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Dipterex Teratogenicity in the Rat, Hamster, and Mouse When Given by Gavage

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Dipterex was teratogenic after administration by gavage (t.i.d.) at a dose level of 480 mg/kg-day to the CD rat on days 6 through 15 of gestation, but not when administered only on days 8 or 10 of gestation. A positive teratogenic response also occurred in the hamster after administration on days 7 through 11 of gestation at 400 mg/kg-day; the apparent no-effect level for the criteria studied was 200 mg/kg-day. Embryotoxicity, but not teratogenicity, occurred after administration at 400 mg/kg-day on day 8 of gestation. In both species, the teratogenicity seen was not merely due to reduced maternal food consumption during the period of exposure. The mouse was less susceptible to Dipterex than were the rat and hamster, but a significant increase in the incidence of cleft palates resulted from exposure on days 10 through 14, or on days 12 through 14 of gestation.

Dipterex (*O*, *O*-dimethyl-l-hydroxy-2,2,2-trichloroethyl phosphonate) was found to be teratogenic when consumed by rats on days 6 through 15 of gestation but not after administration by gavage, once daily (i.d.), for the same period of gestation (*1*). Since drugs, pesticides, and other environmental agents often are tested for teratogenic potential only by gavage, it was considered important to demonstrate whether the teratogenic potential of Dipterex could be detected in the rat by gavage through multiple daily administration as a means of attaining higher daily dose levels of Dipterex more comparable to that achieved through incorporation in the diet.

In the rat the teratogenicity of Dipterex was demonstrated only at dose levels that decreased maternal food consumption and that were far in excess of expected levels of routine human exposure. To determine whether the teratogenicity of Dipterex is unique to the rat, its teratogenic potential also was investigated in the hamster and the mouse.

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Material and Methods

Rats

Nonparous, female rats (CD strain) were housed overnight with mature males of the same strain in quarters lighted for 12 hr of each day and maintained at 20°C (50% RH). Mating was confirmed by detection of spermatozoa in the vaginal lavage the following morning (day 1 of gestation). Between days 1 and 6 of gestation, all rats were caged individually and had free access to water and Wayne Lab Blox. On day 6 of gestation, the rats were assigned to the experimental group or to the pair-fed control group (CPF). Dipterex was given by gavage on days 6 through 15 of gestation at a daily dose on a par with those that were teratogenic in the first study (*1*). Preliminary study revealed that if the rats were gavaged three times daily (t.i.d.) a daily dose of 480 mg/kg was tolerated quite well. The rats of the experimental group were gavaged (t.i.d.) with 160 mg/kg body weight in 10 ml/kg of 0.5% methylcellulose in distilled water; the CPF group was gavaged similarly with only the vehicle.

To determine whether the teratogenicity of Dipterex could be revealed by gavage (t.i.d.) on a single day during organogenesis, additional rats were given Dipterex only on day 8 or 10 of gestation. In this part of the study, the rats in the respective con-

trol groups (Cv) were gavaged with vehicle (t.i.d.), but they were not pair-fed to the experimental groups.

Hamsters

Nonparous golden hamster females arrived from Lakeview Farms, New Jersey on day 3 of gestation. The morning after mating was considered to be day 1 of gestation. They were housed individually in plastic cages with corn cob bedding and had free access to Wayne Lab Blox and water. Room temperature was maintained at 20°C (50% RH) and light was provided for 12 hr of each 24 hr.

Dipterex was administered by gavage (t.i.d.) in 10 ml/kg body weight of distilled water from day 7 to day 11. It was established by preliminary study that the hamster would tolerate 400 mg/kg-day for this period of gestation. The Dipterex solution was freshly prepared each day and the daily dose was administered in thirds at 9:00, 12:30, and 16:00 hr of each exposure day. Concurrent control groups included untreated controls (C) and another group that was given only distilled water by gavage (t.i.d.) at 10 ml/kg-day on days 7 through 11 of gestation (Cv). Lagging one day behind was a third control group (CPF) that was pair-fed to the group given Dipterex at 400 mg/kg-day. The CPF group also was gavaged (t.i.d.) with distilled water on days 7 through 11 of gestation. Food consumption was measured daily, and maternal body weights were recorded on days 3, 7, 9, 11, 13, and 15 of gestation.

