Teratogenic Studies with 2,4,5-T and 2,4-D in the Hamster

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The chlorinated herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been in common use as a weed killer for over 20 years. Recently it has been employed together with 2,4-dichlorophenoxyacetic acid (2,4-D) as a defoliant in Vietnam. In 1964, the National Cancer Institute (1) undertook a screening study of a number of herbicides and other chemicals for carcinogenic and teratogenic potential. Those producing increased proportions of abnormal fetuses were 2,4,5-T and the 2,4-D isooctyl, butyl, and isopropyl esters but not 2,4-D acid (2). The results of that study with the herbicide 2,4,5-T have been reported by Courtney et al. (3). They found that 2,4,5-T was teratogenic and feticidal in two strains of mice when administered at 46.5 and 113 mg/kg either subcutaneously or orally, and in one strain of rats when administered orally (highest dose administered 46.4 mg/kg). Subsequent investigations by Emerson et al. (4) showed that 2,4,5-T was not teratogenic in Sprague-Dawley rats at doses up to 24 mg/kg. The 2,4,5-T used by Courtney and co-workers (3) was contaminated with approximately 30 ppm of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). This contaminant was shown by Sparschu et al. (5) to be teratogenic in rats in amounts as low as 0.5 μg/kg/day. The 2,4,5-T used by Emerson et al. (4) contained < 1 ppm dioxin.

The present work describes the results of our teratogenic studies on hamsters with samples of 2,4,5-T containing varying amounts of dioxin and with samples of 2,4-D from different manufacturers.

Materials and Methods

Primigravid golden Syrian hamsters, Mesocricetus auratus, were used. The animals were shipped to our laboratories from a commercial supplier on the second or third day of pregnancy. In our laboratory they were caged individually and given Purina Chow and water ad libitum. The test compounds were administered daily by oral intubation on days 6 through 10 of gestation according to the method of Robens (6). Groups of 6 hamsters were used as controls and at each dose level for each compound;

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in most cases the experiments were repeated at the same dose levels with a second group of hamsters. The first day after mating was considered day 0 of gestation. On day 14 of gestation the animals were killed by ether; the uterus was then opened and examined for the presence of resorption sites. The fetuses were removed, weighed, and examined for external malformations. Corpora lutea were counted. About one-third of the fetuses were examined further for skeletal anomalies by fixation in alcohol, clearing and staining with Alizarin red. The remaining fetuses were fixed and divided into two groups, one for dissection and the other for histopathological examination.

The sources of the 2,4,5-T and 2,4-D samples are given in Tables 1 and 2, together with the dioxin content of each sample. The vehicle used for all of these compounds was acetone, corn oil, and carboxymethyl cellulose in a ratio of 1:5.8:10, given in amounts of 1 ml per 100 g body weight. Control hamsters were intubated with the vehicle alone on the same days of gestation as the treated hamsters.

All samples utilized in this experiment were analyzed for all dioxins but only the existence of the "tetra" compound could be proven due to limitations in chemical analyses. This does not preclude, however, the existence within the samples of minute, undetectable quantities of other dioxin isomers.

The following conventions were observed. Any dead or resorbed fetus was counted as a dead fetus. A fetus was considered abnormal if it was living but had at least one anomaly of any type. An abnormal litter was said to be any litter in which a minimum of one abnormal fetus was found.

The percentage of fetal viability was computed by obtaining the percentage per litter and calculating the average of the percentages; the non-parametric Mann-Whitney U test was used to compare the percentages with that of controls. Standard chi-square tests were used to compare the abnormalities per litter from treated and control animals. The average weight per hamster was analyzed for statistical significance by the Student "t" test.

Results

2,4-D. All three of the samples of 2,4-D tested were from commercial sources and therefore represent the products used industrially. Terata were produced occasionally, but the percentages were not significantly different from the control percentage (Table 1).

TABLE 1

Teratogenic Effects of 2,4-D in the Hamster

2,4-D Sample[a]	Daily Dose, Days 6-10 (mg/kg)	No. of Dams	Fetuses				% Fetal Viability per Litter	% Abnormalities per Live Litter	% Hemorrhages per Total Live Fetuses	Av. Wt. per Fetus (g)
			Total No.	No. Live	% Total Mortality	Av. No. per Litter				
Control	-	86	975	942	3.4	11.0	96.7	3.5	0.32	1.8
A	100	10	110	98	11.0	9.8	88.9	10.0	0	1.6
	60	11	116	111	4.3	10.1	95.7	18.2	0.9	1.7
	40	11	131	119	9.3	10.8	91.4[b]	0	0	1.7
	20	11	112	106	5.4	9.6	95.6	9.1	7.6	1.8
B	100	8	108	101	6.5	12.6	93.3	12.5	0	1.7
	60	8	108	104	3.7	13.0	96.0	0	0	1.9[c]
	40	7	76	69	9.2	9.9	90.6	0	2.9	1.7
C	100	9	83	74	10.4	8.2	90.9[b]	22.2	0	1.7
	60	8	111	100	9.9	12.5	89.8[b]	12.5	0	1.7
	40	12	144	139	3.6	11.6	95.9	0	0.7	1.7

[a] Samples were obtained from the following sources: A, Dow Chemical Co., "technical" No. 099203, 1964 production; B, Dow Chemical Co., "current production", No. 091940; C, Hercules Powder Co., "current production", No. TB-105. No dioxins could be detected in any sample.

