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

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OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: N-(n-butyl)-N-ethyl-2,6-dinitro-α,α,α-triflluoro-ρ-toluidine (Benefin Technical): Review of a study submitted by the registrant on the developmental toxicity (83-3) of Benefin Technical.

Caswell No.: 130
HED Project No.: 2-0276
MRID Nos: 420391-01

FROM: Walter J. Kozumbo, Ph.D., Toxicologist W.J. Kozumbo 1-29-92
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

TO: Christine Rice/Tom Myers, PM Team 52
Special Review and Reregistration Division
(H7508W)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head J.M. Ioannou 1/30/92
Review Section I, Toxicology Branch II
Health Effects Division (H7509C)

and

Marcia Van Gemert, Ph.D., Branch Chief Marcia Van Gemert 2/4/92
Toxicology Branch II
Health Effects Division (H7509C)

REGISTRANT: Lilly Research Laboratories

ACTION REQUESTED: For reregistration purposes, evaluate a study on the potential of Benefin Technical to produce developmental toxicity in rabbits according to FIFRA guidelines 83-3.

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CONCLUSIONS:

Developmental Toxicity Test in Rabbits (83-3) (MRID # 420391-01): Benefin Technical was administered orally to female rabbits on days 6 to 18 of gestation at the doses of 0, 25, 50, 100 and 225 mg/kg/day. The NOEL for maternal toxicity was established at 50 mg/kg/day, and based on decreases in body-weight gains and food consumptions, the LEL was determined to be 100 mg/kg/day. For developmental toxicity, the NOEL was established at 225 mg/kg/day and the LEL at > 225 mg/kg/day. This study satisfied guideline requirements and was classified as guideline.

Reviewed by: Walter J. Kozumbo, Ph.D. Walter J. Kozumbo 1-29-92
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Stephen C. Dapson, Ph.D. Stephen C. Dapson 1/30/92
Section I, Toxicology Branch II (H7509C)

DATA EVALUATION REPORT

STUDY TYPE:	Developmental Toxicity (Embryo/Fetotoxicity) in Rabbits (83-3)
TOX. CHEM. NO.:	130
MRID NUMBER:	420391-01
TEST MATERIAL:	Benefin (Technical)
STUDY NUMBER:	3130.9
TESTING FACILITY:	Springborn Laboratories, Inc. (SLS) Life Sciences Division 553 North Broadway Spencerville, OH 45887
SPONSOR:	Lilly Research Laboratories P.O. Box 708 Old National Road Greenfield, IN 46140
TITLE OF REPORT:	Teratology Study in Rabbits with Benefin
AUTHORS:	Michael D. Mercieca, B.S.
REPORT ISSUED:	June 3, 1991
CONCLUSIONS:	Pregnant rabbits were orally administered Benefin Technical at dose levels of 0, 25, 50, 100 and 225 mg/kg/day on days 6 to 18 of gestation. The NOEL established for maternal toxicity of Benefin was 50 mg/kg/day. Based on decreased body weight gains and food consumptions, the maternal toxicity LEL was 100 mg/kg/day. The NOEL determined for developmental toxicity was 225 mg/kg/day. The developmental toxicity LEL was higher than 225 mg/kg/day, the highest dose tested.
CLASSIFICATION:	Guideline This study satifies the guideline requirements (83-3) for developmental toxicity in rabbits.

I. MATERIALS

A. Test Material:

Benefin is a solid with the chemical name of N-(n-butyl)-N-ethyl-2,6-dinitro-α,α,α-trifluoro-ϱ-toluidine. The Benefin used in this study was prepared by DowElanco, Indianapolis, IN, (lot no. 231EF4) and was 95.64% pure as determined by chemical analysis on March 9, 1990. Benefin was stored at room temperature in a sealed container. It was mixed by blender with 10% (w/v) aqueous acacia which served as the test vehicle. In the vehicle, benefin was stable for at least 24 h at room temperature and was found to be homogeneously mixed as determined by chemical analysis.