Upon completion of the experiment described above, lower dose levels were added (100, 200, 300 mg/kg-day) to determine the apparent no-effect dose level with respect to the criteria under study, and the 400 mg/kg-day dose level was administered by gavage (t.i.d.) to additional hamsters only on day 8 to determine fetal sensitivity to exposure on a single day of gestation.

Mice

Nonparous, female mice (CD-1 strain) were cohabited overnight with mature males of the same strain (2♀♀/♂) in quarters lighted for 12 hr of each day and maintained at 20°C (50% RH). Mated females detected by the presence of copulatory plugs in their vaginas (considered to be day 1 of gestation), were housed in large plastic cages and had free access to Wayne Lab Blox and water. All females were housed individually beginning with the first scheduled administration of Dipterex and thereafter to permit measurement of food consumption until two days after the last day of exposure to Dipterex. It was established by preliminary study that pregnant mice could tolerate Dipterex in doses up to 600 mg/kg-day for four consecutive days if given by gavage (t.i.d.). On this basis groups of pregnant mice were given Dipterex at a dose level of 600 mg/kg-day on days 6 through 10 and on days 10 through 14 of gestation. Again the apparent no-effect dose level was determined by study of additional dose levels (300, 400, and 500 mg/kg-day) administered for the same periods, and fetal sensitivity to shorter exposure periods was explored by administration of Dipterex at the dose level of 600 mg/kg-day on day 8, days 8 through 10, days 10 through 12, and days 12 through 14 of gestation. The Dipterex solutions were prepared each day in distilled water such that the desired dosage could be administered in 10 ml/kg body weight at 9:00, 12:30, and 16:00 hr daily. Groups of control females (Cv) were given the same volume of distilled water by gavage (t.i.d.) for the same periods of gestation as for the groups given Dipterex; other groups of control females (C) were not gavaged. Maternal body weight was measured on day 1 of gestation, on the first day of Dipterex administration, every other day during dosing, the second day after cessation of Dipterex administration, and on the day of sacrifice.

On day 21 of gestation for the rats, on day 15 for the hamsters, on day 18 for the mice, all females were coded and sacrificed (by CO_2 inhalation for the hamsters, and by CO_2 inhalation followed by cervical dislocation for the rats and mice), and their reproductive status was determined. Implantation sites in each uterine horn were counted and each conceptus was recorded as being alive or dead and as to general condition. Live fetuses were weighed individually, sexed externally and internally, and examined for external malformations. Those weighing 2.0 g or less for rats, 1.0 g or less for hamsters, and 0.5 g or less for mice, and those weighing less than two-thirds the mean of their larger litter mates were termed "stunted." At least one-third of the fetuses of each litter, all "stunted" fetuses, and those having external malformations were dissected and examined for visceral alterations (*2*); their heads were fixed in Bouin's solution and sliced to reveal developmental alterations. All fetuses alive at sacrifice were cleared for visualization of skeletal alterations (*3*).

Statistics

Nonparametric test procedures were used in the data analysis. The litter was considered to be the experimental unit and incidence data were expressed as mean percent affected fetuses per litter. Experimental groups were compared to controls (Cv and CPF) by Mann-Whitney *U* tests (*4*). One-

sided tests were used, and differences significant at the 0.05 level were noted.

Results

Rats

Signs of cholinesterase inhibition were evident after each administration of Dipterex by gavage as was reported previously (*1*). The effects did not accumulate from day to day and as a result only three of the 34 females given Dipterex (t.i.d.) for 10 days died between days 13 and 15 of gestation. None of those given Dipterex only for one day died.

Dipterex administration to rats by gavage (t.i.d.) on days 6 through 15 of gestation was followed by a substantial, although not a statistically significant, decrease in the maintenance of pregnancy, an increase in the fetal mortality rate ($p = 0.06$) among rats in which pregnancy was maintained, and a significant decrease in the body weight of the surviving fetuses versus values for the same criteria among pair-fed control rats (Table 1). Furthermore, an average of 76.4% of the fetuses in each litter of the experimental group was malformed. The malformations occurred in all of the 19 litters exposed to Dipterex and consisted mostly of generalized edema, various types of herniation of the brain and cerebrospinal fluid through the skull, internal hydrocephaly, micrognathia, cleft palate, severely shortened radius and ulna, and hypophalangism and syndactyly. Other alterations noted were hematomas, extensive doubling of the thoracic centra, fusions of the sternebrae and of the ribs, umbilical hernias, small kidneys and common truncus. The malformations seen in the CPF group consisted of two fetuses with wavy ribs and another with doubled thoracic centra; these fetuses were from three litters.

