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Teratogenic Evaluation of 2,4,5-T

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Teratogenic Evaluation of 2,4,5-T

Abstract. *The herbicide 2,4,5-trichlorophenoxyacetic acid is teratogenic and fetocidal in two strains of mice when administered either subcutaneously or orally and in one strain of rats when administered orally. The incidences of both cystic kidney and cleft palate were increased in the C57BL/6 mice as well as the incidence of cleft palate in the AKR mice. The incidence of cystic kidney was also increased in the rats. In addition, an increase in the ratio of liver weight to body weight in the mouse fetus and the occurrence of hemorrhagic gastrointestinal tract in the rat fetus suggest that this compound also has fetotoxic properties.*

The chlorinated herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) is used extensively for weed control (*1*). There have been few reports concerning its pharmacologic and toxicologic properties in animals (*2*, *3*), and there are no data available concerning the effects of this compound on the developing embryo and fetus. Therefore, we evaluated the teratogenic and fetotoxic potential of 2,4,5-T in mice and rats.

Breeding colonies of C57BL/6 and AKR strains of mice were established to supply the mice. Breeding was by random mating. Detection of a vaginal plug indicated day zero of pregnancy. Pregnant rats were procured (Holtzman) with known insemination dates. Detection of sperm indicated day zero of pregnancy. All animals were given free access to chow and water.

The herbicide 2,4,5-T (*4*) was administered either subcutaneously or orally. A solution of 2,4,5-T in 100 percent dimethylsulfoxide (DMSO) in a volume of 100 μl per mouse was used for each subcutaneous administration. For oral administration by stomach tube, 2,4,5,-T was suspended in a honey solution (honey to water, 1:1), volumes of 100 μl per mouse and 200 μl per rat were used.

In the studies with the C57BL/6 strain, 2,4,5-T was administered daily beginning on day 6 of pregnancy and continuing through day 14 or from day 9 through day 17. The mice were killed on day 18 of gestation. In the studies with the AKR strain, 2,4,5-T was administered daily beginning on day 6 of pregnancy and continuing through day 15. These mice were killed on day 19 of gestation. The rats were treated on day 10 through day 15 and killed on day 20 of gestation.

After the animals were killed both mothers and fetuses were examined. In addition, about one-third of the mouse fetuses from each litter were stained with alizarin red S to detect skeletal anomalies.

The following conventions were observed in compiling the data in Tables 1 to 3. If a fetus was either dead or resorbed, it was regarded as a dead fetus. A fetus was classified abnormal if it was alive and had at least one anomaly (regardless of type). Similarly, a litter was classified as abnormal if it contained one or more abnormal fetuses. A fetus was said to have a cystic kidney if at least one of its kidneys was affected. In calculating the ratios of liver to body weight in the mother, maternal body weight was defined as the difference between the weight of the mother on the day it was killed and the gravid uterus weight. Finally, the maternal weight gain was defined as the difference in the corrected maternal body weight on the day it was killed and its weight on day zero of pregnancy.

The percentages for fetal mortality,

DSW 552530

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Table 1. Teratogenic evaluation of 2,4,5-T in mice.

Compound	Vehicle	Dose (mg/kg)	Litters (No.)	Live fetuses per litter (av. No.)	Fetal mortality per litter (%)	Abnormal litters (%)	Abnormal fetuses per litter (%)	Percentage of fetuses per litter with:	
								Cleft palate	Cystic kidney
C57BL/6 strain treated days 6 to 14									
Nontreated	None	None	72	5.8	26	38	11	<1	1
Control	DMSO	*	106	5.5	29	42	12	<1	2
Control	Honey	*	32	7.1	15	41	14	0	1
2,4,5-T	DMSO	21.5	6	7.7	3	50	12	0	0
2,4,5-T	DMSO	113.0	18	4.4	42	86†	57†	22†	41†
2,4,5-T	Honey	46.4	6	8.5	8	100‡	37‡	2	33†
2,4,5-T	Honey	113.0	12	4.8	47†	100†	70†	23†	48†
C57BL/6 strain treated days 9 to 17									
Nontreated	None	None	8	5.1	36	71	31	0	7
Control	DMSO	*	10	6.1	23	30	8	0	0
2,4,5-T	DMSO	113.0	10	7.7	11	100†	77†	29†	60†
AKR strain treated days 6 to 15									
Nontreated	None	None	58	7.1	16	19	5	<1	<1
Control	DMSO	*	72	6.9	15	24	4	<1	<1
Control	Honey	*	12	8.8	9	0	0	0	0
2,4,5-T	DMSO	113.0	14	6.9	23	71†	29†	28†	1
2,4,5-T	Honey	113.0	7	5.3	42‡	100†	55†	55†	0

* Dose, 100 μl per mouse. † $P = .01$. ‡ $P = .05$.

abnormal fetuses, fetuses with cleft palate, and fetuses with cystic kidney were computed by first obtaining the percentage for each litter and then calculating the average of these percentages.

