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

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SEP - 8 1986

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Benefin: 1471-71 (Record Number 164379): Review of Teratology Study with Benefin in Rats
Caswell Number 130, Accession No. 258788

FROM: John H.S. Chen, D.V.M.
Review Section #1
Toxicology Branch/HED (TS-769C)

TO: Robert Taylor, PM #25
Herbicide-Fungicide Branch
Registration Division (TS-767C)

THRU: Robert B. Jaeger, Section Head
Review Section #1
Toxicology Branch/HED (TS-769)

Petitioner:

Eli Lilly and Company
Greenfield, Indiana 46140

Action Requested:

Review and assessment of the teratology study with Benefin in rats, Hazleton Laboratories Ameriac, Inc. Study No. 6180-101, June 18, 1985.

Recommendation:

The registrant should be apprised of the deficiencies noted in this study, which are identified in the detailed review. The study may be upgraded on resolution of these deficiencies.

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Study: Rat Teratology Study with Benefin Hazleton Laboratories Study No. 6180-101, June 18, 1985 (Authors: J.K. Markham and K.M. Macckenize). Accession No. 258788.

Procedure:

The method used to determine the potential maternal, embryotoxic and teratogenic effects of benefin 54521 (Lot No. 231EF4; 97.3% pure; Seven impurities were found, 0.1 - 1.1%) in pregnant female rats is outlined below:

1. Nine-week old female, nonpregnant Crl:CD (SD) BR rats were mated with 11-week old males. The females were checked daily for the presence of a vaginal plug or sperm in the vaginal smear.

2. Four groups of pregnant rats, 25 per group, were treated with Benefin 54521 dissolved in 10% acacia solution by oral gavage at 50, 225, 475, and 1000 mg/kg/day for 9 consecutive days (initiated on gestation day 6 and continuing up to and including day 15 of gestation). To ensure the prescribed dosage levels of the test compound, samples of the test mixtures from the initial preparation (daily prepared) were periodically analyzed.

3. All animals were observed daily for morbundity, death and obvious indications of a toxic effect. Individual maternal body weights were recorded on gestation days 0, 6, 11, 16 and at the time of sacrifice on day 20. Individual food consumptions was recorded for intervals between gestatin days 0 through 6, 6 through 11, 11 through 16, and 16 through 20. All dams were sacrificed on day 20 of gestation. The uterine weight was recorded and the ovaries were examined for gross abnormalities; the number of corpora lutea was recorded. After being examined externally, the uterus was opened along its entire length and the contents were examined.

4. All viable fetuses were examined externally for gross abnormalities and variations. Each fetus was examined for visceral abnormalities or stained for skeletal examination.

5. All statistical analyses were conducted for a minimum significant level of 5% comparing the treated groups to the control group ($P<0.05$). The following analyses were used:

(a) Body weight and food consumption were analyzed by using one-way analysis of covariance (ANCOVA). If this test was significant, Dunnett's test was used to determine significance between the control and treated group;

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(b) One-way analysis of variance (ANOVA) with transformations (e.g., square root, log, reciprocal, are sin, and rank) was done on the following data for pregnant animals: body weight changes between day 0 and 20, gravid uterine weight, the number of corpora lutea and implants, implantation efficiency, and the number and percent of live and resorbed fetuses (Winer, B.J., Statistical principles in Experimental design, McGraw-Hill, New York, 2nd Edition 1971).

(c) The proportion of male and female fetuses with visceral and skeletal variations and anomalies and the proportion of litters with variant and anomalous fetuses was compared with the control groups by the cochran-Armitage test for trend and departure and a Fisher-Irwin (exact) test (Thakur A. et al., Fortran Program for Testing Trend and Homogeneity in Proportions, Comp. Prog. in Bio. Med., 19: 229-233, 1985).

