GS0104 - 139

DATA EVALUATION RECORD

TRICHLORFON

Reproduction and Teratogenic Evaluation in the Mouse of Two Trichlorfon Analogs Administered by Intraperitoneal Injection

CITATION: Scheufler H, Dedek W, Schreider P. 1976. The effect of large doses of demethyltrichlorfon and ethyltrichlorfon on the embryonic development of laboratory mice. Z. Gesamte. Hyg. 22(8):565-569.

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DATA EVALUATION RECORD

STUDY TYPE: Reproduction and teratology study in mice intraperitoneally administered demethyltrichlorfon and ethyltrichlorfon.

CITATION: Scheufler H, Dedek W, Schreider P. 1976. The effect of large doses of demethyltrichlorfon and ethyltrichlorfon on the embryonic development of laboratory mice. Z. Gesamte. Hyg. 22(8):565-569.

ACCESSION NUMBER: Not available.

MRID NUMBER: Not available.

LABORATORY: Biological Institute in the Area Medicine at Martin Luther University. Halle-Wittenberg, Germany.

TEST MATERIAL: I. Demethyltrichlorfon, purity and source not stated. II. Ethyltrichlorfon, purity and source not stated. (Demethyltrichlorfon is a mammalian metabolite of trichlforfon. Ethyltrichlorfon is an analog of trichlorfon).

PROTOCOL:

1. Demethyltrichlorfon and ethyltrichlorfon were studied for their reproductive toxicity and teratogenic potential. Demethyltrichlorfon [O-methyl-(1-hydroxy-2,2,2-trichloroethyl)phosphonate] is a mammalian metabolite of trichlorfon and was utilzed as the sodium salt. Ethyltrichlorfon [O,O-diethyl-(1-hydroxy-2,2,2-trichloroethyl)phosphonate] is structurally related to trichlorfon. The purity and source of the two test materials were not stated.

2. Pregnant mice of the AB Jena-Halle, C57B1, and DBA strains were used as test systems. The number of mice utilized per group is provided in Table 1.

3. Both test materials were administered by intraperitoneal injection. The test materials were dissolved in a 0.9 percent sodium chloride solution and administered at a dose volume of 0.1 ml. The dose levels and days of administration are provided in Table 1. The animals were dosed at approximately the same time each day. [ip injection is an unnatural route of exposure and does not simulate the most probable route of exposure in humans].

TABLE 1. Summary of Test Groups

Group No.	Strain	No. of Mice	Dose Level (mg/kg)	Administration (Days of gestation)
Demethyltrichlorfon				
1	AB J-H	13	240	9
2	AB J-H	23	120	1-14
3	AB J-H	18	240	1-7
4	AB J-H	27	120	7-14
5	C57B1	26	150	1-14
6	C57B1	23	150	7-14
7	DBA	14	120	1-14
8	DBA	23	240	7-14
Ethyltrichlorfon				
1	AB J-H	13	240	9
2	AB J-H	24	120	1-14
3	AB J-H	15	120	7-14
4	DBA	21	240	9
5	DBA	16	120	1-14
6	DBA	16	240	7-14
7	DBA	10	120	7-14
Concurrent Controls				
1	AB J-H	29	Negative Control	--
2	AB J-H	27	Vehicle	1-14
3	DBA	65	Negative Control	--
4	DBA	57	Vehicle	1-14
5	C57B1	35	Negative Control	--
6	C57B1	19	Vehicle	1-14

4. The authors do not state if the dams were clinically observed or if body weights and food consumption were recorded during gestation. The animals were sacrificed on day 18 of gestation by an unspecified method. The number of corpora lutea, and live and dead fetuses were recorded. [Dead fetuses appears to include resorptions.] The live fetuses were weighed. All fetuses were examined for external malformations, macerated with KOH, stained with alizarin red, and examined for skeletal malformations. No visceral examinations were conducted.

