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DATA EVALUATION RECORD

TRICHLORFON

Teratogenic Evaluation of Orally Administered Trichlorfon in the Rat, Hamster, and Mouse

CITATION: Staples RE, Goulding EH. 1979. Dipterex teratogenicity in the rat, hamster, and mouse when given by gavage. Environmental Health Perspectives, 30:105-113.

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DATA EVALUATION RECORD

STUDY TYPE: Teratogenic evaluation of orally administered trichlorfon in the rat, hamster, and mouse.

CITATION: Staples RE, Goulding EH. 1979. Dipterex teratogenicity in the rat, hamster, and mouse when given by gavage. Environmental Health Perspectives, 30:105-113.

ACCESSION NUMBER: Not available.

MRID NUMBER: Not available.

LABORATORY: Environmental Toxicology Branch, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, North Carolina 27709.

TEST MATERIAL: Trichlorfon (Dipterex, 0,0-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate). Supplied by Chemagro, Inc., Kansas City, Missouri. Purity not stated.

PROTOCOL:

1. Trichlorfon, referred to as Dipterex, and consisting of 0,0-dimethyl-1-hydroxy-2,2,2-trichloroethyl phosphonate was studied for its teratogenic potential. The trichlorfon was supplied by Chemagro, Inc., Kansas City, Missouri. The purity and a physical description of the test material was not provided.

2. Three species of test animals were utilized on study as follows:

   a. Nonparous female CD rats.

   b. Nonparous female golden hamsters supplied from Lakeview Farms, New Jersey.

   c. Nonparous female CD-1 mice.

   The number of animals per treatment group is provided in Table 1.

3. The animals were orally administered trichlorfon. The animals were administered 10 ml/kg of the appropriate dose solutions. The vehicle for the rats was 0.5 percent methylcellulose. Distilled water was the vehicle for the hamsters and the mice. One-third the total daily

dosage volume was administered three times per day. This procedure was done to minimize acute maternal toxicity. The times of administration were not specified for the rats. The times of administration were 0900, 1230, and 1600 hours daily for the hamsters and mice. The dose levels and days of administration per group are provided in Table 1.

4. No information pertaining to the recording of clinical observations, body weight, or food consumption data was provided for the rats. The rats were sacrificed on day 21 of gestation by carbon dioxide asphyxiation followed by cervical dislocation. Food consumption was measured daily and maternal body weights were recorded on days 3, 7, 9, 11, 13, and 15 of gestation in the hamsters. The report does not mention clinical observations on the hamsters. The hamsters were sacrificed by carbon dioxide asphyxiation on day 15 of gestation. Maternal body weights for the mice were recorded on day 1 of gestation, the first day of trichlorfon administration, every second day during dosing, the second day after cessation of dosing, and the day of sacrifice. The mice were sacrificed on day 18 of gestation by carbon dioxide asphyxiation followed by cervical dislocation. All animals were caesarean sectioned and the number and placement of implantation sites recorded. Each site was recorded as alive or dead and to its general condition [state of resorption]. Live fetuses were individually weighed, internally and externally sexed, and externally examined. At least one-third of the fetuses in each litter, all stunted fetuses, and fetuses observed to have external malformations were dissected and observed for visceral alterations; the heads were fixed in Bouin's solution and sliced for examination to reveal abnormalities and the decapitated carcasses were stained and evaluated for skeletal abnormalities [Staples technique]. The remaining fetuses were stained and the entire specimen was examined for skeletal abnormalities. The methodology for preparing the specimens for skeletal evaluation was not stated. Stunted fetuses were defined as those weighing 2.0 g or less for rats, 1.0 g or less for hamsters, 0.5 g or less for mice, and those weighing less than two-thirds the mean of the remaining litter-mates.

