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Effect of Purified and Commercial Grade Tetrachlorophenol on Rat Embryonal and Fetal Development[1]

B. A. SCHWETZ, P. A. KEELER AND P. J. GEHRING

Chemical Biology Research, The Dow Chemical Company, Midland, Michigan 48640

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The Effect of Purified and Commercial Grade Tetrachlorophenol on Rat Embryonal and Fetal Development. SCHWETZ, B. A., KEELER, P. A. AND GEHRING, P. J. (1974). *Toxicol. Appl. Pharmacol.* **28**, 146–150. This study evaluated the effects of purified and commercial grade tetrachlorophenol on rat embryonal and fetal development. Dose levels up to and including a maximum tolerated dose were administered po to pregnant Sprague-Dawley rats on days 6 through 15 of gestation. The only fetal anomaly associated with the administration of tetrachlorophenol was delayed ossification of the skull bones—evidence of fetotoxicity, not teratogenicity. The no-effect dose level of these materials for embryotoxicity was 10 mg tetrachlorophenol/kg/day.

Tetrachlorophenol is an antimicrobial and fungicidal agent used for the preservation of wood and other materials. Recently, it has been shown that commercially available preparations may contain small amounts of various nonphenolic contaminants. In particular, hexa-, hepta- and octachlorodibenzo-*p*-dioxin and dibenzofurans have been identified in these commercial preparations. It is not expected that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin would be present in tetrachlorophenol, based on the structure of the chlorophenols formed during the chlorination process. However, the samples used in this study were analyzed for this dioxin using a method with a sensitivity of 0.05 ppm, and none was found.

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin has been shown to be highly toxic to the rat embryo and fetus (Sparschu *et al.*, 1971). Hexachlorodibenzo-*p*-dioxin exhibits similar activity qualitatively but is much less potent. The toxicity of the remaining nonphenolics is either much less than the two agents indicated above or unknown (Schwetz *et al.*, 1973).

The objectives of the study reported herein were to determine whether tetrachlorophenol influenced the development of the embryo and fetus, and whether the nonphenolics in a commercial preparation of tetrachlorophenol may influence the development of the embryo and fetus. These parameters were determined and compared using the *a priori* assumption that a difference in the embryotoxicity and fetotoxicity of purified and commercial grades of tetrachlorophenol would be associated with the nonphenolics in the commercial grade material.

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METHODS

Test materials and animals. The analytical descriptions of the test samples[2] used in this study are given in Table 1.

Pregnant Sprague-Dawley female rats weighing approximately 250 g were used. The day on which sperm were first seen in a vaginal smear was considered day 0 of pregnancy. The animals were housed individually in wire-bottom cages in a room controlled for temperature, humidity, light cycle and noise. Commercial laboratory rat chow and water were provided ad libitum.

TABLE 1

DESCRIPTION OF TEST MATERIALS

	Commercial Grade 2,3,4,6-Tetrachlorophenol	Purified 2,3,4,6-Tetrachlorophenol
Identification	MM-05040-1481-G	Ref. No. 27-87-3
Phenolics (%)[a]		
Pentachlorophenol	27	0.1
Tetrachlorophenol	73	99.6
Trichlorophenol		<0.05
Nonphenolics (ppm)[b]		
Dibenzo-*p*-dioxins		
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin	<0.05	<0.05
Hexachlorodibenzo-*p*-dioxin	28	<0.5
Heptachlorodibenzo-*p*-dioxin	80	<0.5
Octachlorodibenzo-*p*-dioxin	30	<0.5
Dibenzofurans		
Hexachlorodibenzofuran	55	<0.5
Heptachlorodibenzofuran	100	<0.5
Octachlorodibenzofuran	25	<0.5

[a] Determined by gas–liquid chromatography.
[b] Determined by use of an LKB 9000 gas chromatograph—mass spectrometer.

Experimental design. Corn oil solutions of purified and commercial grade tetrachlorophenol were prepared such that the administration of 2.0 ml/kg provided the desired dose of test material. Control rats received 2.0 ml/kg of corn oil po. Dosages were adjusted daily for changes in body weight.