B. Test Animals:

The test animals were female virgin New Zealand White rabbits (108) supplied by Hazleton Research Products, Inc., Denver, PA. The rabbits were caged individually and acclimated for at least 27 days prior to the study in an environment which was controlled for temperature (61-70°F), humidity (40-60%) and a 12-hour light/dark cycle. Relative humidity, however, was outside the specified range by +1 to 30 % on 30 separate occasions. Rabbits in good health were randomly allocated to 5 groups (20 per group) which were allowed to feed (Purina Certified Rabbit Chow® #5322) and drink (deionized tap water) ad libitum. The feed was analyzed by the supplier for contaminants and nutrients, and the water was analyzed for impurities within the year.

II. METHODS

A. Experimental Design and Dosing:

Rabbits were artificially inseminated with 0.5 ml of diluted (physiological saline) semen and then gavaged on days 6-18 with test article (20 rabbits/dose at doses of 0, 25, 50, 100 and 225 mg/kg/day) at a constant dosage volume of 5 ml/kg. Chemical analyses were performed to determine how the actual concentrations of test article compared to the nominal concentrations used for dosing.

The dosing regimen for this study was based on results from two preliminary studies. In the first study, female rabbits (5/dose at doses of 0, 20, 50, 225 and 475 mg/kg/day) were treated with benefin on days 6-18 of gestation and showed signs of maternal toxicity at 475 mg/kg/day as indicated by depressions in food consumption and in body weight gains and by abortions. A second intended definitive study was then performed at the dose levels of 0, 50, 225 and 475 mg/kg/day. Because of severe maternal toxicity occurring at both 225 and

475 mg/kg/day, this study was terminated prior to completion and no developmental data were collected.

B. Measured parameters:

Clinical Observations: Once each day the animals were observed for physical and behavioral abnormalities, and twice each day for moribundity and death. Animals were also observed for toxic effects at 30 min and 2 h following gavage.

Body Weights: Body weights were taken on days 0, 6, 9, 12, 15, 19, 24, and 29 of gestation.

Food Consumption: Daily consumption of food was measured. Food consumption was reported in units of g/animal/day and g/kg/day over various intervals during gestation.

Necropsy: Females dying or aborting during the study or surviving until the end of the study were necropsied for gross morphological changes. When abnormalites were observed, tissues were preserved in 10% neutral formalin solution for possible histological examination.

Uterine and Ovarian Observations: The uterus was removed, weighed and examined externally and internally. Uterine-related observations consisted of the number of viable fetuses, of early and late resorptions, and if no macroscopic evidence of implants, indications of non-visible early embryolethality as evidenced by staining of the uterus with 10% aqueous ammonium sulfide. The ovarian-related observation consisted of the number of corpora lutea. These observations together with the degree of autolysis noted for each non-viable fetus were used to assess the following parameters: Early and late resorptions, dead fetuses, preimplantation losses, and postimplantation losses.

Fetal Morphological Observations: Fetuses were examined for external, visceral and skeletal abnormalities. External examination consisted of measuring weights of all fetuses and the crown-rump length of late resorptions. Visceral examination was performed under a dissecting microscope using the technique as described by Staples (Teratology, 9: A37-A38, 1974). Sex of fetus was determined during this procedure. Skeletons were microscopically examined for abnormalities after the skeletal tissues were prepared by a process that involved fixing (isopropyl alcohol), macerating (1.5% sodium hydroxide solution), staining (Alizarin Res S) and clearing (in glycerin solution).

C. Statistics: See attached p. 18.

D. Compliance: GLP/QA statements were included in the study.

III. RESULTS

Chemical analysis demonstrated that actual concentrations of the test article as suspended in aqueous acacia 10% (w/v) were within 10% of nominal values. One 10 mg/ml concentration that was 12% below nominal value was replaced by a newly made concentration that was within 5% of nominal value.

TABLE 1.

MATERNAL TOXICITY IN PREGNANT RABBITS TREATED DAILY WITH BENEFIN BY ORAL GAVAGE DURING DAYS 6 TO 18 OF GESTATION

N=20	mg/kg/day				
	0	25	50	100	225
maternal deaths (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5)
aborting females no. (%)	0 (0)	0 (0)	0 (0)	0 (0)	3 (15)
few feces	0/0[a]	7/3	0/0	20/6	26/6
no feces	0/0	0/0	0/0	4/1	3/3
urine stain	0/0	0/0	13/1	3/2	6/3
compound -colored urine	0/0	1/1	0/0	0/0	23/6
compound -colored body stain	0/0	0/0	0/0	2/1	12/2

a Total no. of observations in each group/total no. of animals observed with effect at least once.

