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Teratogenic Assessment of 2,4-Dichlorophenol in Fischer 344 Rats

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Teratogenic Assessment of 2,4-Dichlorophenol in Fischer 344 Rats. RODWELL, D. E., WILSON, R. D., NEMEC, M. D., AND MERCIECA, M. D. (1989). *Fundam. Appl. Toxicol.* **13**, 635–640. The potential maternal, embryotoxic, and teratogenic parameters of 2,4-dichlorophenol (2,4-DCP) were evaluated in Fischer 344 rats following oral administration in corn oil on Days 6 through 15 of gestation. Dose levels were 0, 200, 375, and 750 mg/kg/day. Females were sacrificed on Gestation Day 20 and cesarean sections performed. The fetuses were weighed, sexed, measured for crown–rump length, and examined for external malformations. A skeletal examination was conducted on one-half of the fetuses after staining with alizarin red S. The remaining fetuses were fixed in Bouin's solution and examined for visceral anomalies and developmental variations. Maternal body weight gain inhibition occurred in all 2,4-dichlorophenol-treated groups during the treatment period. Four treatment-related deaths occurred in the 750 mg/kg/day group. Additional indicators of maternal toxicity included urogenital staining of the fur at all levels tested, and an increased incidence of hair loss and respiratory rales at the 750 mg/kg/day level. Fetal examinations did not reveal differences in the incidence of external, visceral, or skeletal fetal malformations in any treatment group, when compared with the control group. A slight increase in early embryonic death occurred in the high-dose group only. Fetal weights were lower in the high-dose group than in the control group. Variations in skeletal structure were expressed among fetuses exposed to 750 mg/kg/day during organogenesis. These included delayed ossification of sternal elements and vertebral arches. The reduced fetal weights, intrauterine survival, and retarded ossification may represent a slight degree of embryotoxicity or fetotoxicity in this group. The test material was not teratogenic at any dose level. © 1989 Society of Toxicology.

2,4-Dichlorophenol (2,4-DCP), a chlorinated phenol, is found as a contaminant in both water and soil. Contamination of water occurs from spontaneous reaction of chlorine and phenols following chlorination of water in the waste-treatment process (Exon *et al.*, 1984). Clark *et al.* (1975) have reported that the presence of 2,4-DCP in soil is most likely a result of the decomposition of 2,4-dichlorophenoxyacetic acid (2,4-D). Published data on the effects of 2,4-DCP on the reproduction of laboratory animals and fetal developmental toxicity are limited. Exon *et al.* (1984) exposed groups of sexually mature Sprague–Dawley rats to either 3, 30, or 300 ppm 2,4-DCP in drinking water for 3 weeks *in utero*. Postnatal exposure was continued for 12 weeks for selected offspring. Progeny from maternal animals receiving 300 ppm 2,4-DCP during gestation and postnatally demonstrated enhanced humoral immunity, decreased thymus weights, depressed cell-mediated immunity, and elevated spleen and liver weights. These effects were not significant findings when exposure was limited to the prenatal period only. It was interesting to

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note that continuous exposure of this chlorinated phenol did not disrupt the reproductive function as assessed by conception rates, litter size or weight, pup survival, and weaning. Treatment-related histomorphological lesions were not observed at doses of 300 ppm and less. Schwetz *et al.* (1974) demonstrated that a structurally related compound, pentachlorophenol, induced moderate maternal toxicity and embryolethality in Sprague–Dawley rats exposed to purified or commercially available grades of the agent. Repeated oral doses of 34.7 and 50 mg/kg/day of commercial-grade pentaclorophenol and 30 and 50 mg/kg/day of the purified compound during organogenesis resulted in maternal body weight gain inhibition. Although developmental toxicity, expressed by an increase in early embryonic death and visceral and skeletal variants, was noted, the compound was not considered teratogenic.

In view of the widespread use of 2,4-D as an herbicide, the objective of this study was to assess the potential of 2,4-DCP to produce developmental toxicity in rodents.

METHODS

Chemical. Technical-grade 2,4-dichlorophenol was supplied by Dow Chemical Company as a white solid.

Chemical analyses. The test material was analyzed (data supplied by Industry Task Force on 2,4-D) prior to study initiation by high-performance liquid chromatography (HPLC) and was found to contain 99.2% 2,4-DCP. Dibenzo-*p*-dioxins were not found in the 2,4-DCP sample.

Animals and housing. Cesarean-derived, barrier-sustained male and female Fischer 344 rats were obtained from the Charles River Breeding Laboratories, Inc., Portage, Michigan. The animals were housed individually in suspended stainless-steel cages. Commercial rodent chow (Certified Rodent Chow 5002, Ralston–Purina Co., St. Louis, MO) and tap water were supplied *ad libitum*. Females were housed in an environmentally controlled room that provided at least 12 fresh air changes per hour, the temperature was controlled at 72 ± 3°F, and the relative humidity was maintained at approximately 40%. The animals were identified individually by a metal ear tag imprinted with the animal number. Animals were maintained in accordance with the standards outlined in the *Guide for the Care and Use of Laboratory Animals* (DHEW No. NIH-74-23).