5. The data were analyzed by a one-sided Mann-Whitney test at significance levels of 5 percent ($p \leq 0.05$).

RESULTS:

I. Rats -- The authors reported that signs of cholinesterase activity were observed after each Dipterex administration. The symptoms exhibited, and the number of animals exhibiting the symptoms, were not stated. Three of 34 rats receiving 480 mg/kg Dipterex from days 6-15 of gestation died between days 13 and 15 of gestation, apparently from Dipterex toxicity. The gestation index (number of females with live fetuses/number impregnated) was reduced in this group compared to the pair-fed vehicle controls. The gestation indices were 80 percent in the vehicle control and 65 percent in the

TABLE 1. Summary of Test Groups

Group No.[a]	Species	No. of Animals	Dose Level (mg/kg/day)	Compound Administration Day(s) of Gestation
1	Rat	11	Vehicle-pair-fed	6-15
2	Rat	34	480	6-15
3	Rat	10	Vehicle	8 or 10[b]
4	Rat	14	480	8
5	Rat	14	480	10
6	Hamster	33	Neg. control	7-11
7	Hamster	22	Vehicle	7-11
8	Hamster	5	100	7-11
9	Hamster	25	200	7-11
10	Hamster	10	300	7-11
11	Hamster	30	400	7-11
12	Hamster	16	Pair-fed control	7-11
13	Hamster	16	Vehicle	8
14	Hamster	23	400	8
15	Mouse	13	Neg. control	6-10
16	Mouse	16	Vehicle	6-10
17	Mouse	5	300	6-10
18	Mouse	3	400	6-10
19	Mouse	4	500	6-10
20	Mouse	25	600	6-10
21	Mouse	12	Neg. control	10-14
22	Mouse	23	Vehicle	10-14
23	Mouse	7	300	10-14
24	Mouse	4	400	10-14
25	Mouse	6	500	10-14
26	Mouse	20	600	10-14
27	Mouse	11	Neg. control	8
28	Mouse	13	Vehicle	8
29	Mouse	28	600	8
30	Mouse	11	Neg. control	8-10
31	Mouse	14	Vehicle	8-10
32	Mouse	18	600	8-10
33	Mouse	2	Vehicle	10-12
34	Mouse	4	600	10-12
35	Mouse	14	Neg. control	12-14
36	Mouse	16	Vehicle	12-14
37	Mouse	26	600	12-14

[a] Group numbers assigned by Dynamac.

[b] Concurrent vehicle control for rats dosed on day 8 or day 10 of gestation.

Dipterex group. The gestation indices for the rats receiving 480 mg/kg Dipterex on either day 8 or day 10 of gestation were comparable to the concurrent vehicle control. Weight gains in all groups ranged from 0.2 to 2.0 g. [The authors do not specify if the reported weight gains are daily for the period of dosing. They do not appear consistent with weight gains expected of pregnant rats. The vehicle control weight gain was 0.2 g ± 2.60.] The fetal data are presented in Table 2.

TABLE 2. Effects of Dipterex on Rat Fetuses

	Days 6-15		Day 8 or 10	Day 8	Day 10
	Pair-fed Control	480 mg/kg/day	Vehicle	480 mg/kg/day	480 mg/kg/day
Total number	70	124	105	170	150
Fetal death	14	71	1	5	4
Number stunted	2	1	1	0	0
Weight (g ± S.E.)	3.4 ± 0.1	2.8 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.1
Number malformed	5	86	6	2	2

A significant increase in fetal deaths compared to the controls was observed in litters exposed to Dipterex from days 6-15 of gestation. No effect on fetal death was observed in litters exposed to Dipterex on day 8 or day 10 of gestation. Fetal body weight was significantly decreased in the group receiving Dipterex from days 6-15 of gestation when compared to its control group. The fetal body weights of the groups administered Dipterex once were comparable to the vehicle control group. Nineteen of 19 litters exposed to Dipterex from days 6-15 of gestation contained malformed fetuses. Seventy-six percent of the fetuses were malformed compared to 6 percent in the control group. Three of eight pair-fed vehicle control litters had malformed fetuses. The malformations observed in the fetuses exposed to Dipterex throughout organogenesis consisted 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 pair-fed vehicle control group had "two fetuses with wavy ribs and another with doubled thoracic centra." [The number of malformed pair-fed control fetuses described in the text (3) does not compare with the number reported

in Table 1 of the article (5).] No data on the types of malformations, litters with malformed fetuses, or number of fetuses with a given malformation were reported for the groups receiving Dipterex on either day 8 or 10 of gestation. The number of malformed fetuses were comparable however, between the vehicle control and single exposure Dipterex groups.