Indications of maternal toxicity had occurred that were related to the administration of test article (Table 1). One rabbit from the highest dosed group (225 mg/kg) died on day 18 of gestation. Three other females from the same group were anorectic and aborted on gestation days 20 and 23. Other observations in animals treated at the two highest doses (100 and 225 mg/kg) included a reduction or elimination of feces, and urine or urine stain that were the color of test-article.

TABLE II

BODY WEIGHT GAINS (GRAMS) OF RABBITS TREATED DAILY WITH BENEFIN DURING DAYS 6 TO 18 OF GESTATION					
days	mg/kg/day				
	0	25	50	100	225
0 - 6	258[a] N=17	336 N=16	305 N=15	311 N=18	274 N=16
6 - 19	232 N=17	198 N=16	245 N=15	158 N=18	14 * N=15
19 - 29	189 N=17	188 N=16	201 N=15	163 N=18	275 N=12
0 - 29	679 N=17	722 N=16	751 N=15	631 N=18	656 N=12
0 - 29[b]	316 N=17	290 N=16	274 N=15	223 N=18	241 N=12

a Mean values
b corrected weights = day 0 to 29 body weight gain minus gravid uterus weight
* Statistically significant from vehicle control at $p < 0.01$.

Further indication of maternal toxicity was evinced by data collected on body weight gains (Table II). At the penultimate dose (100 mg/kg), body weight gain appeared to be reduced between gestation day 6 and 19 (down to 158 g from a control value of 232 g), an interval of time nearly coincident with the administration of test article (days 6-18). At the highest dose (225 mg/kg) during the same period of time, a more severe and statistically significant, dose-dependent reduction in body weight gain occurred (down to 14 g) relative to control.

Similarly, food consumption as measured in g/animal/day or g/kg/day (see Table III) was reduced only during a time coincident with treatment of Benefin at 100 and 225 mg/kg. A dose-dependent reduction was likewise observed for food consumption only during the treatment interval between day 6-19 of gestation. For animals treated at 0, 25 and 50 mg/kg/day, no changes were observed in body weights, weight gains or food consumption.

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TABLE III

FOOD CONSUMPTION (GRAMS/KG/DAY) IN PREGNANT RABBITS TREATED DAILY WITH BENEFIN DURING DAYS 6 TO 18 OF GESTATION					
days	mg/kg/day				
	0	25	50	100	225
0 - 6	54[a] N=17	58 N=16	58 N=15	57 N=18	55 N=16
6 - 19	50 N=17	49 N=16	50 N=15	43 N=18	35 * N=15
19 - 29	42 N=17	41 N=16	41 N=15	40 N=18	41 N=12
0 - 29	48 N=17	48 N=16	48 N=15	45 N=18	43 N=12

a Mean values
* Statistically significant from vehicle control at $p < 0.01$.

TABLE IVa

CESAREAN SECTION OBSERVATIONS					
total numbe:	mg/kg/day				
	0	25	50	100	225
females assigned	20	20	20	20	20
insem-inated	20	20	20	20	20
preg-nant (%)	17 (85)	16 (80)	15 (75)	18 (90)	16 (80)
died	0	0	0	0	1
died pregnant	0	0	0	0	1
non-pregnant	3	4	5	2	4
aborted	0	0	0	0	3
pre-mature delivery	0	0	0	0	0