Results:

1. Teratology Range-Finding Study

The preliminary study for dose selection of the test compound (Benefin 54521: Lot #219EF4 95.3% Purity) was conducted on pregnant rats using 5 dose levels of Benefin (50, 100, 225, 475, and 1000 mg/kg/day and one control group (5 rats/group). The general procedures used for the range-finding study were similar to that described in the teratology study.

Under the test conditions reported, survival for animals in this study was 100% in all groups. The mean maternal body weights on gestation days 11 and 16 and corrected weight changes from days 0 through 20 of gestation for the 475- and 1000 mg/kg/day groups were lower than those of the control in a dose-relaed manner. The mean food consumption values between gestation days 6 through 11 for the 475 mg/kg/day group and between gestation days 11 through 16 for the 475- and 1000 mg/kg/day groups were also lower than those of control in a dose-related manner. However, there were no statistically significant differencs in the mean number of corpora lutea or implants, implantation efficiency or the number of percent of live or resorbed fetuses. The reduced food consumption combined with the body weight loss in the treated dams at 1000 mg/kg/day demonstrated a maximum tolerated dose.

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2. Clinical Examination

There were no treatment-related individual clinical observations noted during this study. However, two animals (Nos. C28304 and C28321) in the 1000 mg/kg/day group had alopecia.

3. Mortality

Survival for animals in this study was 100% in all groups.

4. Food Consumption - Mean Daily Food Intake (g)

Dose Levels mg/kg	Day of Gestation 0-6	6-11	11-16	16-20
0	24	26	27	29
50	24	26	27	29
225	24	24	27	30
475	24	22*	25*	29
1000	24	21*	25*	30

* Significantly different from the control P<0.05

Findings: The mean food consumption values between gestation days 6 through 11 and 11 through 16 for the 475- and 1000 mg/kg/day groups were significantly less than those of the control group.

5. Maternal Body Weights

Summary of Mean Body Weight and Weight Change Data

Treatment mg/kg	Body Weight on Days (g) 0	6	11	16	20	Weight Change Between Days (g) 0-6	6-11	11-16	16-20	0-20
0	243	272	299	337	397	30	27	38	60	154
50	242	272	296	333	392	30	25	37	59	151
225	241	267	289	327	388	26	22	38	60	147
475	244	273	286*	322*	383	28	13*	36	61	139
1000	240	269	283*	322*	386	29	14*	39	64	146

* Significantly different from the control at 0.05 level

Findings: The mean maternal body weight on gestation days 11 and 16 and the mean weight changes from days 6 through 11 for the 475 and 1000 mg/kg groups were significantly less (P<0.05) than those of the corresponding control group.

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6. Urine Stains

Urine stains (from light yellow to orange) on the pan paper were observed following the initiation of dosing (Day 10 of gestation) for the 50 mg/kg group and the day following initiation of dosing (Day 7 of gestation) for the remaining treated groups. However, the occurance of this observation began to subside within 48 hours of the end of the dosing period (i.e., day 17 of gestation) and the urine apearance of all animals was normal at the time of cesarean section.

7. Postmortem Observations

Under the individual necropsy observations (number of animals with abnormal necropsy observatgions: control, 2; 50 mg/kg group, 5; 225 mg/kg group, 2; 475 mg/kg, 2; 1000 mg/kg group, 5), dilated renal pelvis was shown in all groups and enlarge hepatic lobes were also noted in all treated groups with similar frequency. There were no treatment-related observations for the pregnant rats at the time of cesarean section.