Experimental design. Females were placed in the home cage of the male for breeding (1 male:1 female). The day sperm were detected in a vaginal smear was considered Day 0 of gestation, at which time the female was returned to an individual cage until sacrifice. Sperm-positive females were allocated at random to one control group and three treatment levels of 34 rats each. The bred females were approximately 22 weeks old at initiation of breeding; body weight values ranged between 179 and 246 g on Day 0 of gestation.

2,4-DCP formulations or vehicle was administered by gastric intubation once daily from Gestation Day 6 through Gestation Day 15. 2,4-DCP suspensions were prepared by mixing the technical-grade material in corn oil at concentrations of 50.0, 93.75, and 187.50 mg/ml. The formulations were administered at 200, 375, and 750 mg/kg body wt/day. Control animals were gavaged with corn oil on an identical regimen at 4 ml/kg. The dams were observed daily for changes in appearance and behavior prior to dosing, pharmacotoxic signs following dosing, and mortality. Body weights of the females were recorded on Gestation Days 0, 6, 10, 12, 15, and 20. Dosages were adjusted based on the most recent body weight of individual dams.

Maternal animals were euthanized by carbon dioxide asphyxiation on Gestation Day 20 and a cesarean section was performed. The order of cesarean section on any given day was randomized in an attempt to equalize fetal developmental variation across groups. The abdominal and thoracic cavities of the dams were opened and organs examined *in situ* for macroscopic changes. The intact uterus was removed, trimmed free of adnexa and weighed. The uterus was opened and the number and location of viable and nonviable fetuses, and early and late resorptions, were recorded. The ovaries were examined, and the number and location of corpora lutea were recorded. The remaining organs and tissues of the dams were discarded. Uteri with no evidence of macroscopic implantation were opened and stained with 10% ammonium sulfide solution (Salewski, 1964) for detection of early resorptions. All fetuses were examined externally, weighed, and measured for crown–rump distance. One-half of the fetuses from each dam were fixed in Bouin's solution for subsequent soft tissue examination by razor sectioning (Wilson, 1965). The remaining one-half of the fetuses were fixed in isopropyl alcohol, macerated in potassium hydroxide, stained with alizarin red S (Dawson, 1926), and examined for skeletal defects. In an effort to minimize bias, specimens were examined without prior knowledge of dose group designation.

Statistical analysis. Statistical analyses were conducted utilizing two-tailed tests for a minimum significance level of $p < 0.05$, considering the litter, rather than the fetus, as the experimental unit. Maternal body weights, body weight gain, total implantations, corpora

lutea, viable fetuses, crown–rump length, and fetal weights among all groups were evaluated initially by a one-way analysis of variance to judge intragroup differences. If the analysis of variance was significant, Dunnett's t test (Steel and Torrie, 1960) was applied to the data, comparing each test group with the control group. The values obtained for resorption sites, dead fetuses, and postimplantational loss were analyzed by the Mann–Whitney U test (Dixon and Brown, 1979). Fetal sex ratios were analyzed by the χ^2 test with Yates' correction factor, and the number of litters with malformations were evaluated by Fisher's exact test (Dixon and Brown, 1979).

RESULTS

Oral administration of 2,4-dichlorophenol resulted in the death of four pregnant females at the 750 mg/kg/day level between Gestation Days 8 and 12. Survival was unaffected by treatment in the 200 and 375 mg/kg/day groups. Dried red material in the area of the eyes, nares, and mouth, hair loss on the abdominal region, and respiratory rales were observed in a majority of animals at the highest tested dosage, but were also seen infrequently in the 375 mg/kg/day group. In addition, a dose-related increase in yellow staining and matting of the urogenital haircoat occurred in all treatment groups when compared with the control.

Maternal body weight means were comparable to that of the control group in the 200 and 375 mg/kg/day groups and decreased throughout the treatment period in the 750 mg/kg/day group (Table 1). When compared with the controls, mean maternal body weight gain was found to be statistically significantly decreased during the treatment period (Gestation Days 6 through 15) at all dose levels tested. This trend continued in all groups for the posttreatment period (Days 15–20), with slight reductions in body weight gain in the low and intermediate groups and statistically significant decreases relative to the control group noted in the 750 mg/kg/day group (Table 1). Correspondingly, gravid uterine weights, corrected body weights, and adjusted body weight change values among treated dams were slightly decreased (data

TABLE 1

MEAN BODY WEIGHTS AND WEIGHT GAIN DURING GESTATION OF DAMS TREATED WITH 2,4-DCP[a]