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TABLE IVb

CESAREAN SECTION OBSERVATIONS					
total # (mean)	mg/kg/day				
	0	25	50	100	225
corpora lutea	184 (10.8)	159 (9.9)	167 (11.1)	197 (10.9)	119 (9.9)
implantations	123 (7.2)	115 (7.2)	125 (8.3)	125 (6.9)	91 (7.6)
live fetuses	99 (5.8)	108 (6.8)	117 (7.8)	118 (6.6)	80 (6.7)
early resorpt.	23 (1.4)	6 (0.4)	5 (0.3)	1 (0.1 *)	10 (0.8)
late resorpt.	1 (0.1)	1 (0.1)	3 (0.2)	6 (0.3)	1 (0.1)
dead fetuses	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
gravid uterus weight	(363.6 g)	(432.4 g)	(477.4 g)	(408.1 g)	(414.9g)
fetal weight	(45.9 g)	(46.3 g)	(43.8 g)	(46.3 g)	(45.4 g)
preim-plantation loss	61 (3.6)	44 (2.8)	42 (2.8)	72 (4.0)	28 (2.3)
postim-plantation loss	24 (1.4)	7 (0.4)	8 (0.5)	7 (0.4)	11 (0.9)
sex M/F	52/47 (3.1/2.8)	50/58 (3.1/3.6)	68/49 (4.5/3.3)	48/70 (2.7/3.9)	43/37 (3.6/3.1

* Statistically significant from vehicle control at $p < 0.05$.

Necropsy showed treatment-related internal effects occurring only in the maternal rabbits which aborted or died before the end of the experiment. The 3 aborting rabbits manifested test article-colored staining of urine and several internal tissues including adipose, thymus and urinary bladder. The rabbit that died during the study had foamy contents in the trachea, mottled lungs, reddened cut areas of the thymus and dark red mammary tissue. No gross abnormalities were observed in the

necropsied rabbits that survived to the end of the study. No changes were observed for animals treated at 0, 25 and 50 mg/kg.

In contrast to positive indications of maternal toxicity cited above, there was no evidence of developmental toxicity observed in this study as a result of the daily administration of test article from days 6 to 18 of gestation (see Tables IVa and IVb). Some of the developmental endpoints examined consisted of the following: numbers of corpora lutea, implantation sites, pre-implantation losses, viable fetuses, dead fetuses, late resorptions, early resorptions, and post-implantation losses; sex ratios; gravid uterus weights; and fetal weights.

The fetuses were examined for external, visceral and skeletal malformations (see attached Table 9 & 10, pp. 40 & 41).. In comparison to negative controls, none of the treated groups was reported to have shown increases in the occurrence of malformations. In fact, there were no malformations (0%) observed after examining 80 fetuses at the highest dose level. By contrast, the negative control group had a total of 9 out of 99 (or 9.1%) of the fetuses with malformations, representing a frequency of malformations that was higher than frequencies observed in any of the treated groups. It should be noted that this 9.1 % rate for negative control rabbits is nearly three times higher than the 3.3 % historical control rate observed upon the examination of 2120 fetuses in the same laboratory (see attached pp. 261-263).

The fetuses were also examined for increases in frequencies of observed external, visceral and skeletal variations (Tables 11 & 12, pp. 42 & 43). The authors reported that the variations found in fetuses of the treatment groups were not statistically different from variations occurring in the negative control group, and thus concluded that the test article induced neither external, visceral nor skeletal variations in fetal rabbits.

It should be pointed out, however, that there was an indication of a possible dose-dependent response occurring in variations of skeletal accessory skull bones (see Table 11 & 12, pp. 42 and 43). The # of skull bone variations/total # of fetuses examined (and % of fetuses with variations) were found to be 1/99 (1.0%), 1/108 (0.9%), 2/117 (1.7%), 2/118 (1.7%) and 4/80 (5.0%) for the doses of 0, 25, 50, 100 and 225 mg/kg/day, respectively. When these variations were expressed in terms of the % of litters affected out of the total # of litters examined, a similar dose-dependent increase was observed that ranged between 5.9% and 27.3% for control group and highest dosed group, respectively. None of the variations observed in any of the treatment groups was found to be

statistically significant relative to negative controls; and historical control groups for this variation ranged between 0 and 3.5% of fetuses/control group, and between 0 and 29.4% of litters/control group.

IV. DISCUSSION

The developmental toxicity of Benefin Technical was investigated in rabbits treated during days 6 through 18 of gestation with doses of 0, 25, 50, 100 and 225 mg/kg/day. The authors concluded that the NOEL for maternal toxicity was 50 mg/kg/day and that the NOEL for developmental effects was 225 mg/kg/day.