5. A formula given by Haseloff and Hoffmann (Haseloff OW, Hoffmann HJ. 1965, Textbook of Statistics, Walter de Gruyter and Co., Soviet Occupied Berlin) was used to calculate significance. The formula was chosen as it allows comparison of percentages. The authors combined the data from the treated groups of each test article and compared the combined calculations of the treated groups against the controls. The authors stated that this procedure was used because they were interested only in determining if the test articles were active.

RESULTS:

I. Demethyltrichlorfon--Results are summarized in Table 2. Administration of demethyltrichlorfon did not affect the percentage of the AB Jena-Halle or DBA mice that were pregnant. [This measurement may be reflective of mating success and not the activity of the test article as it was not stated by the authors if this parameter was the number of mated mice that were found pregnant at sacrifice or the number of mice impregnated.] However, the percentages of pregnant C57B1 mice administered the compound were reduced. The number of implantation sites in all strains of mice administered demethyltrichlorfon from days 1-7 or 1-14 of gestation [encompassing implantation] were comparable to their respective controls and treatment groups not dosed until days 7-14 (after implantation). The number of resorptions per litter was increased in the AB Jena-Halle strain at both dose levels and all durations of administration when compared to the negative and vehicle controls. No increases in the number of resorptions were observed in the DBA and C57B1 strains when compared to their control groups.

The numbers of live fetuses per litter were comparable to the controls in the demethyltrichlorfon treated groups of the C57B1 and DBA strains. The number of live AB Jena-Halle fetuses was reduced at 240 mg/kg when administered from day 1-7 of gestation. The number of live fetuses per litter was comparable to the vehicle control in the three remaining demethyltrichlorfon treated groups. This is misleading because of the variation between the treated and control groups in the number of implantation sites. A comparison of the ratio of live fetuses to implantation sites indicates that this ratio was reduced at 240 mg/kg on day 9 of gestation (0.79) and 120 mg/kg on days 7-14 of gestation (0.80) when compared to the vehicle controls (0.95). This ratio for the 120 mg/kg group dosed from days 1-14 of gestation (0.90) was comparable to the vehicle controls. Fetal body weights were comparable in the DBA and

TABLE 2. Effect of Demethyltrichlorfon on the Prenatal Mouse

Dose Level	Gestation Days of Administration	Percent Pregnant	No. of Corpora Lutea	No. of Implantations	No. of Live Fetuses	No. of Resorptions	Post-Implantation Losses (Percent)	Total Losses (Percent)	Fetal Weight(g)
I. AB Jena-Halle Strain									
Neg. Control	-	74	11.6	10.2	9.5	0.5	6.4	14.0	1.24
Veh. Control	1-14	74	12.9	9.5	9.0	0.5	4.7	29.6	1.60
240 mg/kg	9	92	11.6	10.6	8.4	2.2	20.5	27.3	1.13
120 mg/kg	1-14	61	10.9	9.9	8.9	1.1	10.0	18.4	1.14
240 mg/kg	1-7	94	12.3	9.2	7.1	2.1	22.9	42.1	1.08
120 mg/kg	7-14	81	11.9	10.8	8.6	2.1	20.7	28.0	1.15
II. C57B1 Strain									
Neg. Control	-	80	9.4	8.1	6.4	1.7	21.2	31.9	0.86
Veh. Control	1-14	89	8.4	7.9	6.6	1.2	20.0	24.6	0.90
150 mg/kg	1-14	65	8.5	7.9	7.0	0.9	11.8	17.7	0.90
150 mg/kg	7-14	70	8.5	7.5	6.3	1.1	16.6	26.6	0.86
III. DBA Strain									
Neg. Control	-	77	9.0	7.7	7.0	0.7	10.1	22.6	1.14
Veh. Control	1-14	61	10.0	7.5	6.3	1.1	15.7	17.1	1.08
120 mg/kg	1-14	59	9.4	8.9	7.9	1.0	11.3	16.0	1.09
240 mg/kg	7-14	83	8.3	8.2	7.3	0.9	11.4	12.1	1.11

C57B1 strains between all demethyltrichlorfon and control groups. All of the AB Jena-Halle groups treated with demethyltrichlorfon had reduced fetal body weights when compared to the negative controls. The fetal body weight for the vehicle control group appears exceptionally heavy and may represent a typographical error in the article. No data were presented on fetal malformations. The authors stated that the "DBA mice showed an increased number of fetuses with ectopias [heart outside of the thoracic cavity] that were extremely rare in the controls. The small number of these malformations, however, does not permit a final judgement."