	Dose (mg/kg/day)							
	0		200		375		750	
	x	wt gain	x	wt gain	x	wt gain	x	wt gain
Day 0 (wt gain 0–6)	196 ± 6	7 ± 3 (28)	195 ± 7	8 ± 4 (31)	196 ± 8	8 ± 4 (31)	197 ± 12	8 ± 3 (27)
Day 6 (wt gain 6–10)	203 ± 5	7 ± 4 (28)	203 ± 8	6 ± 4 (31)	204 ± 8	7 ± 5 (31)	204 ± 12	−1 ± 9[d] (27)
Day 10 (wt gain 10–12)	210 ± 7	4 ± 2 (28)	209 ± 9	5 ± 3 (31)	211 ± 7	4 ± 4 (31)	203 ± 13[c]	1 ± 9[c] (26)
Day 12 (wt gain 12–15)	214 ± 8	10 ± 4 (28)	214 ± 9	7 ± 4[d] (31)	215 ± 9	6 ± 4[d] (31)	206 ± 16[c]	4 ± 5[d] (24)
Day 15 (wt gain 15–20)	225 ± 9	39 ± 10 (28)	221 ± 10	37 ± 10 (31)	221 ± 10	37 ± 8 (31)	211 ± 13[d]	30 ± 11[c] (23)
Day 20 (wt gain 0–20)	263 ± 16	68 ± 13 (28)	258 ± 16	62 ± 14 (31)	258 ± 14	62 ± 11 (31)	241 ± 17[d]	45 ± 16[d] (23)
— (wt gain 6–15)	—	22 ± 6 (28)	—	18 ± 6[c] (31)	—	17 ± 7[c] (31)	—	7 ± 6[d] (23)
Day 20 (wt gain 0–20)[b]	221 ± 9	25 ± 6 (28)	219 ± 11	24 ± 9 (31)	219 ± 9	23 ± 7 (31)	208 ± 16	11 ± 9 (22)

[a] Data in grams, $x \pm$ SD, number of animals in parentheses.
[b] Corrected body weight exclusive of the gravid uterus and contents.
[c] Significantly different from control at the 0.05 level.
[d] Significantly different from control at the 0.01 level.

TABLE 2

SUMMARY OF CESAREAN SECTION DATA FROM DAMS TREATED WITH 2,4-DCP[a]

Parameter	Dose (mg/kg/day)			
	0	200	375	750
Number of females pregnant/ number mated	28/34	31/34	31/34	27/34
Number with viable fetuses/ number pregnant	27/28	31/31	31/31	22/27
Viable fetuses	251 (9.0 ± 2.9)	238 (7.7 ± 3.2)	252 (8.1 ± 2.5)	162 (7.0 ± 3.7)
Postimplantation loss	23 (0.8 ± 0.9)	15 (0.5 ± 0.7)	17 (0.5 ± 0.8)	32 (1.4 ± 2.7)
Early resorptions	23 (0.8 ± 0.9)	14 (0.5 ± 0.7)	15 (0.5 ± 0.7)	27 (1.2 ± 2.7)
Late resorptions	0 (0.0 ± 0.0)	1 (0.0[b] ± 0.2)	2 (0.1 ± 0.2)	4 (0.2 ± 0.4)
Implantation sites	274 (9.8 ± 2.5)	253 (8.2 ± 3.4)	269 (8.7 ± 2.7)	194 (8.4 ± 3.3)
Corpora lutea	342 (12.2 ± 2.2)	360 (12.0 ± 1.2)	385 (12.4 ± 1.7)	290 (12.6 ± 1.5)
Fetal weight (g)	(3.1 ± 0.2)	(3.1 ± 0.2)	(3.1 ± 0.2)	(3.0 ± 0.2)
Crown–rump length (cm)	(3.3 ± 0.2)	(3.3 ± 0.1)	(3.2 ± 0.1)	(3.2 ± 0.2)
Gravid uterine weight (g)	(43 ± 12)	(39 ± 16)	(39 ± 12)	(36 ± 15)
Sex ratio (M:F)	126:125	114:124	116:136	78:85

[a] Means ± SD are given in parentheses. No values are significantly different from control at the 0.05 level. Data from four dams that died were not tabulated.
[b] Value between 0.0 and 0.05.

not analyzed statistically) at the high-dose level only (Tables 1 and 2).

Cesarean section data and reproductive performance are summarized in Table 2. No effects related to test material administration were observed in those variables assessed at cesarean section (viable litter size, implantation or resorption sites, corpora lutea, sex ratios, fetal weights, and crown–rump distance) in the 200 and 375 mg/kg/day groups. The mean for fetal weights was reduced in the 750 mg/kg/day dosage group, in comparison with the control group. Similarly, intrauterine survival was slightly lower in the 750 mg/kg/day group than the control group, most likely due to an increase in early embryonic death. None of these differences was statistically significant.