On the other hand, administration by gavage (t.i.d.) of the same daily dose of Dipterex on day 8 or 10 of gestation significantly decreased maternal food consumption, on a temporary basis (one day period), but did not have a significant adverse effect on the remaining criteria studied (Table 1).

Hamsters

Ten minutes after administration of Dipterex at 400 mg/kg-day by gavage (t.i.d.) the hamsters exhibited signs of cholinesterase inhibition, e.g., ptyalism, exophthalmos, loss of equilibrium, and muscular tremors. In general, they recovered by the time the next dose was to be administered, but three of the 30 females given Dipterex (days 7 through 11) at this level died; one after the first and two after the third day of dosing. None of the control animals died before scheduled sacrifice.

The maintenance of pregnancy was not adversely affected by Dipterex administration on days 7 through 11 of gestation, but food consumption and maternal weight gain were significantly reduced below the values for both the Cv and CPF groups and the incidence of fetal death, stunted, and mal-

Table 1. Effect of Dipterex administration by gavage (t.i.d.) to C-D rats for various periods of gestation.

	Days 6-15				
	CPF[a]	480 mg/kg-day[b]	Day 8 or 10 Cv[c]	Day 8, 480 mg/kg[b]	Day 10, 480 mg/kg[b]
Females					
Total number	11	34	10	14	14
Mortality (%)	1(9)	3(9)	1(10)	0	0
Number pregnant (%)	8(80)	20(65)[d]	9(100)	13(93)	12(86)
Food intake, g[e]	17.5 ± 0.94	17.4 ± 0.54	24 ± 0.6	21 ± 0.8[f]	23 ± 0.5[f]
Weight gain, g[e]	1 ± 4.4	1 ± 4.7	0.2 ± 2.60	2.0 ± 3.72	—
			0.5 ± 3.01	—	1.5 ± 1.82
Fetuses					
Total number	70	124	105	170	150
Fetal death, % ± SE	14(21 ± 8.6)	71(38 ± 6.5)[g]	1(0.9 ± 0.93)	5(2.6 ± 1.62)	4(2.5 ± 1.96)
Number stunted	2	1	1	0	1
Weight, g	3.4 ± 0.11	2.8 ± 0.11[f]	3.9 ± 0.13	3.9 ± 0.07	3.8 ± 0.06
Number malformed (%)	5(6 ± 4.1)	86(76.4 ± 8.4)[f]	6(6 ± 4.7)	2(1 ± 0.8)	2(2 ± 1.7)

[a] CPF: a group given only the vehicle by gavage on days 6 through 15 of gestation and pair-fed to the corresponding group given Dipterex.
[b] Daily dosage of Dipterex given in mg/kg-day.
[c] Cv: a combination of two groups of rats given only the vehicle by gavage on day 8 or 10 of gestation.
[d] One female had 13 resorptions but no fetuses.
[e] Included the period gavaged plus two more days ($\bar{X}$ ± SE).
[f] $p < 0.05$ compared to controls.
[g] $p = 0.06$.

Table 2. Effect of Dipterex administration by gavage (t.i.d.) to hamsters on days 7 through 11 of gestation.