[b] Significant at $P < 0.05$.

[c] Significant at $P < 0.01$.

TABLE 2

Teratogenic Effects of 2,4,5-T in the Hamster

2,4,5-T Sample, Dioxin Content (ppm)[a,b]	Daily Dose, Days 6-10 (mg/kg)	No. of Dams	Fetuses				% Fetal Viability per Litter	% Abnormalities per Live Litter	% Hemorrhages per Total Live Fetuses	Av. Wt. per Fetus (g)
			Total No.	No. Live	% Total Mortality	Av. No per Litter				
Control	-	86	975	942	3.4	11.0	96.7	3.5	0.32	1.8
A, 45	100	9	103	0	100.0	0	0 [c]	-	-	-
	80	9	125	7	94.4	0.8	5.3[c]	100.0[c]	42.9	1.6
	40	10	144	37	74.3	3.7	25.8[c]	33.3[c]	75.7	1.7
	20	12	130	88	32.3	7.3	68.1[c]	25.0[d]	28.4	1.7
B, 2.9	100	6	87	77	11.4	12.8	88.5[d]	50.0[c]	13.0	1.7
	80	8	92	83	9.8	10.4	90.1[d]	12.5	2.4	1.7
	40	7	69	64	7.2	9.1	93.1	0	0	1.7
C, 0.5	100	11	131	56	57.2	5.1	40.2[c]	40.0[c]	7.6	1.5[c]
	80	8	94	53	43.6	6.6	58.3[c]	40.0[c]	12.5	1.6[c]
	40	9	126	121	4.0	13.4	95.9	11.1	2.5	1.6[c]
	20	11	153	140	8.5	12.6	90.8[d]	0	8.6	1.7[d]
D, 0.1	100	6	68	36	47.1	6.0	57.2[c]	0	5.6	1.6[d]
	80	12	141	94	33.3	7.8	68.3[c]	0	2.1	1.7
	40	7	82	80	2.4	11.4	97.8	0	0	1.8

E, ND	100	8	87	38	56.3	6.3	53.1[c]	36.4[c]	0	1.5[c]
	80	11	137	96	29.9	8.7	69.1[c]	0	4.2	1.5[c]
	40	12	150	134	10.7	11.2	88.2[c]	0	1.5	1.5[c]
F, ND	100	6	64	44	31.3	7.3	71.4[c]	40.0[c]	6.8	1.6[d]
G, ND	100	5	60	42	30.0	8.4	68.3[c]	0	16.7	1.6[c]

[a] Samples were obtained from the following sources: A, K & K Chemical Co.; B, Monsanto Chemical Co., No. NL-07-020; C, Dow Chemical Co., "technical", No. 120449; D, Dow Chemical Co., "technical", current production No. 120110; E, Eastman Kodak Co. (sample recrystallized in FDA); F, Dow Chemical Co., "pure", No. T-00206-2; G, Hercules Powder Co., No. X-17394.

[b] ND indicates no detectable dioxin.

[c] Significant at $P < 0.01$.

[d] Significant at $P < 0.05$.

Fetal viability per litter decreased significantly in the two highest doses of sample C and at 40 mg/kg of sample A, but the decreases were not clearly dose-related. Hemorrhages per total liveborn were consistently increased at the low dose levels. The effects of 2,4-D on the average weight of the offspring were negligible.

2,4,5-T. The results of the administration of 2,4,5-T and dioxin contaminants are shown in Table 2. At 100 mg/kg, 2,4,5-T without detectable dioxin greatly increased fetal mortality, the incidence of hemorrhage in the liveborn (except for sample E), and the number of malformations among the liveborn (except for sample G); fetal viability per litter was significantly decreased. After dosage, the fetal responses ranged from lack of abnormalities and hemorrhages to significant differences, suggesting that there was considerable variation among the compounds obtained from different manufacturers.

At 100 mg/kg, fetotoxicity increased with dioxin content, except at 2.9 ppm. However, fetal viability per litter was significantly depressed at all four levels of dioxin. At 100 mg/kg, abnormalities per live litter were significantly increased both in rats given dioxin-contaminated samples and in those given 2,4,5-T without dioxin contaminants, except for samples D & G. The percentage of hemorrhages per total liveborn was increased with administration of 2,4,5-T with and without dioxin contaminants, but no clear correlation with dioxin level could be established.

