TOXICOLOGY BRANCH: DATA REVIEW

Caswell No.: 385
Shaughnessy No.: 057901

Chemical: Trichlorfon

Study Type: Teratology in rats

Citation: Staples, R.E., R.G. Kellman and J.K. Hoseman. 1976. Developmental toxicity in the rat after ingestion or gavage of organophosphate pesticides (Dipterex, Imidan) during pregnancy. Environ. Health Persp. 13: 133-140.

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Test Material: Dipterex technical (98.5%), lot#405006 supplied by Chemagro Corp., suspended in aqueous 0.5% methylcellulose (for gavage administration) or incorporated in the diet.

Procedures: Groups of pregnant CD rats were administered test substance from day 6 through day 15 of gestation, either by gavage at 50, 75, 150, 200 or 250 mg/kg/day (9, 9, 16, 26 and 6 dams, respectively) or in the diet at levels of 76, 145, 375, 432 or 519 mg/kg/day (respectively: 9, 9, 17, 21 and 19 dams), and observed for clinical signs daily throughout the study. Control groups received either the gavage vehicle or untreated diet; a portion of each control was matched by weight to three dietary or gavage dose levels and pair-fed during the period of Dipterex administration (9, 16 and 13 dams at the 145, 432 and 519 mg/kg/day dose levels, respectively; 9, 16 and 8 at the 75, 200 and 250 mg/kg/day doses). Body weights were obtained before treatment began (days 1 and 6 of gestation), as well as during trichlorfon administration (days 8, 10, 12, 14 and 15) and twice following the treatment period (days 17 and 21). Food consumption was recorded daily during treatment and for two days thereafter.

All rats were sacrificed on day 21 of gestation by cervical dislocation and the uterine contents were examined. The number and distribution of implantation sites were recorded as live fetuses, dead fetuses [appeared to include resorptions] and their general condition. Live fetuses were individually weighed, sexed, and examined for external malformations. Stunted fetuses were defined as fetuses weighing 2.0 g or less, or weighing less than two-thirds the average of the remaining littermates. At least one-third of the fetuses per litter, plus all stunted and externally abnormal fetuses, were dissected and examined for visceral abnormalities. [The authors did not specify if the fetuses examined for visceral malformations were decapitated which

is standard procedure with the Staples technique]. The carcasses of all fetuses examined viscerally, and the remaining live fetuses, were processed with KOH and Alizarin Red S and examined for skeletal malformations. The skeletons of dead, non-marcerated, fetuses were examined but these data were not included by the authors. [The authors stated that they combined these data from the dietary and vehicle groups because these data were similar].

The Mann-Whitney U test was used to compare experimental groups to the control groups. Jonckheere's test* was employed to test the significance of a dose-response relationship. The pair-fed controls were compared to the animals receiving Dipterex by Wilcoxon's sign-rank test. Because of the rapid weight gains observed in 21-day old fetuses, rank analysis of covariance was employed to adjust fetal weight for time of sacrifice and the number of fetuses per litter. The litter was used as the experimental unit and significance was noted at the 5 percent level utilizing one-sided tests.

Results: A combined summary of results from this study is presented here as Table 1. The authors combined the data from the vehicle (gavage) and dietary control groups.

In the gavage study, significant increases in maternal mortality were observed. Three, six, and seven dams died at 150, 200, and 250 mg/kg/day, respectively. No deaths occurred at levels less than 150 mg/kg/day. Food consumption was significantly depressed among all Dipterex treated groups except the high dose. The maternal weight gains (days 6-17 of gestation) of the Dipterex-treated groups were also comparable to the weight gain for the controls. The weight gains during this period for the pair-fed controls were less than their Dipterex-treated counterparts. The numbers of dead and stunted fetuses in the Dipterex-treated groups were comparable to the controls. Although fetal body weights among treated groups were significantly less than the control fetal body weights at dose levels of 75 mg/kg/day and greater, no such reduction was evident in the pair-fed controls. The percentage of malformed fetuses per treatment group was comparable between the Dipterex and control groups. A total of 14 anomalies was reported among 640 fetuses from 54 litters, all minor skeletal variations consisting of eleven doubled vertebral centra and three incompletely ossified skull were bones [specific skull bones not identified].

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*Jonckheere, A.R. 1954. A distribution-free k-sample test against ordered alternatives. Biometrica 41: 133.