In a preliminary tolerance study using nonpregnant rats, groups of 5 rats each received 3, 10, 30, 100 or 300 mg/kg/day of commercial grade tetrachlorophenol for 10 consecutive days. Body weights were recorded at 3-day intervals during the treatment period and at weekly intervals following treatment. Animals were observed daily for signs of toxicity. In the teratologic study, dose levels of 10 and 30 mg/kg/day of commercial grade or purified 2,3,4,6-tetrachlorophenol were administered by gavage to groups of 20–40 bred rats on days 6–15 inclusive of gestation. All rats were observed daily and were weighed on days 6, 13 and 21 of gestation.

[2] The test samples were supplied by the Dow Chemical Co., Midland, Michigan 48640.

Fetal examination. All pregnant females were sacrificed by carbon dioxide anesthesia on day 21 of gestation. After exteriorizing the uterine horns through a midline incision in the abdominal wall, the number and position of live, dead and resorbed fetuses were noted. The umbilical cords were clamped and severed distally. After being weighed and the sexes noted, the fetuses were examined for external anomalies. The crown–rump length of the fetuses was measured with a vernier caliper. Each litter was divided equally into 2 subgroups for preservation and subsequent examination. One subgroup, preserved in Bouin's solution, was examined by the method of Wilson (1965) for evidence of soft tissue anomalies. The second subgroup, preserved in alcohol, was cleared and stained with alizarin red-S (Dawson, 1926) for examination of the skeletons for evidence of abnormalities.

Statistical evaluation. The binomial test for proportions was used to evaluate the frequency of anomalies and resorptions within the fetal population and between litters (Goldstein, 1964). Maternal and fetal body weight and body measurements were statistically analyzed by an analysis of variance and Tukey's test (Steel and Torrie, 1960). In all cases, the level of significance was $p < 0.05$.

RESULTS

The maximum tolerated dose of commercial grade tetrachlorophenol following 10 days of administration in a preliminary tolerance study was 30 mg/kg/day. Signs of toxicity and death were observed at 100 and 300 mg/kg/day.

In the teratologic study, maternal weight gain during gestation was not affected by the administration of 10 or 30 mg of commercial grade or purified tetrachlorophenol/kg/day on days 6–15 of gestation. No signs of maternal toxicity were observed among dams treated with any dose of either sample during gestation or at the time of cesarean section and autopsy on day 21 of gestation.

Resorptions and Fetal Body Measurements

As shown in Figs. 1 and 2, administration of 10 or 30 mg of commercial or purified tetrachlorophenol/kg/day had no effect on resorptions, fetal body weight or fetal crown–rump length.

Fetal Anomalies

No anomalies were seen upon gross examination of litters of dams treated with either dose of commercial grade or purified tetrachlorophenol. The only skeletal anomaly which was observed at a statistically significant incidence was delayed ossification of the skull bones. At 30 mg commercial grade tetrachlorophenol/kg/day, the incidence was significantly increased both among the fetal population (26%, 23/88) and among litters (50%, 8/16). At 30 mg of purified tetrachlorophenol/kg/day, the incidence was significantly increased among the fetal population (17%, 18/104) but was not significantly increased among litters (35%, 7/20). The incidence of delayed ossification of the skull bones among the control animals was 8% (14/173) of the fetal population and 19% (6/31) of the litters. The incidence of this anomaly at 10 mg/kg/day of either compound was not different from controls. The only soft tissue anomaly which occurred at an increased incidence among the fetal population and litters was subcutaneous edema. This

anomaly was associated with the administration of 10 mg commercial grade tetrachlorophenol/kg/day (16%, 14/87 of the fetuses; 50%, 8/16 of the litters) as well as 10 mg purified tetrachlorophenol/kg/day (27%, 27/101 fetuses; 62%, 10/16 litters). The incidence of subcutaneous edema among controls was 7% (12/170) of the fetal population and