II. Hamsters — Hamsters administered 400 mg/kg Dipterex from days 7-11 of gestation exhibited excessive salivation, protruding eyes, tremors, and loss of equilibrium within 10 minutes after intubation. Recovery occurred within 24 hours in an unspecified number of the animals. One of the high dose hamsters died after one administration of Dipterex and two died after three days of dosing. Two animals at 200 mg/kg died of unspecified causes. No deaths occurred at 300 mg/kg or in the control groups. The observed clinical signs of Dipterex toxicity among the hamsters receiving 300 mg/kg/day or less of Dipterex were not described; however, they were present at 200 mg/kg. The gestation indices for all Dipterex-treated groups were similar to the control groups. The daily weight gain during dosing was significantly reduced among the 300 mg/kg (9.3 g) and 400 mg/kg (5.7 g) groups when compared to the vehicle control females (17.8 g). This weight gain for the 400 mg/kg dose level was also significantly reduced when compared to its pair-fed controls (12.5 g). Food consumption was significantly reduced at the 300 and 400 mg/kg dose levels (8.6 g/day and 7.0 g/day, respectively) when compared to the vehicle controls (10.7 g/day). The number of live fetuses/litter was significantly reduced at the 400 mg/kg dose level (8.6) when compared to either the vehicle control (10.4) or pair-fed controls (11.3). Fetal deaths were significantly increased at 400 mg/kg when compared to the vehicle or pair-fed controls. Seventy-seven dead fetuses were found in 19 of 27 400 mg/kg litters. Ten vehicle control fetuses in 8 of 20 litters and 11 pair-fed control fetuses in 6 to 16 litters were dead. Fetal body weights were significantly reduced at the 300 mg/kg (1.75 g) and 400 mg/kg (1.57 g) when compared to the vehicle controls (1.89 g). The fetal body weights at 400 mg/kg were significantly less than the fetal body weights of the pair-fed controls (1.86 g). The incidence of fetal malformations is presented in Table 3. A significant increase in the number of litters with malformed fetuses and the number of malformed fetuses was observed at 400 mg/kg. Edema, cleft palate, and skin folds from the limbs to the torso ["patagium", specific limbs not stated] were the most frequently occurring malformations among the 400 mg/kg dose level.

No description of clinical signs of toxicity is given for hamsters administered 400 mg/kg Dipterex on day 8 of gestation. The body weight gain (4.1 g) and daily food consumption (8.9 g/day) were less than those of the vehicle control group (6.0 g and 10.2 g/day, respectively). The gestation index for the two groups were comparable. No difference in the number of live fetuses/litter between the 400 mg/kg (10.8) and vehicle controls (11.1) was

TABLE 3. Incidence of Malformations Among Fetuses of Hamsters given Dipterex by Gavage on Days 7 through 11 or on Day 8 of Gestation

	Days 7 through 11							Day 8	
	Negative Control	Vehicle Control	100 mg/kg/day	200 mg/kg/day	300 mg/kg/day	400 mg/kg/day	Pair-fed Control[a]	Vehicle Control	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	
Total	1/1	3/2	1/1	4/4	1/1	13/10[b,c]	1/1	1/1	4/4

[a] Group gavaged and pair-fed to the Dipterex group given 400 mg/kg.

[b] $p<0.05$ compared to vehicle control.