	C[a]	Cv[b]	100 mg/kg-day	200 mg/kg-day	300 mg/kg-day	400 mg/kg-day	CPF[c]
Females							
Total number (deaths)	33	22	5	25(2)	10	30(3)	16
Number pregnant	31	20	5	22	10	27	16
Food intake, g[d]	10.8 ± 0.48	10.7 ± 0.31	10.8 ± 0.31	10.6 ± 0.22	8.6 ± 0.50[e]	7.0 ± 0.38[e]	7.2 ± 0.47[e]
Weight gain, g[d]	17.1 ± 0.99	17.8 ± 0.95	18.2 ± 2.20	15.5 ± 1.40	9.3 ± 3.14[e]	5.7 ± 2.10[ef]	12.5 ± 1.51[e]
Fetuses							
Total number	339	208	49	238	105	225	181
Number per female	10.9 ± 0.36	10.4 ± 0.42	10.0 ± 0.71	10.8 ± 0.35	10.5 ± 0.70	8.6 ± 0.55[ef]	11.3 ± 0.45
Fetal deaths (no. dead/no. litters)	17/12	10/8	5/2	13/8	16/5	77/19	11/6
Fetal death, %	17(4.9 ± 1.34)	10(4.6 ± 1.46)	5(9.1 ± 4.98)	13(5.0 ± 1.48)	16(12.6 ± 5.08)	77(24.5 ± 4.50)[ef]	11(5.5 ± 2.28)
Number stunted	1	3	0	2	1	12	0
Weight, g	1.90 ± .026	1.89 ± 0.042	2.02 ± 0.075	1.89 ± 0.037	1.75 ± 0.059[e]	1.57 ± 0.047[ef]	1.86 ± 0.040
Number malformed	1	3	1	4	1	13	1
Percent malformed	0.39 ± 0.390	1.29 ± 0.928	2.20 ± 2.200	1.45 ± 0.673	1.00 ± 1.00	8.98 ± 3.947[ef]	0.44 ± 0.440

[a] C: group not gavaged.
[b] Cv: group gavaged (t.i.d.) with distilled water for the days indicated.
[c] CPF: group gavaged and pair-fed to the Dipterex group given 400 mg/kg.
[d] Included period gavaged plus two more days ($\bar{X}$ ± SE).
[e] $p < 0.05$ compared to Cv.
[f] $p < 0.05$ compared to CPF.

formed fetuses was significantly increased among the litters of the Dipterex group (Table 2). Most of the malformations consisted of edema, cleft palate, patagium, and fused ribs (Table 3). The hemimelic fetus had no radius or ulna in the left front limb, and the tibia in the left hind limb was only one-half the length of that in the right hind limb; the distal portion was missing. In addition, this stunted fetus had a cleft palate, bilateral cleft lip, micrognathia, and both fused and doubled thoracic and lumbar centra. The pulmonary trunk of another fetus led from the right ventricle under the aortic arch and inserted into the right subclavian artery instead of into the descending aorta via the ductus arteriosus. The ectrodactyly seen in both the Cv and Dipterex groups consisted of missing thumbs.

Administration of Dipterex on days 7 through 11 of gestation at a dose level of 300 mg/kg-day also resulted in significantly lower food consumption, maternal weight gain, and fetal weight upon comparison to the values for the Cv group (Table 2). Fetal lethality was increased, but the difference from that of the control group was not statistically significant. Only one malformed fetus was seen among the fetuses of the group given Dipterex at 300 mg/kg-day, but it had several severe malformations (Table 3).

In the group given Dipterex at 200 mg/kg-day, two females died between the end of the dosing period and scheduled sacrifice. During the dosing period signs of cholinesterase inhibition, while still present, were not as severe as those seen after administration at 400 mg/kg-day. Additional statistically significant differences were not seen between the 200 mg/kg group and the Cv group (Tables 2 and 3).

After Dipterex administration only on day 8 of gestation at 400 mg/kg-day, maternal food consumption was significantly decreased and fetal lethality was significantly increased upon comparison to the corresponding Cv group (Table 4). None of the remaining criteria studied differed significantly between the two groups.

Mice

Dipterex was administered to mice at 600 mg/kg-day, but even at this dose level overt signs of cholinesterase inhibition were not noted. Four of the females given Dipterex at this level for some period of gestation died before scheduled sacrifice but no more than one occurred in each group. Higher dose levels were not studied because after administration at 600 mg/kg-day for five days both maternal food intake and rate of gain in body weight were significantly reduced (Table 5).

Although maternal food consumption and weight gain were significantly reduced after administration of the highest levels of Dipterex on days 6 through 10 of gestation, adverse effects were not detected on maintenance of pregnancy, fetal survival, or on incidence of stunting (Table 5). Fetal weight was significantly reduced at dose levels of 400 mg/kg-day or more, but the total incidence of developmental malformations was not increased above control values (Tables 5 and 6). Administration of

Table 3. Incidence of alterations among fetuses of hamsters given Dipterex by gavage (t.i.d.) on days 7 through 11 or on day 8 of gestation.