No deaths occurred among dams administered Dipterex in the diet. The authors did not state if any other clinical signs of toxicity were observed, but as noted in the table, average daily food consumption decreased significantly at all doses except the LDT, and significantly decreased body weight gains were recorded at the two highest dose levels. The weight gains of the Dipterex groups consuming 375 mg/kg/day or less were similar or greater than the controls, but pair-fed controls had body weight gains that were less than their Dipterex counterparts. Although the percentage of dead fetuses was significantly greater than controls in litters from dams consuming 432 mg/mg/day, there was no increase in fetal mortality in the 432 mg/kg pair-fed control litters. The percentage of fetal deaths was also increased at 519 mg/kg/day, but less than at 432 mg/kg/day, and in the 519 mg/kg pair-fed controls was comparable to the percentage in the high-dose group. Small incidences of stunted fetuses were observed in 2 of 3 pair-fed control groups and at 432 and 519 mg/kg. The fetuses from dams consuming the two highest doses had significantly reduced body weights compared to the controls. The fetal body weights of the 432 mg/kg pair-fed controls was significantly greater than their dosed counterpart; on the other hand, the 519 mg/kg pair-fed controls fetuses and the high-dose fetuses had comparable fetal body weights. [All teratology data, however, were presented on a fetus basis and no data were presented on a litter basis.] Compared to the controls, significant increases in the percent of fetuses with anomalies of any type occurred at levels of 145 mg/kg/day and greater of Dipterex. The percentages of malformed fetuses from and dams consuming 145 mg/kg/day was similar to the percentage in its pair-fed control counterparts. At 432 and 519 mg/kg/day, however, major malformations were observed. [The authors combined the teratology data for the two highest dose levels.] In the two highest dose levels, 109 of 396 fetuses were malformed: 33 instances of the "skull and central nervous system (execephaly, meningocoele, hydrocephaly), 26 of the limb (syndactyly, markedly shortened radii and ulnae, missing digits), 14 micrognathiae, 19 cleft palates, 9 facial hematomas, 17 cases of generalized edema, 11 fused sterna, 92 doubled vertebral centra, 7 wavy ribs, and four great vessel defects." "Seven other malformations" occurred only once. At 375 mg/kg/day, the anomalies were less numerous and severe, consisting mainly of minor skeletal variations ("13 doubled vertebral centra, three wavy ribs, two fenestrated [incompletely ossified] supraoccipital bones, andd one umbilical hernia"). Data from pair-fed controls (tabulation attached) indicated that the increased incidence of anomalies observed among offspring from dams fed 375 mg/kg trichlorfon or more did not result solely from reduced food consumption by these animals.

Conclusions: The administration of trichlorfon to pregnant CD dams from day 6 to day 15 of gestation produced mortality at dose levels of 150 mg/kg/day and greater, decreased food consumption at all dose levels except the high dose, but did not affect maternal weight gain from day 6 to day 17 of gestation. Based on the data reported, Dipterex was maternally toxic at dose levels of 150 mg/kg/day and higher, an effect not observed among the pair-fed controls which also had smaller weight gains than their intubated counterparts. Although fetal body weights were decreased among the dams dosed with Dipterex at 75 mg/kg/day or greater, this depression did not occur among the pair-fed controls despite the decreased maternal weight gain observed in the pair-fed controls. These data indicated that Dipterex induced fetal toxicity when orally administered, and that the effects observed are not a result of decreased food intake. However, the teratology data presented by the authors did not include the incidence of litters with malformed fetuses, nor the number of fetuses and litters affected by a given malformation. Despite this deficiency, the low incidence of malformed fetuses and the inconsequential nature of the anomalies observed indicate to this reviewer that Dipterex was not teratogenic when orally administered at dose levels as great as 200 mg/kg/day. [Only two litters were produced at 250 mg/kg/day, and the data at this dose level was considered insufficient for concluding that Dipterex was teratogenic.]

Dietary administration of Dipterex reduced maternal body weight (days 6-17 of gestation) among dams consuming 432 and 519 mg/kg/day, and decreased food consumption in dams consuming 145 mg/kg/day or more; however, at 145 mg/kg/day and 375 mg/kg/day, concurrent reductions in body weight gains did not occur, nor in the 145 mg/kg/day pair-fed controls. These data suggest that the decreased food consumption at 145 and 375 mg/kg/day was not evidence of overt maternal toxicity. [It could be indicative of an umpalatable diet.] Based on the data reported, dietary consumption of Dipterex was maternally toxic with a LEL of 432 mg/kg/day and a NOEL of 375 mg/kg/day. Dietary consumption of Dipterex did not affect the dam's ability to maintain pregnancy, decreases in fetal body weights were observed when the dams consumed 432 and 519 mg/kg/day. This reduction in fetal body weight was not evident in the 432 mg/kg/day pair-fed control, however, indicating that the observed effect was compound-induced. Although the percentage of dead fetuses was increased at 432 and 519 mg/kg/day, the significance of this finding is

