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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

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OCT 30 1984

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT: EPA Reg. No. 3125-58; Disulfoton (Di-Syston)®:
Embryotoxic and Teratogenic Effects in Rats.
Accession No. 250786
Caswell No. 341

TO: George LaRocca
Product Manager (15)
Registration Division (TS-767)

THRU: Christine F. Chaisson, Ph.D. *C.F. Chaisson 10/26/84*
Head, Review Section IV
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: George Z. Ghali, Ph.D. *G. Ghali 10/26/84*
Toxicology Branch
Hazard Evaluation Division (TS-769)

Registrant: Mobay Chemical Corporation
Stiwell, Kansas 66085 *WAB 10/30/84*

Action Requested:

Review and evaluation of a teratology study in the rat.

Conclusions and Recommendations:

Under the conditions of this study, the oral administration of 1 mg/kg of disulfoton daily from days 6 to 15 of gestation did not produce teratogenic effects in the fetus of the Sprague-Dawley rat. Based on the incomplete ossification of the parietals and sternbrae, disulfoton was demonstrated to be fetotoxic with an LEL of 1.0 mg/kg and a NOEL at 0.3 mg/kg.

The study is classified as Core-minimum data.

Teratology Study in Rats

Lamb, D.W. and Hixson, E.J. (1983). Embryotoxic and teratogenic effects of Disulfoton. An unpublished report on Study Number 81-611-02 submitted by Mobay Chemical Corp. May 13, 1983.

Test Chemical:

Disulfoton technical 98.2%.

Experimental Protocol:

1. One hundred seventy 50- to 60-day old female CD rats supplied by Charles River Breeding Laboratories, Inc. were used for the study. An unspecified number of adult male CD rats, received from the same supplier and maintained as a breeding colony were used for mating purposes only. The females were acclimated to laboratory conditions for seven day prior to study initiation.
2. Disulfoton was suspended in a Carbowax (polyethylene glycol 400) vehicle to provide dose levels of 100, 300, and 1000 ug/kg/day. The rationale for selecting the dosages was not stated. A positive control group received hydroxyurea at 350 mg/kg at 0.25 percent of the body weight on days 9, 10, and 11 of gestation. The dosages of the vehicle and test material were administered daily by gavage at 0.25 percent of the body weight from day 6 to 15 of gestation. Dose volumes were based on the day 6 of gestation body weights.

The dosing solutions were analyzed for disulfoton content by gas chromatography approximately weekly. The disulfoton content of the analyzed solutions was reported to range from 74 to 106 percent of nominal content. A stability test determined that the stock solution from which the dosing solutions were prepared was stable for 21 days at room temperature.

3. The rats were housed in a room controlled for temperature (69-74°F), relative humidity (35-55 percent), and light (12 hour light/dark cycle). The females were individually housed in stainless steel wire mesh cages. Purina Laboratory Rodent Chow and water were available ad libitum.

4. The females were mated to the males overnight, but the method of selecting mating pairs, the ratio of females to males, and the duration of mating were not stated in the Mobay report. The females were examined each morning for the presence of sperm in a vaginal smear and to determine the stages of estrus. The day that sperm were found was designated at day 0 of gestation, at which time the females were randomly assigned to treatment groups using a computer generated weight-stratified design. Twenty-five mated females were assigned to each of the five treatment groups.
5. Individual body weights were recorded on gestation days 0, 6, 13, 16, and 21. Food consumption was determined for gestation days 0-6, 6-13, and 13-21. The dams were observed daily for appearance, health, and evidence of "abortion" and premature delivery. Plasma and RBC cholinesterase activities were determined in five dams/group bled by orbital sinus puncture on day 15 of gestation. An automatic blood chemistry analyzer was used to determine the enzyme activities. The Mobay report did not specify the method employed to select the dams.
6. The rats were sacrificed on day 21 of gestation by carbon dioxide asphyxiation and examined for gross lesions. The uterus and ovaries were removed in toto and weighed. The ovaries were examined for the number of corpora lutea and the uterus was opened and the contents were examined. The number and distribution of live, dead, and resorbed fetuses were determined. The placentas were examined for gross abnormalities and each fetus was weighed, sexed, and examined for gross external abnormalities.