	Days 7 through 11						Day 8		
	C[a]	Cv[b]	100 mg/kg-day	200 mg/kg-day	300 mg/kg-day	400 mg/kg-day	CPF[c]	Cv[b]	400 mg/kg
Number of fetuses/number of litters examined									
External alterations	356/31	218/20	54/5	251/22	121/10	302/27	192/16	161/14	225/22
Visceral alterations	144/31	83/20	21/5	95/22	43/10	119/27	73/16	63/14	96/22
Skeletal alterations	356/31	218/20	54/5	251/22	121/10	302/27	192/16	161/14	225/22
Number of fetuses affected/number of litters affected									
External examination									
Edema						3/2			
Cleft lip						1/1			
Cleft palate					1/1	3/3			
Ectrocardia				1/1					
Micrognathia						1/1			
Microphthalmia		1/1							
Hemimely						1/1			
Ectrodactyly		1/1			1/1	2/1			
Patagium						4/4			
Tailed kinked		1/1							
Hematoma		1/1							
Visceral examination									
Pulmonary trunk displacement						1/1			
Innominate, absent	1/1		1/1						
Pancreas agenesis					1/1				
Spleen agenesis					1/1				
Kidney, small				1/1	1/1	1/1			1/1
Lungs, small					1/1				
Skeletal examination									
Vertebrae centra fused		1/1				1/1			
Vertebrae centra doubled						1/1			
Vertebrae centra misaligned						1/1			
Ribs fused				2/2		2/2		1/1	3/3
Ribs branched							1/1		
Sternebrae fused				1/1		1/1		1/1	
Total	1/1	3/2	1/1	4/4	1/ 1	13/10[d,e]	1/1	1/1	4/4

[a] C: group not gavaged.
[b] Cv: group gavaged (t.i.d.) with distilled water for the days indicated.
[c] CPF: group gavaged and pair-fed to the Dipterex group given 400 mg/kg.
[d] $p < 0.05$ compared to Cv.
[e] $p < 0.05$ compared to CPF.

Dipterex on days 10 through 14 was accompanied by similar maternal and fetal effects, however, the incidence of stunting was significantly increased at the 600 mg/kg-day dose level and at both the 500 and 600 mg/kg-day levels the incidence of development alterations was significantly increased (Table 7). The increase in developmental alterations consisted largely of cleft palates (Table 6); in the 500 mg/kg dose group only one of the cleft palates occurred in fetuses weighing less than 0.75 g, but in the 600 mg/kg dose group three of the five fetuses with cleft palates were stunted and only one weighed more than 0.75 g. In the 300 mg/kg dose group all alterations except the doubled centra occurred in only one fetus.

After administration of Dipterex to mice at 600 mg/kg for three or less days of gestation a statistically significant increase in cleft palate occurred among nonstunted fetuses in the group dosed on

Table 4. Effect of Dipterex administration by gavage (t.i.d.) to hamsters on day 8 of gestation.

	Cv[b,a]	400 mg/kg
Females		
Total number (deaths)	16	23
Number pregnant	14	22
Food intake, g[b]	10.2 ± 0.51	8.9 ± 0.36
Weight gain, g[b]	6.0 ± 1.28	4.1 ± 0.82
Fetuses		
Total number	155	237
Number per female	11.1 ± 0.67	10.8 ± 0.54
Fetal death (no. dead/no. litters)	6/4	18/13
Fetal death, %	6(3.3 ± 1.35)	18(7.1 ± 1.64)[c]
Number stunted	1	2
Weight, g	1.91 ± 0.043	1.87 ± 0.033
Number malformed	1	4
Percent malformed	0.64 ± 0.640	1.76 ± 0.855

[a] Cv-group gavaged (t.i.d.) with distilled water on day 8.
[b] Included day 8 plus two more days ($\overline{X}$ ± SE).
[c] $p < 0.05$ compared to Cv.

Table 5. Effect of Dipterex administration by gavage (t.i.d.) to CD-1 mice on days 6 through 10 of gestation.

