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Acute Dermal Toxicity of Tetrachlorophenols in the Rat

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Tetrachlorophenol isomers (primarily the sodium salt) are used extensively in the forest industry as a slimicide and to prevent sap staining in fresh cut lumber (JONES, 1981; AHLBORG & THUNBERG, 1980). It was estimated that in Canada during 1981, 750 metric tons of tetrachlorophenol was used for this purpose (JONES, 1982). Although there is some limited information available on the acute oral and intraperitoneal toxicity of these chemicals, no data were available on their acute dermal toxicity. Since the dermal route would likely constitute a major route of exposure occupationally, it was considered important to obtain some acute dermal toxicity information on these chemicals.

MATERIALS AND METHODS

Two tetrachlorophenol isomers (2,3,4,5-TCP and 2,3,5,6-TCP), their respective phenates, and a commercial tetrachlorophenol were used in this study. The two tetrachlorophenol isomers were obtained from Aldrich Chemical Co., Milwaukee, Wisc., and had a stated purity of 98%. Both chemicals were purified by recrystallization and had a final purity of greater than 99%. Identity and purity of the chemicals were determined by GC using both flame ionization and electron-capture detectors. Confirmation of the specific isomers found in the commercial mixture was carried out using NMR techniques. Also, a sample of the commercial tetrachlorophenol was subjected to GC-MS analysis in order to identify possible dioxin contamination. The respective phenates of the compounds were prepared by first dissolving the isomers in ethanol, adding an equimolar amount of sodium hydroxide and then evaporating the alcohol. The commercial tetrachlorophenol was obtained as a gift from Dr. J. Singh, Agriculture Canada, Ottawa, Ontario.

For the first study, male Sprague-Dawley rats (255-300 g) and female Sprague-Dawley rats (218-267 g) were randomly divided into 6 groups containing 10 animals each in the following manner: group 1, vehicle control; group 2, 2,3,5,6-tetrachlorophenol; group 3, 2,3,4,5-tetrachlorophenol; group 4, 2,3,4,5-tetrachlorophenate; group 5, 2,3,5,6-tetrachlorophenate; group 6, commercial tetrachlorophenol. All treated groups received the above chemicals at 2000 mg/kg bw. The procedure used for determining the dermal toxicity of the above chemicals followed the procedures as recommended and documented by OECD (1982). In short, the materials

were dissolved in ethanol and applied to a shaved area of skin, approximately 8 cm square. The chemical was left in contact with the skin for 24 hours and at that time any residue was removed by washing the area with ethanol. All animals were observed at least daily, and if any deaths occurred, the animals were necropsied to attempt to determine cause of death and to see if any organs were grossly affected. All animals were killed after a 14 day observation period and subjected to gross pathological examination.

For the second study, 120 male (255-325 g) and 120 female (194-245 g) Sprague-Dawley rats were divided into 12 groups as follows: group 1, vehicle control; group 2, negative control. Groups 3,4,5,6,7 received 200, 400, 600, 800 and 1000 mg/kg b.w. of 2,3,4,5-tetrachlorophenate respectively. Groups 8,9,10,11 and 12 received 200, 400, 600, 800 and 1000 mg/kg b.w. of commercial tetrachlorophenol respectively. The treatment of the animals and the observation period were identical to that described above for the first study. LD_{50} values were calculated using the method of FINNEY (1971).

In the third study 5 g of 2,3,4,5-tetrachlorophenol was dissolved in a mixture of ethanol (200 ml), water (50 ml), and 1M sodium hydroxide (100 ml). The solution was extracted with three 50 ml portions of chloroform. The extracts were combined, washed twice with 1M sodium hydroxide (50 ml) and water (50 ml), and dried over anhydrous sodium sulfate. The solvent was evaporated to dryness and the residues were dissolved in 5 ml ethanol. An aliquot of the solution was analyzed by GC and GC-MS for the presence of chlorinated dibenzodioxins. The remainder was applied to the shaved skin of a group of 5 female rats at 2 ml/100 g bw. Another group of female rats serving as a positive control was painted with ethanolic solution of the 2,3,4,5-isomer at 0.2 g/100 g bw. Post-administration observations and necropsies were carried in a manner similar to those described in the previous two experiments.

RESULTS AND DISCUSSION

The mortality results for the first study are shown in Table 1.

The clinical signs preceding death included a period of hyperactivity, usually for 1-2 hours followed by a period of hypoactivity for a few hours followed by neuromuscular weakness, convulsions and death. At necropsy, no specific tissues were grossly affected and no direct cause of death could be ascertained. Based on the data, it was concluded that the dermal LD_{50} of 2,3,5,6- and 2,3,4,5- tetrachlorophenol and 2,3,4,5-tetrachlorophenate were greater than 2000 mg/kg and thus no further

testing was deemed necessary on these chemicals. Since both 2,3,5,6-tetrachlorophenate and the commercial preparation demonstrated considerable toxicity at 2000 mg/kg, a second study was initiated to determine their exact LD_{50}'s.

Table 1. Mortality after administering tetrachlorophenols dermally at 2000 mg/kg.

Chemical	Mortality (No. Dead/No. Tested)	
	Males	Females
Vehicle (Ethanol)	0/10	0/10
2,3,5,6-tetrachlorophenol	0/10	2/10
2,3,4,5-tetrachlorophenol	1/10	0/10
2,3,5,6-tetrachlorophenate	7/10	10/10
2,3,4,5-tetrachlorophenate	0/10	1/10
Commercial tetrachlorophenol	7/10	10/10

The results of the second study are summarized in Table 2.