2,4,5-T without dioxin, given at dose levels of 40 and 80 mg/kg, increased the level of embryonic mortality and the numbers of liveborn with hemorrhages; the fetal viability per litter was significantly decreased in a dose-related manner. Contamination with dioxin at these dose levels further increased the levels of embryonic mortality and the number of liveborn with hemorrhages; fetal viability per live litter was significantly decreased in a dose-related manner. Abnormalities per live litter were clearly related to the levels of dioxin in the 2,4,5-T. 2,4,5-T with no detectable dioxin produced no malformations below 100 mg/kg.

After administration of 2,4,5-T containing no detectable dioxin or dioxin at 0.1 and 0.5 ppm, the average weight per fetus was significantly decreased. At dioxin levels of 2.9 and 45 ppm, the apparently normal weights of the fetuses were due to edema.

Tables 3 and 4 show the types of terata produced. Bulging eyes (absence of eyelid) and delayed head ossification accounted for the majority of the terata from dioxin containing 2,4,5-T, while fused ribs were seen with the greatest frequency among the terata produced by 2,4-D.

TABLE 3

Fetal Anomalies[a] Associated with Administration of 2,4,5-T

2,4,5-T Sample, Dioxin Content (ppm)[b]	Daily Dose, Days 6-10 (mg/kg)	Exencephaly	Eye Abnormalities	Delayed Head Ossification	Hind Limb Deformities	Cleft Palate	Ectopic Heart	Fused Ribs
Control	-	-	-	-	-	-	-	3
A, 45	80	-	4	1	-	-	-	-
	40	-	5	-	-	-	-	-
	20	-	-	3	-	-	-	-
B, 2.9	100	-	2	-	-	1	1	-
	80	-	-	-	-	-	-	1
C, 0.5	100	1	-	1	-	1	-	-
	80	-	-	2	-	-	-	-
	40	1	-	-	-	-	-	-
E, ND	100	-	-	6	1	-	-	-
F, ND	100	1	2	2	-	-	-	-

[a] Fetuses were also examined for ear abnormalities, but none were found.

[b] See Table 2 for sources of 2,4,5-T. No terata were observed for sample D, containing 0.1 ppm dioxin, or for sample G, with no detectable dioxin.

TABLE 4

Fetal Anomalies[a] Associated with Administration of 2,4-D

2,4-D Sample[b]	Daily Dose, Days 6-10 (mg/kg)	Delayed Head Ossification	Ear Abnormalities	Fused Ribs
Control	-	-	-	3
A	100	-	1	-
	60	-	-	3
	20	-	-	1
B	100	1	-	-
C	100	-	-	3
	60	-	-	2

[a] Fetuses were also examined for exencephaly, eye abnormalities, hind limb deformities, cleft palate, and ectopic heart, but none were found.

[b] See Table 1 for sources of 2,4-D. No sample contained detectable amounts of dioxins.

The control hamsters had a very low fetal mortality and there were only three malformed fetuses from a total of 942 live fetuses, or 0.3%. With this same strain of hamsters, Robens (7) found four malformed fetuses in 1081 control hamsters or 0.4%.

Discussion

2,4-D. The incidence of fetal anomalies resulting from the administration of the three commercial samples of 2,4-D was low. The lowest dose causing effects, 60 mg/kg, would approximate 600 ppm in the diet, and the maximum human dietary exposure to 2,4-D from permitted tolerances is 0.3 ppm.

2,4,5-T. The fetal anomalies resulting from 2,4,5-T administration in the hamsters were clearly related to fetal head development. They were, namely, eye abnormalities (absence of eyelids), delayed head ossification, and exencephaly. The results differ from those found by Courtney et al. (3) in rats and mice. Courtney et al. found cleft palates in mice, but they were rarely found in our study with hamsters (seen in only two animals).

Hemorrhagic gastrointestinal tracts in the hamster fetuses were a prominent effect of 2,4,5-T administration, but could not be clearly linked to dose level of the compound or the dioxin content. It is possible that such hemorrhages reflect a toxic effect on fetal organs as opposed to a developmental effect.

The relationship between human exposure to low levels of 2,4,5-T and of 2,4-D and the production of congenital abnormalities in different animal species is difficult to assess. There is a wide margin of safety between the exposure levels of 2,4,5-T permitted by registered uses existing prior to April 1970 on food crops and the levels producing teratological changes in the hamsters used here. Since April, these uses have been cancelled so that the amount of 2,4,5-T now in use has still further decreased the possible hazard to humans.

Summary

Commercial samples of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were feticidal and teratogenic in the golden Syrian hamster when administered orally on days 6 - 10 of organogenesis, and the incidence of effects increased with the content of the impurity, 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin). Abnormalities per live litter were clearly related to dose levels of 2,4,5-T in combination with dioxin. With dioxin, the abnormalities caused by 2,4,5-T consisted chiefly of absence of eyelid and delayed head ossification. Dioxin contamination increased the level of hemorrhages in the liveborn, and also produced marked edema.

Terata were produced occasionally with 2,4-dichlorophenoxyacetic acid (2,4-D) and the fetal viability per litter decreased, but neither effect was clearly dose-related. Fused ribs were seen with the greatest frequency among the terata produced by 2,4-D.

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