	C[a]	Cv[b]	300 mg/kg-day	400 mg/kg-day	500 mg/kg-day	600 mg/kg-day
Females						
Number pregnant	13	16	5	3	4	25
Food intake, g[c]	6.3 ± 0.16	6.2 ± 0.12	5.2 ± 0.20[d]	6.1 ± 0.55	5.4 ± 0.25[d]	5.3 ± 0.11[d]
Weight gain, g[c]	4.6 ± 0.56	3.3 ± 0.44	1.4 ± 0.80	2.1 ± 0.98	1.7 ± 1.06[d]	1.6 ± 0.35
Fetuses						
Total number (%)	144(11.1)	187(11.7)	53(10.6)	39(13.0)	46(11.5)	270(10.8)
Fetal death, %	12.7 ± 3.54	11.6 ± 2.60	3.7 ± 2.26	15.4 ± 1.80	4.4 ± 2.70	9.9 ± 2.00
Number stunted (%)	0	1(0.53)	1(1.89)	0	1(2.17)	1(0.37)
Weight, g	0.89 ± 0.016	0.87 ± 0.012	0.88 ± 0.067	0.74 ± 0.009[d]	0.84 ± 0.027[d]	0.82 ± 0.015[d]
Number malformed (%)	1(1.0 ± 0.96)	1(0.7 ± 0.69)	2(3.1 ± 3.08)	0	1(3.1 ± 3.12)	3(2.1 ± 1.28)

[a] C; group not gavaged.
[b] Cv: group gavaged (t.i.d.) with distilled water for days indicated.
[c] Included the period gavaged plus two more days ($\overline{X}$ ± SE).
[d] $p < 0.05$.

Table 6. Incidence of alterations among the fetuses of CD-1 mice given Dipterex by gavage (t.i.d.) on days 6-10 of gestation.

	Days 6-10						Days 10-14					
	C	Cv	300 mg/kg-day	400 mg/kg-day	500 mg/kg-day	600 mg/kg-day	C	Cv	300 mg/kg-day	400 mg/kg-day	500 mg/kg-day	600 mg/kg-day
Number of fetuses/number of litters examined												
External alterations	144/13	187/16	53/5	39/3	46/4	270/25	135/12	267/23	91/7	44/4	67/6	205/20
Visceral alterations	57/13	67/16	20/5	14/3	16/4	100/25	52/12	101/23	33/7	16/4	27/6	88/20
Skeletal alterations	144/13	187/16	53/5	39/3	46/4	270/25	135/12	267/23	91/7	44/4	67/6	205/20
External examination												
Cleft palate	1/1	1/1				1/1		1/1	1/1		3/2*	5/5*
Exencephaly												1/1
Ablepharia												1/1
Umbilical hernia												1/1
Kinked tail								1/1	1/1			
Clubbed limbs									1/1			1/1
Hematoma			2/1								1/1	
Visceral examination												
Hydrocephaly									1/1			1/1
Testes small							1/1					
Lungs small												
Skeletal examination												
Vertebrae fused arch										1/1		
Vertebrae doubled centra									1/1			
Ribs missing					1/1							
Ribs wavy						2/2						
Sternebrae fused												
Total affected	1/1	1/1	2/1	0	1/1	3/3	1/1	2/1	2/2	1/1	4/3*	7/7*

days 12-14 of gestation. Three fetuses in the group given Dipterex only on day 8 of gestation had several structural alterations, but their incidence did not differ significantly from the Cv group in which three nonstunted litter mates had cleft palates.

Comparison of the females given 10 ml/kg of water t.i.d. (Cv) for various periods of gestation to those not gavaged or handled several times a day during pregnancy (C) revealed no significant change in food consumption, maternal weight gain, maintenance of pregnancy, fetal survival, or fetal development to day 18 of gestation (Tables 5 to 10).

Discussion

In a previous study (*3*), Dipterex was found to be teratogenic in the rat if consumed at dose levels of 432 or 519 mg/kg body weight/day on days 6 through 15 of gestation, but not if given by gavage once daily for the same period even at doses that were lethal to

Table 7. Effect of Dipterex administration by gavage (t.i.d.) to CD-1 mice on days 10 through 14 of gestation.