questionable because the percentage of dead fetuses was greater at 432 mg/kg/day than at 519 mg/kg/day, and the 519 mg/kg pair-fed controls also produced an increased percentage of dead fetuses. Based on the reduction in fetal body weights, Dipterex consumed in the diet induced fetotoxicity with a LEL of 432 mg/kg/day and a NOEL of 375 mg/kg/day.

The failure of the authors to present the teratology data on a litter basis (number of litters with malformed fetuses, number of litters with a given malformation present), and to present the number of fetuses with a given anomaly prevents the determination a NOEL and LEL. In spite of this deficiency, the occurrence of malformations in 109 of 396 fetuses in the 432 and 519 mg/kg/day consumption groups indicated that the dietary administration of Dipterex was teratogenic at these levels. The lack of increased malformations in the pair-fed controls further suggested that the malformations were Dipterex-induced and not the result of maternal stress.

CORE Classification: Supplementary Data.

The following deficiencies were noted:

°The teratology data were not presented as litters with malformed fetuses, nor as the number of litters and fetuses with a given malformation.

°The oral intubation phase of the study contained only one dosage group with 20 or more pregnant females.

°In the dietary study only one dosage group had 20 or more pregnant females.

TABLE 1. Effect of Dipterex Administration by Diet or Gavage in Pregnant Rats and their Offspring

	Diet (mg/kg)						Dietary Control Pair Fed (mg/kg)[a]		
Females	0[b]	76	145	375	432	519	145	432	519
Total Number	47	9	9	17	26	24	9	18	14
Percent Dead	0	0	0	0	0	0	0	0	0
Percent Pregnant (number)	94 (44)	100 (9)	100 (9)	100 (17)	81 (21)	79 (19)	100 (9)	89 (16)	92 (13)
Average Daily Food Consumption (g) (day 6-15 gestation)	24	23	22^{s-}	22^{s-}	20^{s-}	20^{s-}	22	20	20
Average Daily Weight gain (g) (day 6-17 gestation)	45	53	58	49	37^{s-}	27^{s-}	43^{p-}	28	97
Fetuses									
Total Number	474	117	118	181	216	180	180	199	97
Percent Dead	6	2	4	6	18^{s+}	10	5	5^{p-}	13
Percent Stunted	0	0	0	0	0.3	0.5	1	1	0
Weight (g)	4.0	3.9	3.8	4.0	3.5^{s-}	3.5^{s-}	3.8	3.7^{p+}	3.6
Percent Malformed	1	0	4^{s+}	11^{s+}	30^{s+}	30^{s+}	2	3^{p-}	2^{p-}

[a] Refer to dose administered to corresponding experimental group.

[b] Combined data of dietary controls and vehicle control.

s^+ or s^- = Signifies significantly greater than or less than the respective control ($p < 0.05$).

p^+ or p^- = Signifies significantly greater than or less than the corresponding pair-fed experimental group ($p < 0.05$).

Chart continue

Females	Gavage (mg/kg)					Gavage Control Pair Fed (mg/kg)[a]		
	50	75	150	200	250	75	200	250
Total Number	9	9	18	30	11	9	16	10
Percent Dead	0	0	17^{s+}	20^{s+}	64^{s+}	0	0	0^{p-}
Percent Pregnant (number)	100 (9)	100 (9)	100 (16)	100 (26)	81 (6)	100 (9)	89 (16)	92 (8)
Average Daily Food Consumption (g) (days 6-15 gestation)	22^{s-}	21^{s-}	21^{s-}	20^{s-}	23	21	20	21
Average Daily Weight gain (g) (day 6-17 gestation)	41	42	42	42	47	38	33^{p-}	20
Fetuses								
Total Number	98	120	159	242	21	114	170	82
Percent Dead	12	5	4	3	0	1	12	3
Percent Stunted	0	0	0	0	0	0	0	0
Weight (g)	3.9	3.7^{s-}	3.8^{s-}	3.5^{s-}	2.3^{s-}	4.1^{p+}	3.7	4.2
Percent Malformed	4	3	3	2	0	0	1	6