Table 2. Dermal LD_{50} values for 2,3,5,6-tetrachlorophenate and commercial tetrachlorophenol in male and female rats.

Chemical	LD_{50} values (mg/kg bw)	
	Males	Females
2,3,5,6-tetrachlorophenate	294 (208-378)[1]	469 (376-562)
Commercial tetrachlorophenol	485 (366-617)	565 (464-672)

[1]The values in parenthesis represent the 95% confidence limits.

The clinical symptoms in animals that died from treatment were the same as the ones described for the first study. In general, those animals that died did so within 6 hours of application of the material to the skin. It was obvious from the data generated in these studies that 2,3,5,6-tetrachlorophenate was the most toxic of all the chemicals tested followed by the commercial tetrachlorophenol. In male rats, the relative potency of commercial tetrachlorophenol compared to 2,3,5,6-tetrachlorophenate was 0.61 with 95% confidence limits of 0.41 and 0.86 respectively. In female rats, the relative potency of commercial tetrachlorophenol compared to 2,3,5,6-tetrachlorophenate was 0.83 with 95% confidence limits of 0.63 and 1.08 respectively. There was no dramatic difference in toxicity related to the sex of the animals tested.

Of the chemicals tested only 2,3,4,5-tetrachlorophenol and its respective phenate caused dermatosis and resulted in large and hard scar tissue being formed.

The analytical results (NMR) indicated that the only tetrachlorophenol isomer that could be identified in the commercial mixture was 2,3,4,6-tetrachlorophenol. However, this technique would only detect the other isomers if they were present in excess of 5-10% of the product. Routine GC techniques did not offer any further elucidation of the possible presence of the other tetrachlorophenol isomers since they all had virtually the same retention time. However, GC-MS and HPLC analysis showed that there was about 5-10% contamination with pentachlorophenol. The GC-MS analysis showed that there was no significant contamination of the commercial product with polychlorinated dibenzodioxins (1.0 ppm).

In the third study all animals painted with 2,3,4,5-tetrachlorophenol developed dermatosis associated with large scar tissues. In contrast those animals treated with the materials after extraction with sodium hydroxide showed no dermatological changes indicating dermatosis was elicited by the 2,3,4,5-isomer rather than neutral fractions such as chlorinated dibenzodioxins.

Previous acute oral and intraperitoneal toxicity information on tetrachlorophenols in mice indicate that the 2,3,5,6-tetrachlorophenol was the most toxic of the three isomers tested (AHLBORG & THUNBERG). In our studies we found 2,3,5,6-tetrachlorophenate to be the most toxic chemical applied dermally to rats. The clinical symptoms associated with the acute dermal exposure to 2,3,4,6-tetrachlorophenate or the commercial tetrachlorophenol are quite similar to those observed with pentachlorophenol (AHLBORG & THUNBERG, 1980) and with acute oral exposure to 2,3,4,6-tetrachlorophenol (KOZAK et al., 1979). The actual cause of death could not be determined but it is likely that death was due to either respiratory failure as was reported for 2,3,4,6-tetrachlorophenol (KOZAK et al., 1979) or heart failure as was the case with pentachlorophenol (AHLBORG & THUNBERG, 1980).

In the present study we were not able to test a pure sample of 2,3,4,6-TCP because it was no longer commercially available. However, since the commercial tetrachlorophenol product consisted of at least 90% of the 2,3,4,6 isomer, the acute toxicity data obtained with this substance most likely reflects the acute toxicity of 2,3,4,6-tetrachlorophenol. Recent work carried out by HATTULA et al. (1981) reports on the acute and subacute effects of orally administered 2,3,4,6-tetrachlorophenol in the rat. They determined the acute toxicity of this chemical to be approximately 360 mg/kg, which is slightly lower than the value we obtained in rats treated dermally with our commercial mixture.

The dermatosis observed in this study with 2,3,4,5-tetrachlorophenol and its phenate are not a unique observation. Dermatosis has been reported in man as a result of exposure to tetrachlorophenol and its sodium salt (AHLBORG & THUNBERG, 1980) but the specific cause was attributed to the presence of dimeric contaminants rather than the tetrachlorophenol itself. However, we considered the dermatosis observed in our study was due to the tetrachlorophenol itself. The purity of the isomer was greater than 99%. The GC and GC-MS analysis showed the levels of chlorinated dibenzodioxin (presumably dimeric contaminants) were below 1 ppm. When 2,3,4,5-tetrachlorophenol was removed by extraction with sodium hydroxide, the residues that remained produced no skin changes. The lack of skin effects noted with the 2,3,5,6- and the commercial (2,3,4,6-) tetrachlorophenols is in agreement with previously reported dermal effects of those two isomers (KOZAK et al., 1979).

The increased toxicity shown by the 2,3,5,6-tetrachlorophenate occurred in both sexes of animals and might be attributable to an increase in the dermal absorption. Skin uptake studies using radiolabeled materials would probably be able to determine whether or not there were differences in the efficiency of dermal absorption between tetrachlorophenols and their sodium salts.

Occupational exposure to tetrachlorophenols occurs almost exclusively in the lumber industry in British Columbia (JONES, 1981). The dipping, spraying and pressure treatment of the lumber exposed workers to both tetrachlorophenol and pentachlorophenol via the pulmonary and dermal routes and have led to dermatological, respiratory and neurological complaints (STERLING et al., 1982). The data presented here indicate that commercial (2,3,4,6-) tetrachlorophenol and 2,3,5,6-tetrachlorophenate are only one third as toxic as pentachlorophenol in acute mammalian dermal exposures (NIOSH, 1980).

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