	C[a]	Cv[b]	300 mg/kg-day	400 mg/kg-day	500 mg/kg-day	600 mg/kg-day
Females						
Mortality					1	1
Number pregnant	12	23	7	4	6	20
Food intake, g[c]	6.8 ± 0.24	6.6 ± 0.16	6.4 ± 0.34	6.2 ± 0.35	6.0 ± 0.22[d]	5.5 ± 0.11[de]
Weight gain, g[c]	11.0 ± 0.59	10.0 ± 0.44	8.6 ± 0.96	7.1 ± 1.01[d]	6.0 ± 0.95[d]	5.3 ± 0.48[de]
Fetuses						
Total number	135(11.2)	267(11.6)	91(13.0)	44(11.0)	67(11.2)	205(10.2)
Fetal death, %	12.9 ± 1.70	9.3 ± 1.64	12.5 ± 7.12	16.8 ± 4.34	13.3 ± 3.79	16.1 ± 2.40
Number stunted (%)	0	0	0	0	0	4(1.95)
Weight, g	0.95 ± 0.028	0.94 ± 0.013	0.86 ± 0.019[d]	0.84 ± 0.041[d]	0.80 ± 0.026[d]	0.80 ± 0.017[de]
Number malformed (%)	1(0.7 ± 0.69)	2(0.8 ± 0.79)	2(2.0 ± 1.33)	1(2.5 ± 2.50)	4(6.5 ± 3.56)[d]	7(4.0 ± 1.28)[d]

[a] C: group not gavaged.
[b] Cv: group gavaged (t.i.d.) with distilled water for the days indicated.
[c] Included the period gavaged plus two more days ($\overline{X}$ ± SE).
[d] $p < 0.05$.
[e] Significant dose response.

Table 8. Effect of Dipterex administration by gavage (t.i.d.) to CD-1 mice for day 8 and days 8-10 of gestation.

	Day 8			Days 8-10		
	C[a]	Cv[b]	600 mg/kg-day	C[a]	Cv[b]	600 mg/kg-day
Females						
Mortality			1			1
Number pregnant	11	13	28	11	14	18
Food intake, g[c]	6.3 ± 0.37	5.7 ± 0.16	5.6 ± 0.11	6.9 ± .24	6.7 ± .33	5.9 ± .18[d]
Weight gain, g[c]	0.54 ± 0.217	0.08 ± 0.224	0.07 ± 0.142	3.0 ± .30	2.4 ± .36	1.4 ± .38
Fetuses						
Total number	122(11.1)	149(11.4)	293(10.5)	121(11.0)	156(11.1)	200(11.1)
Fetal death, %	13.6 ± 2.48	10.7 ± 1.96	18.2 ± 3.02	12.3 ± 1.84	7.8 ± 2.45	8.6 ± 1.82
Stunted, (%)	0	0	2(0.68)	0	0	1(0.50)
Weight, g	0.96 ± 0.023	0.91 ± 0.023	0.88 ± 0.013	0.93 ± 0.037	0.93 ± 0.037	0.86 ± 0.023
Malformed, (%)	0	5(3.5 ± 2.42)	4(2.1 ± 1.26)	1(0.8 ± 0.75)	1(0.9 ± 0.89)	0

[a] C: group not gavaged.
[b] Cv: group gavaged (t.i.d.) with distilled water for the days indicated.
[c] Included the period gavaged plus two more days ($\overline{X}$ ± SE).
[d] $p < 0.05$.

Table 9. Effect of Dipterex administration by gavage (t.i.d.) to CD-1 mice on days 10-12 and days 12-14 of gestation.

	Days 10-12		Days 12-14		
	Cv[a]	600 mg/kg-day	C[b]	Cv[a]	600 mg/kg-day
Females					
Mortality		1			
Number pregnant	2	4	14	16	26
Food intake, g[c]	6.3 ± 0.60	5.0 ± 0.07	7.2 ± 0.31	6.8 ± 0.14	6.1 ± 0.18[d]
Weight gain, g[c]	5.3 ± 1.00	1.2 ± 0.72[d]	6.9 ± 0.82	5.3 ± .59	4.2 ± .47
Fetuses					
Total number	23(11.5)	49(12.2)	156(11.1)	185(11.6)	306(11.8)
Fetal death, %	17.8 ± 3.55	14.3 ± 4.74	15.3 ± 3.92	9.7 ± 2.40	14.1 ± 2.0
Number stunted (%)	0	0	1(0.64)	0	1(0.33)
Weight, g	0.92 ± 0.045	0.82 ± 0.026[d]	0.96 ± 0.021	0.95 ± 0.020	0.86 ± 0.015[d]
Number malformed (%)	0	0	0	1(0.4 ± 0.44)	4(1.2 ± 0.58)

[a] Cv: group gavaged (t.i.d.) with distilled water for the days indicated.
[b] C: group not gavaged.
[c] Included the period gavaged plus two more days ($\overline{X}$ ± SE).
[d] $p < 0.05$.