Fifty percent of the fetuses in each litter were selected by an unspecified method and were fixed in Bouin's solution. After fixation, the fetuses were examined for soft-tissue abnormalities by Wilson's technique. The remaining fetuses were fixed in 70 percent ethanol, examined, internally, eviscerated, prepared for staining with Alizarin Red S, and following destaining of nonossified tissue, were examined for skeletal abnormalities.
7. A Chi-square analysis utilizing SAS computer software was used to test body weights, food consumption, litter data, and external, soft tissue, and skeletal abnormalities for statistical significance. The fetal malformation data were analyzed for each individual abnormality and total abnormalities using both the fetus and the litter as the basic sample unit. Unit otherwise stated, the use of the word "significant" in this evaluation implies a statistical connotation with $p < 0.05$ as reported by Mobay.

RESULTS:

Clinical Observations and Mortalities: Alopecia was observed on two dams in the 100 ug/kg dose group. No abnormal clinical observations were observed among the remaining dams. There were no deaths prior to sacrifice on day 21 of gestation and at necropsy of the dams, no gross lesions were observed except for a pale liver and mottled kidneys in one female receiving 100 ug/kg disulfoton and mottled kidneys in a dam receiving 300 ug/kg disulfoton.

Body Weights and Food Consumption: The days 0, 6, 13, and 21 of gestation mean body weights of disulfoton-treated dams were similar to the negative control dams. Mean corrected body weight gains were similar among the disulfoton and negative control groups; however, this correction was not defined. While it is understood that the maternal body weight was "corrected" by subtracting the gravid uterus weight, the gestation day to which it was corrected was not indicated.

Food consumption was similar among the disulfoton and negative control groups during the three intervals.

Group mean data were not presented for the positive controls.

Cholinesterase Activities: The mean plasma and RBC cholinesterase activities for the dams receiving 300 and 1000 ug/kg disulfoton were significantly depressed when compared to the negative controls. Plasma cholinesterase activity, compared to the negative controls, was depressed 41 and 90 percent for the 300 and 1000 ug/kg groups, respectively. The low dose group RBC (9 percent) and plasma (6 percent) cholinesterase activities were similar to the negative control group.

Reproductive Indices: A summary of the reproduction indices is presented in Table 1. A comparison of the mean numbers of corpora lutea, early resorptions, late resorptions, dead fetuses, and mean fetal body weights indicated no differences between the disulfoton dose groups and the negative controls. The positive control was similar to the negative control for these parameters.

A slight, non-significant decrease in the mean numbers of implantation sites and live fetuses per litter was observed when the disulfoton and positive control dams were compared to the control. The mean numbers of fetuses/litter with gross external abnormalities were similar between the disulfoton and negative control groups while the positive controls had a greater than 10-fold increase when compared to the negative controls.

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TABLE 1. Summary of Reproduction Indices

	Dose Level (μ g/kg)				
	Negative Control	Positive Control ^a	100	300	1000
Dams inseminated	25	25	25	25	25
Dams pregnant	22	24	24	24	24
Dams delivering early	1	0	0	0	0
Dams c-sectioned	21	24	24	24	24
Mean no. of corpora lutea	14.7	14.6	14.5	14.6	14.4
Mean no. of implants	14.3	13.4	13.3	12.8	13.4
Mean no. of early resorptions	0.9	0.9	0.6	0.7	0.7
Mean no. of late resorptions	0.0	0.0	0.0	0.1	0.0
Mean live fetuses/litter	13.4	12.3	12.6	11.8	12.7
Mean fetal body weight (g) ^b	5.6	5.1	5.3	5.6	5.5
Mean fetuses/litter with external abnormalities	0.4	5.1	0.5	0.3	0.5

^a 350 mg/kg hydroxyurea on days 9, 10, and 11 of gestation.

^b Mean weights calculated from the individual fetal body weight by Dynamac.

