4CG
Control	18.4 ± 1.1	18.4 ± 1.1
50 ppm	24.7 ± 2.7[b]	27.0 ± 6.0[b]
500 ppm	20.7 ± 3.7	24.4 ± 5.9[b]
5000 ppm	26.2 ± 7.1[b]	20.3 ± 1.3[b]

a. Data represent mean ± SD of six animals.
b. Statistically significant at $p \leq 0.05$ as compared to control group.

Serum sorbital dehydrogenase activity was not affected by 3CG or 4CG at any dose level. Hematological results were normal and not altered by 3CG or 4CG. All animals appeared healthy throughout the experiment with no gross abnomalities being observed on necropsy. No histological change was observed in any tissue.

The analysis of tissue samples indicated that no significant level (0.2 ppm) of 3CG was found in any tissue of rats, a result showing this compound was either rapidly metabolized and/or excreted. Low levels of 4CG were found in the liver and kidney of rats on 500 ppm and 500 ppm diet. (Table 2) The accumulation of 4CG in these tissues appears to be in a dose-related manner.

The data presented above indicate that 3CG and 4CG are moderately toxic to the rats.

TABLE 2

Tetrachloroguaiacols (ppm)

	500 ppm	5000 ppm
Liver	0.66 ± 0.18	6.4 ± 1.6
Kidney	0.74 ± 0.10	7.9 ± 2.0

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