8. Cesarean Section Observation - Mean Values

Observations	Benefin (mg/kg)				
	0	50	225	475	1000
Number of animals on test	25	25	25	25	25
Number (percent) pregnant	24(96)	24(96)	24(96)	24(96)	25(100)
Corpora lutea	16	16	18	15	17
Implants	16	15	15	14	16
Implantation efficiency	96.8	97.1	88.7	92.5	91.6
Live fetuses	14.7	14.5	14.7	13.6	15.4
Percent live fetuses	100	100	100	100	100
Fetal viability	94.8	93.3	95.0	94.5	96.7
Sex Ration (M/M+F)	50.6	51.3	48.3	47.0	51.5
Male Fetal Weight (g)	3.6	3.5	3.5	3.5	3.5
Female Fetal Weight (g)	3.3	3.3	3.3	3.3	3.3
Resporptions*	0.8	1.0	0.8	0.8	0.5
Percent resorptions	5.2	6.7	5.5	5.5	3.3

* All were early resorptions except for one late resorption in Animal No. C26848 in the 50 mg/kg group.

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Findings: There were no significant differences (P>0.05) in the mean number of corpora lutea, or implants, implantation efficiency, fetal weights, sex ratio, or the numbr of percent of live or resorbing fetuses. All fetuses were also survived during this study.

9. Number of Fetuses with External Abnormalities

One fetus (No. 4) from Animal No. C28236 in the 50-mg Benefin/kg group had atailia. Another fetus (No. 3) from Animal No. C28274 in the 225-mg Benefin/kg group had a thread-like tail and no anus. However, this fetal external abnormalities were isolated incidents and are not considered treatment related.

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10. Number of Fetuses and Litters with Anomaly - Summary of Incidences (Males and Females)

	Fetuses					Litters				
Dose Levels (mg/kg)	0	50	225	475	1000	0	50	225	475	1000
Number Examined	185	179	182	167	198	24	24	24	24	25
Skeletally										
Skull										
Reduced ossification	4	12	6	12	9	4	6	5	5	9
Unossified hyoid	1	0	1	0	1	1	0	1	0	1
Vertebrate										
Absent vertebra/centra	1	1	2	1	0	1	1	2	1	0
Unossified vertebra/ centra	1	0	4	0	4	1	0	1	0	2
27 Presacral	0	0	0	0	1	0	0	0	0	1
25 Presacral	1	0	1	0	0	1	0	1	0	0
13 Presacral	0	1	0	0	0	0	1	0	0	0
Sternebra										
Bipartite	0	0	0	1	1	0	0	0	1	1
Ribs										
Wavy	1	0	0	0	0	1	0	0	0	0
Bent	0	3	0	0	0	0	1	0	0	0
Rudimentary	5	7	2	7	5	4	6	2	5	5
Extrafull unilateral	2	4	1	5	1	2	3	1	5	1
Reduced/interrupted ossification	1	0	0	0	0	1	0	0	0	0
7th Cervical	0	0	0	1	1	0	0	0	1	1
Absent	0	1	0	0	0	0	1	0	0	0
Pectoral Girdle										
Bent/malformed scapulae	0	0	0	1	0	0	0	0	1	0
Pelvic Girdle										
Malformed ileum	0	0	1	0	0	0	0	1	0	0
Total Number W/Skeletal Malformation	17	29	18	28	23	16	19	14	19	21
Number Examined	167	168	117	157	187	24	24	24	24	25
Viscerally										
Head										
Cleft palate	0	1	0	0	0	0	1	0	0	0
Aphakia	0	0	1	0	0	0	0	1	0	0
Circulatory										
Absent innominate	0	0	1	1	0	0	0	1	1	0
Accessory left subclavian	0	0	0	0	1	0	0	0	0	1
Gastrointestinal										
Dark brown-red diffuse areas or liver	0	0	5	1	4	0	0	5*	1	4
Urogenital										
Undescended testis	0	0	0	0	0	2	2	0	0	0
Total Number W/Soft Tissue Malformation	0	1	7	2	5	0	3	7	2	5

* Significantly different from the control at 0.05 level.

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Findings: The number of litters with dark brown-red diffuse areas on the liver was significantly greater in the 225 mg/kg group when compared to the control group. The diffuse areas on the liver appeared to be hemorrhagic regions. There was no evidence of a doe-response relationship for this incidence of liver observations. No other significant differences were observed between the litters of treated groups and the litters of control group.