Maternal toxicity was manifested by statistically significant alterations observed in body weight gains (Table II) and food consumptions (Table III) at the 225 mg/kg/day dose level. These changes coincided with the days when the test article was administered (on days 6 to 18 of gestation), thereby indicating a test article-related effect. Although not statistically significant, marked reductions in body weight gains and food consumptions also occurred at 100 mg/kg/day over the same dosing interval. The dose-response relationships observed for these parameters and their eventual return to control levels point to 50 mg/kg/day as the NOEL and 100 mg/kg/day as the LEL for maternal toxicity in this study. The absence of clinical observations involving feces reduction and compound-colored staining (see Table 1) at doses lower than 100 mg/kg/day would also suggest that 50 mg/kg/day is a reasonable NOEL for maternal toxicity. In spite of the dose-related effect observed in urine staining (Table 1), only one animal at 50 mg/kg/day was affected. An observation of urine staining involving just one animal is, by itself, insufficient evidence for reducing the NOEL for maternal toxicity to 25 mg/kg/day.

Because in this study no significant increases in malformations or variations were observed at any dose level and because a reasonable number and variety of developmental parameters were assessed, a NOEL for the developmental toxicity of Benefin Technical in rabbits of 225 mg/kg/day may be justified.

This conclusion is reached in spite of a concern with the data regarding malformations (Tables 9 & 10, pp. 40 & 41). That is, the high incidence of malformations observed in concurrent negative controls of this study (9.1%) versus a substantially lower mean historical control value (3.3%) could conceivably mask some treatment-related effects. This doesn't seem to be the case, however. If the historical controls provided by the sponsor (see attached pp. 261-263) are used in evaluating this study, a similar conclusion is reached regarding a lack of

test article-induced developmental effects in rabbits. This conclusion stems primarily from the fact that the highest dosed animals in this study were completely without malformations, thereby precluding the existence of possible dose-dependent relationships needed to establish a clear effect.

A NOEL for this study of 225 mg/kg/day of Benefin was also supported by the data on variations (Tables 11 & 12, pp. 42 & 43). This support comes in spite of a dose-dependent trend observed in irregularities of accessory skull bones. The fact that the highest dose (225 mg/kg/day) of Benefin resulted in a slightly higher frequency in this variation than was seen in the highest historical control group (5.0% versus 3.5%, respectively) enhanced the concern over possible irregularities in skull bones. On the other hand, however, these concerns were mitigated by the fact that the 5.0% incidence observed at the highest dose was not determined to be statistically different (based on the statistical methods used in this study) from a concurrent control value of 1.0%. Because this control value actually fell well within the historical range of control values (0 to 3.5%) for this particular variation, there was little chance that statistical significance was being masked by a higher than usual control value. Another mitigating factor is that the % of litters displaying this variation at the highest dose was somewhat less than the highest historical negative control values (27.3% versus 29.4%, respectively). Thus, based on the relatively marginal increase of skull bone variations above historical control level, the greater weight normally given to concurrent rather than historical controls in determining toxic effects, and the lack of other significant skeletal variations, it seems reasonable to conclude that an incidence of 5% in skull bone variations at the highest dose is not a test article-related effect.

It should also be noted that the statistically significant increase in bent hyoid arches observed at the intermediate dose of 50 mg/kg/day was not considered to be related to test article because incidences at the two higher doses were non-significant and actually less than the concurrent negative control value (which was well within the range of historical control values).

The chemical analytical data supplied in this study indicated that the test article was sufficiently pure, stable and homogenous. Signed statements of GLP/QA were included in the report.

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V. CONCLUSIONS

Pregnant rabbits were orally administered Benefin Technical at dose levels of 0, 25, 50, 100 and 225 mg/kg/day on days 6 to 18 of gestation. The NOEL established for maternal toxicity of Benefin was 50 mg/kg/day. Based on decreased body weight gains and food consumptions, the maternal toxicity LEL was 100 mg/kg/day. The NOEL determined for developmental toxicity was 225 mg/kg/day. The developmental toxicity LEL was higher than 225 mg/kg/day, the highest dose tested.

VI. CORE CLASSIFICATION

Guideline

This study satifies the guideline requirements (83-3) for developmental toxicity in rabbits.

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