The incidence of malformed fetuses in the 2,4-dichlorophenol-treated groups was comparable to that of the control group (Table 3). An increase in the number of fetuses and litters with unossified sternebrae (Nos. 1, 2, 3, and/or 4) or delayed ossification of vertebral arches occurred in the 750 mg/kg/day group (Table 4). The number of litters was statistically significantly higher in this group than in the control group. No other significant differences were observed between the 2,4-dichlorophenol-treated groups and the control group.

DISCUSSION

Dams receiving repeated oral doses of 2,4-DCP at 200, 375, or 750 mg/kg/day experienced toxic effects. Four of thirty-four (12%) of the dams administered 750 mg/kg/day 2,4-dichlorophenol died after receiving two to six doses. Mortality did not occur in the 200 and 375 mg/kg/day groups. Although changes in behavior and general demeanor were not observed at any of the selected dosages, a number of changes in the general appearance of the dams was noted. These included excremental staining of the fur, respiratory rales, various degrees of alopecia, and the appearance of nasal and ocular porphyrin. The incidence of these symptoms was generally, but

TABLE 3

NUMBER OF FETUSES (LITTERS) WITH MALFORMATIONS FROM DAMS TREATED WITH 2,4-DCP[a]

	Dose (mg/kg/day)			
	0	200	375	750
Total malformations[b]				
Number with external malformations	1 (1)	2 (2)	3 (2)	0
Number with soft tissue malformations	0	0	1 (1)	0
Number with skeletal malformations	3 (3)	1 (1)	3 (3)	3 (2)
Total number with malformations	4 (4)	3 (3)	6 (4)	3 (2)
Number examined externally	251 (27)	238 (31)	252 (31)	162 (22)
Microphthalmia and/or anophthalmia	1 (1)	1 (1)	2 (2)	0
Exencephaly	0	0	1 (1)	0
Cleft palate	0	1 (1)	0	0
Number examined viscerally	121 (27)	118 (29)	126 (31)	82 (21)
Retina(s) folded	0	0	1 (1)	0
Number examined skeletally	130 (27)	120 (31)	126 (31)	80 (22)
Sternebra(e) malaligned (severe)	3 (3)	1 (1)	3 (3)	2 (1)
Pubis unossified	0	0	0	1 (1)

[a] Data from four dams that died were not tabulated.
[b] The number of litters with malformations from the 2,4-DCP groups is not significantly different from control at the 0.05 level.

not exclusively, higher with increasing dosage. At all doses studied, significant inhibition of body weight gain was observed during the period of administration, which continued for the remainder of gestation.

Treatment with 2,4-dichlorophenol was

TABLE 4

NUMBER OF FETUSES (LITTERS) WITH DEVELOPMENTAL VARIATIONS FROM DAMS TREATED WITH 2,4-DCP[a]

	Dose (mg/kg/day)			
	0	200	375	750
Number examined externally	251 (27)	238 (31)	252 (31)	162 (22)
No findings	251 (27)	238 (31)	252 (31)	162 (22)
Number examined viscerally	121 (27)	118 (29)	126 (31)	82 (21)
Renal papilla(e) not developed and/or distended ureters	0	0	1 (1)	0
Dilation of the brain ventricles	5 (3)	3 (2)	0	4 (4)
Number examined skeletally	130 (27)	120 (31)	126 (31)	80 (22)
Sternebrae 5 and/or 6 unossified	55 (23)	60 (24)	68 (27)	35 (15)
Sternebrae 1, 2, 3, and/or 4 unossified	0	0	1 (1)	4 (4)[b]
Sternebrae malaligned (slight or moderate)	22 (14)	23 (15)	23 (18)	9 (7)
Seventh cervical rib(s)	0	4 (3)	1 (1)	2 (2)
Reduced ossification of the vertebral arch(es)	0	0	0	6 (6)[b]

[a] Data from four dams that died not tabulated.
[b] Significantly different from control at the 0.05 level.

neither embryolethal nor fetotoxic at doses of 375 mg/kg/day and less. The degree of early embryonic death was elevated and mean fetal weights were lower in the high-dose group, when compared with the concurrent control group but not when compared with historical values from this laboratory (data not presented). Delayed ossification of sternal elements and vertebral arches also occurred infrequently. Biologically significant differences in the types and incidences of fetal anomalies observed were not apparent on comparing the controls with the 2,4-DCP experimental groups. Thus, it is concluded that 2,4-dichlorophenol was not teratogenic at the doses tested. The statistically significant increases in skeletal variations, seen at 750 mg/kg/day, were considered to be consistent with the maternal toxicity and not a selective effect on the embryos/fetuses.

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