11. Number of Fetuses and Litters with Variation - Summary of Incidences (Males and Females)

	Fetuses					Litters				
Dose Levels (mg/kg)	0	50	225	475	1000	0	50	225	475	1000
Number Examined Skeletally	185	179	182	167	198	24	24	24	24	25
Vertebrae										
Centra abnormalities	10	6	5	11	18	7	5	4	7	12
Sternebra										
Unossified	10(M) 24(F)	17(M) 23(F)	21(M) 26(F)	*12(M) 25(F)	22(M) 34(F)	14	16	15	15	21
Total Number W/Skeletal Variation	44	46	52	48	74	21	21	19	22	33
Number Examined Viscerally	167	168	171	157	187	24	24	24	24	25
Urogenital										
Distended ureter	13	21	10	7	2	9	13	9	4	2
Dilated renal pelvis	12	24	21	9	9	10	16	14	8	7
Total Number W/Soft Tissue Variation	25	45	31	16	11	19	29	23	12	9

* Significantly different from the control at 0.05 level (for the males only).

Findings: Although there was a significantly greater incidence of unossified sternebra found in males of the 225 mg/kg group, no evidence of a dose-response relationship was observed. No other significant differences were observed between the fetuses of treated groups and the fetuses of control group.

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12. Total Number of Abnormal Fetuses

		Benefin (mg/kg)				
		0	50	225	475	1000
Number of feuses examined		185	179	182	167	198
		167	168	171	157	187
	Total	352	347	353	324	385
Number of abnormal fetuses		17	29	18	28	23
		0	1	7	2	5
		44	46	52	48	74
		25	45	31	16	11
	Total	86	121	108	94	113
Percent of abnormal fetuses		24.4 (86/352)	34.9 (121/347)	30.6 (108/353)	29.0 (94/324)	29.4 (113/385)

Evaluation and Conclusion:

1. The parameters which were unaffected by the treatment of Benefin in pregnant rats are clinical observation, maternal survival, postmortem examination, conception rate, fetal sex ratio, preimplantation loss, postimplantation loss, and the number or percent of live or resorbed fetuses. Urine staining, observed in the Benefin-treated groups from days 7 through 17 of gestation, were not dose-related.

2. The treatment-related decreases of maternal weights were observed in the treated pregnant female groups receiving 475 and 1000 mg/kg Benefin. These results were correlated well with the significant decreases of food consumption values in the same treated groups during this study.

3. Although there were no treatment-related increases of the number of abnormal fetuses (i.e., fetuses with skeletal and soft tissue variations and anomalies) noted in the Benefin-treated groups, the number of unossified sternebra was significantly greater for male fetuses of dams treated with 225 mg/kg Benefin when compared to the control group. The number of fetuses and litters with dark brown-red diffuse areas on the liver was also significantly greater in the 225 mg/kg group. However, the total number of fetuses with skeletal and soft tissue variations and anomalies in the treated and control groups accumulated in the Table on page 9 (i.e., total abnormal fetuses: control group, 67; 50 mg/kg group, 94; 225 mg/kg group, 89; 475 mg/kg group, 75; 1000 mg/kg group, 90) were less than the total number of abnormal fetuses given in the Table 7 (summary of fetal skeletal observations) on page 21 and in the Table 8 (summary of fetal soft tissue observations) on page 24. This discrepency should be clarified.

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4. In addition, details regarding the visceral and skeletal examination techniques in this study must be provided in this report.

Since the submitted information in this report are inconclusive, the study is judged supplementary in the present form. Until the reporting deficiencies and data gaps cited in our conclusions #3 and #4 are clarified and resolved, this study is rated supplementary.

Classification of Data - Supplementary

Maternal Toxicity NOEL = 225 mg/kg

Developmental Toxicity NOEL = To be determined.

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