II. Ethyltrichlorfon--Results are summarized in Table 3. The percentage of pregnant AB Jena-Halle mice treated with 120 mg/kg of the test article was comparable to the controls. This percentage was reduced in the group receiving 240 mg/kg. The percentage of pregnant DBA mice varied greatly between groups ranging from 86 percent in the 240 mg/kg group dosed on day 9 of gestation to 44 percent at the same dose level but with compound administration from days 7-14 of gestation. [Please refer to the comment under demethyltrichlorfon results regarding the percentage of pregnant mice]. Among the AB Jena-Halle mice exposed to ethyltrichlorfon prior to or during implantation (day 6 of gestation) the number of implantation sites was fewer than the vehicle control. This is artifactual due to fewer corpora lutea in the 120 mg/kg dose level animals dosed on days 1-14. A comparison of the ratio of implantation sites to corpora lutea for the 120 mg/kg dose group receiving the test article on days 1-14 (0.69) to the vehicle control group (0.74) indicated no difference.

Among the AB Jena-Halle mice injected with 240 mg/kg of ethyltrichlorfon on day 9 of gestation, the number of resorptions and live fetuses was comparable to the controls; however, the fetal body weight was reduced. Increased incidence of resorptions with a decreased number of live fetuses was observed at 120 mg/kg with both dosing regimens. Fetal body weights were also reduced compared to the controls.

Among the DBA mice, increased resorptions and decreased live fetuses were observed when the compound- treated groups were compared to the controls with the exception of the DBA mice treated with 120 mg/kg ethyltrichlorfon on days 7-14 of gestation. The reproductive parameters of this group that were examined were all comparable to the controls. Among the remaining three ethyltrichlorfon treated groups, no dose-response relationship was evident. The ratio of live fetuses to implantations was greater in the 240 mg/kg group dosed from days 7-14 (0.69) than in the group dosed at 240 mg/kg on day 9 (0.58). This ratio was comparable between the groups treated with 240 mg/kg of ethyltrichlorfon on days 7-14 of gestation and the group receiving 120 mg/kg on days 1-14 of gestation (0.79 and 0.71, respectively). The fetal body weights of all DBA mouse groups were comparable. No data on malformations were provided for ethyltrichlorfon with the exception of the percentage of DBA mice with "encephalias" [exact meaning unknown] and ectopias. At 240 mg/kg, 9.1 percent of the fetuses exposed on day 9 had these malformations and the percentage was not given for fetuses exposed from days 7-14 of gestation. At 120 mg/kg, 2.4 percent of the fetuses exposed on days 1-14 were malformed, while 4.0 percent of these exposed on days 7-14 were malformed. The authors state that such

TABLE 3. Effect of Ethyltrichlorfon on the Prenatal Mouse

Dose Level	Gestation Days of Administration	Percent Pregnant	No. of Corpora Lutea	No. of Implantations	No. of Live Fetuses	No. of Resorptions	Post-Implantation Losses (Percent)	Total Losses (Percent)	Fetal Weight(g)
I. AB Jena-Halle Strain									
Neg. Control	-	74	11.6	10.2	9.5	0.5	6.4	14.0	1.24
Veh. Control	1-14	74	12.9	9.5	9.0	0.5	4.7	29.6	1.60
240 mg/kg	9	62	13.8	11.3	10.4	0.9	2.8	24.6	1.06
120 mg/kg	1-14	67	10.7	7.4	4.4	2.8	41.5	50.7	1.04
120 mg/kg	7-14	67	13.2	10.5	7.4	3.0	29.5	43.9	1.03
II. DBA Strain									
Neg. Control	-	77	9.0	7.7	7.0	0.7	10.1	22.6	1.14
Veh. Control	1-14	61	10.0	7.5	6.3	1.1	15.7	17.1	1.08
240 mg/kg	9	86	10.4	8.4	4.9	3.6	42.1	53.2	1.00
240 mg/kg	7-14	44	7.8	7.0	4.8	2.2	31.0	37.6	1.09
120 mg/kg	1-14	75	10.1	8.4	6.0	2.4	28.8	40.9	1.08
120 mg/kg	7-14	64	10.4	8.0	7.1	1.4	16.7	31.5	1.07