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TABLE 2. Incidences of Major Fetal Abnormalities and Minor Abnormalities Affecting Greater than One Litter

	Dose Level ($\mu\text{g/kg}$)				Positive Control ^a
	Negative Control	100	300	1000	
I. External					
Hematoma	7/281(4) ^b	7/302(5)	4/283(4)	10/305(6)	8/296(5)
Runts	0/281	3/302(3)	1/283(1)	1/305(1)	2/296(2)
Total fetuses affected ^c	9/281	12/302	7/283	11/305	123/296
Total litters affected ^c	6/21	10/24	6/24	7/24	23/24
II. Soft Tissue					
Blood in nasal cavity	0/142	8/144(6)	3/136(2)	5/146(3)	4/140(2)
Esophagus distended	3/142(2)	9/144(8)	6/136(5)	6/146(3)	9/140(8)
Microphthalmia	0/142	0/144	1/136(1)	0/146	40/140(15)
Exencephaly	0/142	0/144	1/136(1)	0/146	0/140
Hydrocephalus(lateral)	0/142	0/144	1/136(1)	0/146	34/140(12)
Hydrocephalus (3rd)	0/142	0/144	1/136(1)	0/146	6/140(4)
Aortic arch malformed	0/142	1/144(1)	0/136	0/140	0/140
Slight hydronephrosis	44/142(14)	33/144(14)	41/136(19)	38/136(16)	42/140(18)
Total fetuses affected ^c	46/142	46/144	49/136	47/146	86/140
Total litters affected ^c	15/21	19/24	20/24	16/24	23/24
III. Skeleton					
Incomplete ossification of frontal bones	25/152(9)	14/156(8)	11/147(7)	16/154(9)	67/157(17)
Incomplete ossification of the parietals	5/152(1)	3/156(2)	2/147(2)	3/154(3)	11/157(7)
Incomplete ossification of intraparietals	1/152(1)	0/156	1/147(1)	8/154(4)	7/157(6)
Basioccipital dorsally displaced	1/152(1)	1/156(1)	2/147(1)	1/154(1)	3/157(2)
Incomplete ossification of maxillary process	7/152(2)	2/156(2)	1/147(1)	3/154(1)	1/157(1)
Hyoid unossified	0/152	5/156(2)	0/147	0/154	5/157(3)
Hyoid incompletely ossified	1/152(1)	8/156(3)	0/147	1/154(1)	5/157(3)
Enlarged fontanel	15/152(6)	6/156(5)	6/147(5)	3/154(3)	39/157(16)
Sternebrae incompletely ossified	3/152(3)	4/156(2)	1/147(1)	10/154(6)*	8/157(24)
Unossified sternebrae	1/152(1)	1/156(1)	3/147(3)	0/154	5/157(5)
Vertebral centra lobed	1/152(1)	9/156(6)	10/147(7)	3/154(2)	38/157(14)
Vertebral centra split	1/152(1)	8/156(3)	3/147(3)	7/154(3)	27/157(13)
Displaced lumbar vertebrae	0/152	0/156	0/147	1/154(1)	1/157(1)
Extra ribs	0/152	0/156	0/147	5/154(2)	93/157(21)
Paw incompletely ossified	58/152(16)	88/156(21)	71/147(22)	82/154(20)	96/157(22)
Total fetuses affected ^c	76/152	96/156	82/147	97/154	140/157
Total litters affected	18/21	22/24	22/24	22/24	24/24

^a 350 mg/kg hydroxyurea on days 9, 10, and 11 of gestation.^b Litters affected.^c Includes all abnormalities observed.* Significantly different than the control $p \leq 0.05$.

Fetal Evaluation: The incidences of major malformations and malformations affecting more than one litter are presented in Table 2. Major malformations are defined as malformations that are not comparable with life or that occur rarely in Sprague-Dawley rats.

Hydroxyurea, the positive control, produced increased external (raised cranium and reduced eye bulges), soft tissue (microphthalmia, hydrocephalus, and depressed olfactory bulbs), and skeletal (reduced cranial ossification, lobed and split vertebral centra, and extra ribs) abnormalities when compared to the negative control.