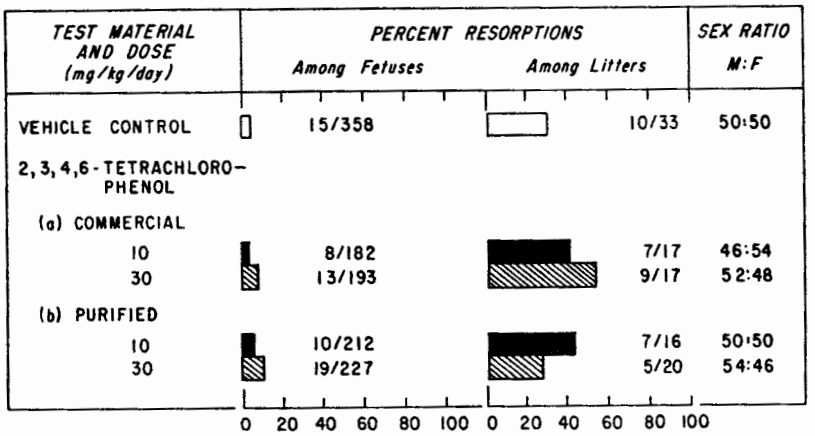

FIG. 1. Effect of tetrachlorophenol on the incidence of fetal resorptions and on the sex ratio of survivors. The number of resorbed fetuses per total number of implantations and the number of litters with 1 or more resorptions per total number of litters is indicated. No values were significantly different from control values by the binomial expansion test, $p < 0.05$.

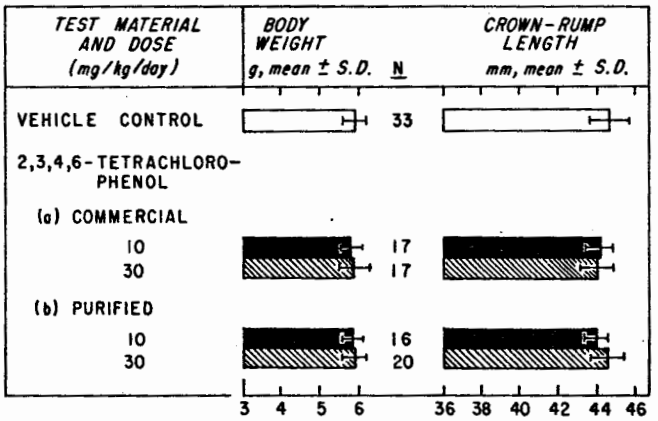

FIG. 2. Effect of tetrachlorophenol on fetal body measurements. Values are the mean of litter means; N equals the number of litters. No values were significantly different from control values by an analysis of variance and Tukey's test, $p < 0.05$.

18% (6/33) of the litters. Subcutaneous edema was not caused by 30 mg/kg/day of either compound. Since the incidence was not dose-related, the statistical significance may have been related to chance rather than treatment with either the commercial grade or purified tetrachlorophenol.

DISCUSSION

Commercial grade and purified tetrachlorophenol were neither embryolethal nor teratogenic at dose levels up to and including the maximum tolerated dose. Fetotoxicity was observed at the highest dose level. There was essentially no difference in the degree of toxicity associated with the two grades of tetrachlorophenol—purified versus commercial grade. The only evidence of fetotoxicity, delayed ossification of the skull bones, was caused by both forms of tetrachlorophenol. This anomaly occurs normally in all control populations in our laboratory and reflects either a mild, nonspecific toxicity or stress without definitive consequence.

The no-effect dose level of commercial grade and purified tetrachlorophenol for embryotoxic effects was 10 mg/kg/day when administered on days 6 through 15 of gestation. The only fetotoxic effect observed at 10 mg/kg, subcutaneous edema, was not observed at 30 mg/kg.

These studies show that the amounts of the nonphenolics contained in commercial grade tetrachlorophenol do not contribute significantly to the effects of this material on the developing rat embryo and fetus. Embryotoxic and fetotoxic responses, but not teratogenic responses, were observed at the maximum tolerated dose level of tetrachlorophenol.

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