The percentage of abnormal litters provides a measure of the prevalence of abnormal fetuses across litters, while the percentage of abnormal fetuses per litter gives an indication of the prevalence of abnormal fetuses within litters.

The control animals were those that were used in a large study during the 3-year period in which 2,4,5-T was evaluated. The data from the control groups treated with DMSO or honey were compared with the data for the nontreated control group. Then the results from animals treated with 2,4,5-T in either DMSO or honey were compared to the appropriate control data. Standard corrected 2×2 chi-square tests (*5*) were used to compare the results from animals treated with 2,4,5-T with the appropriate control data for the proportion of litters containing abnormal fetuses.

The distribution of the percentage of abnormal fetuses per litter for litters treated with 2,4,5-T was compared with the appropriate control distribution by use of the nonparametric Mann-Whitney U test (*5*). Also, this test was used for comparing the percentage of fetal mortality, cleft palate, cystic kidney, and enlarged renal pelvis per litter. This test requires that the proportion of dead or abnormal fetuses per litter is independent from litter to litter but requires no assumption about the frequency distributions of these proportions.

Initial analysis of the data indicated that anomalies were not randomly distributed across all litters but tended to cluster within litters. Many litters possessed no anomalies, whereas all of the fetuses in some litters were abnormal. Since fetuses within the same litter tend to be more alike, pooling the data across litters before performing statistical tests is not appropriate. The experimental unit (*6*) is that entity to which treatments are applied, in this case the pregnant animal. Hence, all calculations of averages and all statistical tests were performed on the independent responses of the litters.

The administration of DMSO or honey to mice or rats did not adversely affect the development of the fetuses. The incidence and type of naturally occurring anomalies observed in the animals treated with DMSO or honey did not show an increase compared with the nontreated group. Fetuses of the control mice, when stained with alizarin, showed very few skeletal anomalies. No skeletal anomalies were detected by staining in the treated mice. For both mice and rats, there were no differences in the average number of implantations in the control and experimental litters. A few values for treated animals were less than those of their appropriate controls. These differences were not statistically significant including the 3 percent fetal mortality observed in the C57BL/6 mice receiving 21.5 mg of 2,4,5-T per kilogram of body weight (Table 1). This value of 3 percent reflects a period of low fetal mortality (9 percent) observed in the control mice during the first few months of the study. This difference in mortality is not statistically significant. There were no other significant changes in these control data during the 3-year period.

The administration of 2,4,5-T to C57BL/6 mice on days 6 through 14 at a dose of 113 mg/kg produced significant increases in percentage of abnormal litters and percentage of abnormal fetuses per litter (Table 1). The anomalies produced by 2,4,5-T were almost exclusively cystic kidney and cleft palate.

Similar results were obtained regardless of whether the compound was administered subcutaneously or orally. A dose of 46.4 mg/kg adminis-

Table 2. Liver weight study. The 2,4.5-T (113 mg/kg) was administered daily subcutaneously in DMSO from day 9 through day 17 of gestation in C57BL/6 mice.

Treatment	Fetal			Maternal	
	Liver wt (g)	Body wt (g)	Liver wt/ body wt	Weight gain (g)	Liver wt/ Body wt
None	0.047	0.810	0.058	6.00	0.069
DMSO	.046	.818	.056	5.99	.068
2,4,5-T	.057*	.738†	.076‡	4.65	.120‡

* $P = .05$. † $P = .10$. ‡ $P = .01$.

DSW 552531

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Table 3. Teratogenic evaluation of 2,4,5-T in rats.

Compound	Vehicle	Dose (mg/kg)	Litters (No.)	Live fetuses per litter (av. No.)	Fetal mortality per litter (%)	Abnormal litters (%)	Abnormal fetuses per litter (%)	Percentage of fetuses per litter with:	
								Enlarged renal pelvis	Cystic kidney
Nontreated	None	None	7	9.9	11	43	9	9	0
Control	Honey	*	14	8.7	1	57	12	12	<1
2,4,5-T	Honey	4.6	8	8.2	12	88	36†	18	21
2,4,5-T	Honey	10.0	7	7.1	28‡	86	46†	17	30†
2,4,5-T	Honey	46.4	6	2.7	59‡	67	60§	27	33§

* Dose, 200 μl per rat. † $P = .05$. ‡ $P = .01$. § The sample size was possibly too small to show a significant difference.

tered orally did not produce a significant increase in fetal mortality or an effect on palatal development, but it did cause a significant increase in the percentages of fetuses with cystic kidney. Administration of 2,4,5-T subcutaneously at a dose of 21.5 mg/kg did not affect the viability or development of the fetuses. Thus, a dose-response relation for the fetocidal and teratogenic properties of 2,4,5-T in mice is suggested for both routes of administration.