malformations occur in DBA control mice in less than 1 percent of the fetuses. No data on malformation in the control animals used in the study were given.

CONCLUSIONS:

I. Demethyltrichlorfon--Three different strains of mice (AB Jena-Halle, C57B1, and DBA) were intraperitoneally administered demethyltrichlorfon at doses of 120 to 240 mg/kg on day 9 of gestation or through prolonged periods of gestation.

The ommission of data on maternal toxicity and malformations prevents this reviewer from reaching conclusions on the maternal toxicity or teratogenicity of demethyltrichlorfon. The data on reproductive parameters indicated that demethyltrichlorfon was not a reproductive hazard to the DBA or C57B1 strains of mice under the conditions tested. The demethyltrichlorfon produced reproductive toxicity at 240 mg/kg with exposures of one day (day 9 of gestation) and at 120 mg/kg with exposure during days 1-14 and 7-14 of gestation. The toxicity was expressed as increased resorptions and decreased numbers of live fetuses with reduced body weights.

II. Ethyltrichlorfon--Two strains of mice (AB Jena-Halle and DBA) were administered ethyltrichlorfon intraperitoneally at 120 and 240 mg/kg on day 9 of gestation or through prolonged periods of gestation.

The ommission of data on maternal toxicity prevents any conclusions on the maternal toxicity of ethyltrichlorfon. In the AB Jena-Halle strain, administration of 240 mg/kg on day 9 produced no effects on embryo survival but did produce reduced fetal body weights. Ethyltrichlorfon produced reproductive toxicity in this strain at 120 mg/kg when administered from days 7-14 or 1-14 of gestation. Increased resorptions with decreased numbers of live fetuses were observed. The body weights of the fetuses were reduced. The reproductive data presented for the DBA mice is conflicting in nature and prevents definitive conclusions on the reproductive toxicity in DBA mice. More adverse reproductive effects (increased resporptions and fewer live fetuses) were observed in litters exposed to 240 mg/kg on day 9 of gestation than in litters exposed to 240 mg/kg from days 7-14 of gestation.

The data on the teratogenicity of ethyltrichlorfon, when provided, was insufficient to provide definite conclusions. The data provided for the DBA mice (percentage of malformed fetuses) suggested that the test article may have been teratogenic in that strain.

CORE CLASSIFICATION:

I. Demethyltrichlorfon--Supplemental. The following deficiencies were noted:

- o No data on maternal toxicity or teratogenicity were provided.
- o The compound was administered by intraperitoneal injection. This is an unnatural route of exposure and does not simulate the most probable route of exposure in humans.
- o No visceral examinations were performed on the fetuses.
- o The dose levels and duration of dosing were inconsistent and variable within a given strain. This prevented the determination of any dose-response relationship.

II. Ethyltrichlorfon--Supplemental. The following deficiencies were noted:

- o No data on maternal toxicity were provided. No data on teratogenicity were provided except for the DBA mice. These data were insufficient for reaching definite conclusions on teratogenicity.
- o The compound was administered by intraperitoneal injection. This is an unnatural route of exposure and does not simulate the most probable route of exposure in humans.
- o No visceral examinations were performed on the fetuses.
- o The number of animals in 5 of 7 ethyltrichlorfon treated groups was less than 20.
- o Only two dose levels were utilized and the duration of dosing varied between the dose groups.