[c] $p<0.05$ compared to pair-fed control.

observed; however, the number of dead fetuses at 400 mg/kg was significantly greater. Eighteen dead fetuses in 13 of 22 litters was observed compared to 6 dead fetuses in 4 of 14 vehicle control litters. The incidence of fetal malformations is presented in Table 3. One 400 mg/kg fetus had small kidneys and three fetuses from three separate 400 mg/kg litters had fused ribs. One fetus with fused ribs was the only malformation observed in the vehicle control group.

III. Mice — No Dipterex-related abnormal clinical observations were reported for any of the dose regimens. The mice were not dosed throughout organogenesis; however, maternal toxicity was observed at dose levels of 500 and 600 mg/kg/day from days 6–10, 10–14, 8–10, 10–12, or 12–14 of gestation. The maternal toxicity consisted of reduced body weight gain and/or food consumption. Reduced body weight gain was also observed among the dams receiving 400 mg/kg Dipterex from days 10–14 of gestation. No increase in fetal death or the number of stunted fetuses was observed at any dose level regardless of the period of Dipterex administration. Fetuses exposured to 400, 500, or 600 mg/kg Dipterex from days 6 to 10 of gestation had significantly reduced weights when compared to the vehicle control fetuses. Fetuses exposed to Dipterex from days 10–14 of gestation had significantly reduced body weights at dose levels of 300, 400, 500, and 600 mg/kg. Fetal body weights were significantly reduced at 600 mg/kg when dosing occurred on days 10–12 of gestation. No differences in fetal body weight were observed at 600 mg/kg when exposure occurred on day 8 or days 8–10 of gestation. The incidences of fetal malformations are presented in Tables 4 and 5. The number of litters with malformed pups, number of malformed pups, and the number of malformations were comparable at all dose levels to the controls when Dipterex was administered on days 6–10, day 8, days 8–10, or days 10–12 of gestation. Significant increases in the number of litters with fetuses having cleft palate and the number of fetuses with cleft palate were observed at the 500 and 600 mg/kg dose levels when Dipterex was administered from days 10–14 of gestation and at 600 mg/kg when Dipterex was administered from days 12–14 of gestation.

CONCLUSIONS:

I. Rats — Acute maternal toxicity including death was evident at 480 mg/kg. Administration of Dipterex throughout organogenesis reduced the ability of the dam to maintain pregnancy and produced increased fetal death with a decrease in fetal body weight. Although administration of Dipterex at 480 mg/kg throughout organogenesis produced signs of reproductive toxicity and was fetotoxicity, the presence of maternal toxicity and lack of lower dose levels prevents this reviewer from determining if the reproductive and fetal toxicity observed was a direct result of

TABLE 4. Incidence of Malformations Among Fetuses of CD-1 Mice given Dipterex by Gavage on Days 6-10 or 10-14 of Gestation

	Days 6-10						Days 10-14					
	Negative Control	Vehicle Control	300 mg/kg/day	400 mg/kg/day	500 mg/kg/day	600 mg/kg/day	Negative Control	Vehicle Control	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 alteration	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 alteration	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*

*$p \leq 0.05$ compared to vehicle control.

TABLE 5. Incidence of Malformations Among Fetuses of CD-1 Mice given Dipterex by Gavage on Day 8, Days 8-10, Days 10-12, and Days 12-14 of Gestation

	Day 8			Day 8-10			Days 10-12		Days 12-14		
	Negative Control	Vehicle Control	600 mg/kg/day	Negative Control	Vehicle Control	600 mg/kg/day	Vehicle Control	600 mg/kg/day	Negative Control	Vehicle Control	600 mg/kg/day
Number of fetuses/number of litters											
External alterations	122/11	149/13	293/28	121/11	156/14	200/18	23/1	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	12/11	156/14	200/18	23/2	49/4	156/14	185/16	306/26
External examination											
Cleft palate		3/1									4/4[a]
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] $p<0.05$ compared to vehicle control.