Table 10. Incidence of alterations among the fetuses of CD-1 mice given Dipterex by gavage (t.i.d.) on day 8, days 8-10, days 10-12, and days 12-14.

	Day 8			Days 8-10			Days 10-12		Days 12-14		
	C[a]	Cv[b]	600 mg/kg	C[a]	Cv[b]	600 mg/kg-day	Cv[b]	600 mg/kg-day	C[a]	Cv[b]	600 mg/kg-day
Number of fetuses/number of litters											
External alterations	122/11	149/13	293/28	121/11	156/14	200/18	23/2	49/4	156/14	185/16	306/26
Visceral alterations	50/11	55/13	117/28	47/11	61/14	80/18	8/2	18/4	61/14	72/16	114/26
Skeletal alterations	122/11	149/13	293/28	121/11	156/14	200/18	23/2	49/4	156/14	185/16	306/26
External examination											
Cleft palate		3/1									4/4[c]
Exencephaly			1/1								
Ablepharia			1/1								
Umbilical hernia											
Kinked tail											
Clubbed limbs											
Hematoma											
Visceral examination											
Hydrocephaly			1/1								
Testes small											
Lungs small			1/1								
Skeletal examination											
Vertebrae fused arch										1/1	
Vertebrae doubled centra											
Ribs missing											
Ribs wavy					1/1						
Sternebrae fused			1/1	1/1							
Total affected	0	3/1	3/3	1/1	1/1	0	0	0	0	1/1	4/4

[a] C: group not gavaged.
[b] Cv: group gavaged (t.i.d.) with distilled water for the days indicated.
[c] $p < 0.05$.

some of the dams (150 to 250 mg/kg-day). In the current study a positive teratogenic response roughly equivalent to that obtained in the former study was achieved by administration of Dipterex by gavage (t.i.d.). By dividing the daily dose into three equal portions, given roughly 3 hr apart, Dipterex could be administered at 480 mg/kg-day on days 6 through 15 of gestation without causing a significant increase in maternal lethality. Use of a pair-fed control group demonstrated that the teratogenicity seen was not due to decreased food intake during the dosing period or to the stress of extended t.i.d. administration. The positive teratogenic response was not obtained if Dipterex was given (t.i.d.) for only one day of gestation (on day 8 or 10). Hence, the positive teratogenicity seen after administration of this chemical with a transient pharmacologic action also could be demonstrated after administration by gavage, provided the daily dose was given in fractions. This is an important consideration for those responsible for determining the teratogenic potential of environmental chemicals.

For the hamster, the maximum dose of Dipterex tolerated by gavage (t.i.d.) on days 7 through 11 of gestation was 400 mg/kg-day. A positive teratogenic response was obtained at this dose level which included edema, cleft palates, digital malformations, fused ribs, and even one case of the rarely seen absence or shortening of the long bones of the extremities. The incidence of malformed fetuses per litter was not as high as seen in the rat but the spectrum of malformations obtained was very similar in both species. Again comparison to a pair-fed control group revealed that the malformations were not merely due to reduced food intake. Statistically, the apparent "no-effect" dose was 200 mg/kg-day, in that significant differences from the control group were not obtained in the dams or their offspring for the criteria under study. Embryotoxicity, but not teratogenicity, was evident after administration of Dipterex at 400 mg/kg-day by gavage (t.i.d.) on day 8 of gestation. Fused ribs were seen, but they occurred in three littermates among the 22 litters exposed as well as in one fetus from the control group. Hence, Dipterex had to be given by gavage for more than one day to demonstrate its teratogenicity in the hamster.

The mouse was less susceptible to Dipterex than the rat or hamster upon consideration of the dosage administered without display of cholinesterase inhibition. The specific reason for this species differ-

ence was not ascertained in the present study, but the trend also was evident in the offspring. A positive teratogenic response was obtained at the highest doses tested but only when the Dipterex was given on days 10 through 14 or 12 through 14 of gestation. Dipterex related malformations consisted mostly of cleft palates present in one-quarter to one-sixth of the litters exposed, respectively.

Therefore, the teratogenicity of Dipterex was not unique to the rat but was present also in the hamster and to a lesser extent in the mouse. In each species adverse effects on the conceptus occurred only at doses that decreased maternal food consumption, however, at least in the rat and hamster, the teratogenicity was not merely due to the decreased level of food consumed.

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