In mice treated with 2,4,5-T on days 6 through 14 there was a significant decrease in the incidence of naturally occurring anomalies. These consist of microphthalmia followed by anophthalmia and are in accord with other C57BL/6 colonies (*7*). Although the fetuses from mice treated on days 6 through 14 had fewer naturally occurring anomalies, the fetuses from mice treated on days 9 to 17 did exhibit these anomalies. Thus, it appears that the interval of days 6 to 9 of gestation is one of the sensitive periods of development with respect to 2,4,5-T. Two other sensitive periods are during development of the palate and kidney since they are so highly affected. The occurrence of these two anomalies are statistically unrelated.

Administration of 2,4,5-T to the C57BL/6 mice on days 9 to 17 of gestation produced a significant increase in the ratio of liver to body weight in both the mother and the fetus (Table 2). The significant increase in the ratio of fetal liver to body weight reflects both an increase in fetal liver weight and a decrease in fetal body weight. The significant increase in the ratio of maternal liver to body weight suggests a change in activity of drug metabolizing enzymes of the endoplasmic reticulum (*8*). The Mann-Whitney U test was used to compare the animals administered 2,4,5-T with the appropriate controls given DMSO.

Thus, in the C57BL/6 mice, 2,4,5-T is fetocidal and teratogenic and is capable of producing an increase in the ratio of liver to body weight.

Treatment of mice of the AKR strain with 2,4,5-T in honey produced a significant increase in fetal mortality. The incidence of cleft palate was increased with both routes of administration. However, 2,4,5-T did not produce an increased incidence of cystic kidney in this strain. There was no effect of 2,4,5-T administration in this strain on the maternal weight gain with either route of administration. However, the ratio of liver to body weight in the mother was increased with either route of administration.

In addition, hybrid litters resulting from mating C57BL/6 females with AKR males were evaluated. The administration of 113 mg/kg in DMSO from days 6 through 14 of gestation produced a high incidence of both cystic kidney and cleft palate. There was no effect on maternal weight gain.

The oral administration to rats of 2,4,5-T at a dose of 10.0 or 46.4 mg/kg on day 10 through day 15 of gestation produced a significant increase in fetal mortality (Table 3). The two lower doses (4.6 and 10.0 mg/kg) produced a significant increase in the percentage of abnormal fetuses. These fetuses displayed a high incidence of cystic kidney. At the highest dose (46.4 mg/kg), the marked increase in fetal mortality reduced the population of live fetuses to a small sample. However, cystic kidneys were observed. In a limited study, the administration of 2,4,5-T at doses of 21.5 or 46.4 mg/kg from day 6 through day 15 of gestation was highly fetocidal.

At all doses studied in the rat, hemorrhagic gastrointestinal tracts were observed in the fetuses. The percentages of fetuses per litter with hemorrhagic gastrointestinal tracts showed a dose-response relation—that is, 3, 56, and 83 percent at doses of 4.6, 10.0, and 46.4 mg/kg, respectively. None were observed in the fetuses from the control animals. Drill and Hiratzka (*2*) have reported that dogs which received 2,4,5-T in the diet showed some necrosis and inflammation of the intestinal mucosa. The hemorrhagic gastrointestinal tracts observed in the rat fetuses is probably a toxic effect of 2,4,5-T on the fetal organ as opposed to a developmental defect.

These studies show that 2,4,5-T adversely affects the development and viability of the mouse and rat fetus.

Note added in proof: The sample of 2,4,5-T used in this study contained approximately 30 ppm of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin). Dioxin as well as purified 2,4,5-T are currently being investigated for their teratogenic and fetotoxic potential.

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4. The 2,4,5-T was produced by the Diamond Alkali Co., 98 percent, Tech., melting point 149° to 151°C.
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9. These results are from a large study designed to screen selected compounds for teratogenic effects in mice which was performed at the Bionetics Research Laboratories, Division of Litton Industries, under contract Nos. PH 43-64-57 and PH 43-67-735 from the National Institutes of Health. During this study, K.D.C. was a staff member of the Bionetics Research Labs., Inc., and H.L.F. was a member of the National Cancer Institute. We thank Dow Chemical Co. for the analysis of 2,4,5-T.

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