Dipterex activity or secondary to maternal toxicity. Administration of 480 mg/kg Dipterex throughout organogenesis produced severe teratogenic effects in the fetus. Despite the severity of the malformations produced and their widespread occurrence, the presence of acute maternal toxicity in the only dose level tested does not allow for the distinction between Dipterex-related and maternal stress-related terata. A single exposure to 480 mg/kg Dipterex on either day 8 or day 10 of gestation produced no indications of reproductive toxicity or teratogenicity.

II. Hamsters -- The authors reported clinical signs of organophosphate toxicity at dosages of 200 mg/kg/day and greater. The symptoms reported at 400 mg/kg/day were indicative of severe acute organophosphate toxicity. Decreased food consumption and maternal body weight gain were observed at 300 and 400 mg/kg/day. These data indicate that Dipterex was acutely toxic to the pregnant hamster with a LEL of 200 mg/kg and a NOEL of less than 200 mg/kg. A dose level of 100 mg/kg/day contained only 5 animals at which no signs of maternal toxicity were noted.

Increased fetal death with a concurrent decrease in the number of live fetuses/litter was detected at 400 mg/kg/day when Dipterex was administered over the period of organogenesis (days 7-11 of gestation). Decreased fetal body weights were observed at the 300 and 400 mg/kg/day dose levels. The reproductive parameters from the controls pair-fed to the 400 mg/kg/day treatment group indicated that the fetal effects produced were not resultant from decreased food consumption. Based on the data reported, the LEL for reproductive toxicity (fetal toxicity) in the hamster is 300 mg/kg and the NOEL is 200 mg/kg.

Major malformations were detected in fetuses from dams exposed to 400 mg/kg/day of Dipterex during organogenesis. The dams at this dose level were exhibiting symptoms of severe organophosphate intoxication which may have enhanced the teratogenic capacity of Dipterex. The malformation data from the pair-fed controls indicate that the fetal malformations did not result from decreased food intake. The occurrence of cleft palate in three of 27 400 mg/kg litters and in one of 10 300 mg/kg litters indicates that at least cleft palate is produced by a teratogenic action of Dipterex; however, the small number of litters at 300 mg/kg and the lack of a dose-response relationship to other malformations or total malformations prohibit a definitive conclusion on teratogenicity. [However, coupled with the cleft palate findings in mice, seems to indicate that Dipterex is teratogenic in hamsters].

III. Mice -- The failure to administer Dipterex to the mice throughout the entire period of organogenesis (days 6-14 of gestation) prevents this reviewer from making conclusions regarding LEL's and NOEL's. Administration of Dipterex at 500 and 600 mg/kg/day from days 6-10, 8-10, 10-12, or 12-14 of gestation produced maternal toxicity in the mouse. Dose levels of 400, 500, and 600 mg/kg/day produced maternal toxicity when administered from days 10-14 of gestation.

Administration of Dipterex at levels of 400 mg/kg/day from days 6-10 of gestation or at levels of 300 mg/kg/day from days 10-14 of gestation reduced fetal body weight. Exposure to Dipterex for shorter periods of time produced reduced fetal body weights at 600 mg/kg/day. These data indicate that Dipterex is a reproductive hazard (fetotoxic) in the mouse.

Administration of Dipterex to the pregnant mouse prior to day 10 of gestation produced no indications of teratogenicity at dose levels of 600 mg/kg or less. Dipterex was teratogenic when administered at day 10 of gestation or later during organogenesis. Administration of 500 and 600 mg/kg/day on days 10-14 of gestation or 600 mg/kg/day on days 12-14 of gestation produced cleft palates in mouse fetuses.

CORE CLASSIFICATIONS:

I. Rat — Supplemental
The following deficiencies were noted:

- o Only one dose level (480 mg/kg) of Dipterex was administered.
- o The dose level administered was acutely toxic and produced maternal death.

II. Hamster — Minimum

III. Mouse — Supplemental
The following deficiencies were noted:

- o No mice were dosed throughout the entire period of organogenesis (Days 6-14 of gestation).
- o Many of the treatment groups contained less than 20 pregnant females.