The incidences of external and soft tissues abnormalities were similar when the disulfoton-group fetuses and litters were compared to the controls. In the 300 ug/kg dose group the occurrence of microphthalmia, exencephaly, and hydrocephaly was restricted to one litter and, although not verifiable, probably one fetus.

A slight, nonstatistically significant increase when compared to the negative controls was observed in the number of high dose fetuses and litters with extra ribs and with incomplete ossification of the intraparietals.

The numbers of fetuses and litters with lobed or split vertebral centra were increased non-significantly in the three disulfoton dose groups. This increase did not appear to be related to the dosage. A significant increase in the number of fetuses with incompletely ossified sternbrae was observed at the high dose level, but the Mobay report did not indicate whether the number of affected high dose litters (6) also was significantly greater than the negative controls (3 litters).

DISCUSSION:

Oral administration of disulfoton did not produce any overt signs of maternal toxicity. The test material produced significant decreases in RBC and plasma cholinesterase activities of maternal animals. Although the decreases in the enzyme activities were not manifested clinically, the extent of enzyme inhibition strongly supports the consideration that higher doses than 1000 ug/kg could have been lethal to the dams. Consequently, it is considered that a maximum acceptable test dose was used.

There were no statistically significant effects on the reproductive parameters that were examined. However, despite the absence of significance, there was a decrease in the number of implantation sites/litter among the disulfoton-dosed dams. Implantation occurs on day 6 of gestation, the first day of dose administration, which indicates that the pre-implantation losses may be related to disulfoton. However, this seems unlikely for two reasons. A dose-related increase in pre-implantation losses was not observed, and the ratio of implantation sites to corpora lutea determined in the disulfoton-treated groups was not usual for Sprague-Dawley rats. Indeed, the pre-implantation loss observed among the negative controls is small than normally encountered and this gives rise to the appearance of an increased pre-implantation loss among the disulfoton dams.

The nonsignificant decrease in the numbers of live fetuses/litter observed among the disulfoton-dosed dams does not appear to be compound-related. The observed decrease resulted from the previously discussed decrease in implantation sites. A disulfoton-induced embryoletality would be expected to have manifested itself by a decrease in live fetuses/litter and a concurrent increase in resorptions or dead fetuses per litter.

Disulfoton did not produce increased incidences of soft tissue or external abnormalities in the fetuses. Among the 1000 ug/kg fetuses, increased incidences of incompletely ossified parietal bones and sternebrae were observed. A statistically significant increase in the number of 1000 ug/kg litters with fetuses having incompletely ossified sternebrae was also observed.

These abnormalities, while apparently related to the test material are considered to be indicative of retarded development and, therefore, a fetotoxic effect rather than a teratogenic effect.

A slight, nonsignificant increase in the number of 1000 ug/kg fetuses with extra ribs was observed. In the absence of any major abnormalities among the dose group fetuses, the slight increase in extra ribs did not clearly indicate a disulfoton-related teratogenic effect on skeletal formation.

Inconsistencies were detected in the summary data for fetal mean body weights presented in the Mobay report. The fetal mean body weights given in Table VI (Cumulative Litter Data) of the Mobay report did not always agree with the values given in Table 3A (Litter Data Summary) or with the mean fetal body

weights calculated by this reviewer from the individual fetal weights. For example, 4.9 g was reported in Table VI as the fetal mean body weight for the negative controls while the Table 3A value and this reviewer's calculation from the individual data both agreed on a mean of 5.6 g. Furthermore, the mean fetal weights for the 100 and 1000 ug/kg groups, and the positive control group presented in Table VI and Table 3A of the report did not agree with the means calculated from the individual data. The tabulated fetal mean body weight data presented in this evaluation (Table 1) were recalculated and corrected by this reviewer from individual animal data.

CONCLUSIONS:

Under the conditions of this study, the oral administration of 1000 ug/kg of disulfoton daily from days 6 to 15 of gestation did not produce teratogenic effects in the fetus of the Sprague-Dawley rat. Based on the incomplete ossification of the parietals and sternebrae, disulfoton was demonstrated to be fetotoxic with an LEL of 1000 ug/kg and a NOEL at 300 ug/kg.

CORE CLASSIFICATION: Minimum Data.