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

004443

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM:

SUBJECT : TERRACIL. Registration Standard Data Call-In. EPA Registration Number 352-317. Teratogenicity and Mutagenicity Studies. Caswell No. 821A. Accession No. 255763.

TO : Robert Taylor (25). Registration Division (TS-767)

FROM : Roland A. Gessert, D.V.M.; Veterinary Medical Officer
Toxicology Branch, Section I. TS-769.

Roland A. Gessert
May 10, 1985

THRU : R. Bruce Jaeger, Toxicology Branch Section Head
Section 1; TS-769.

5/10/85

REGISTRANT: E. I. duPont de Nemours & Co.
Wilmington, Deleware

The registrant, E. I. duPont de Nemours & Co., submitted the following studies as requested for the registration standard data call in:

1. Unscheduled DNA Synthesis/Rat Hepatocytes in Vitro

2. CHO/HGPRT Assay for Gene Mutation

3. In Vivo Assay for Chromosome Aberrations in Rat Bone Marrow Cells

4. Embryo-Fetal Toxicity and Teratogenicity Study of Terbacil by Gavage in the Rabbit

CONCLUSIONS:

1. The reports of all the mutagenicity studies are deficient due to missing pages and tables. Mutagenicity studies reviewed by John Chen and R. Gessert.

2. The embryo-fetal toxicity and teratogenicty study in the rabbit demonstrates:

   a. TERABICIL is not teratogenic in the rabbit

   b. The NOEL for maternal toxicity and embryotoxicity is 200 mg/kg

   c. The data are Core Minimum.

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EMBRYO-FETAL TOXICITY AND TERATOGENICITY STUDY OF TERBACIL BY GAVAGE IN THE RABBIT. Haskell Laboratory Report Number 528-83. By Howard M. Solomon, et al. February 21, 1984.

MATERIAL TESTED: Terbacil, IND-732-53. 96.1% pure.

VEHICLE: 0.5% aqueous suspension of methyl cellulose 1500 centipoise, supplied by Callahan Chemical Company, Palmyra, New Jersey.

ANIMALS: 5-month old nulliparous female New Zealand White rabbits obtained from Hazleton-Dutchland Laboratories; Denver, PA. Males of same strain from same supplier.

PROCEDURE: 21 days after arrival the does were given 25 USP units HCG/kg body weight IV and inseminated 1 to 3 hours later. Day of insemination was designated day 0 of gestation. Only healthy does were placed on study, and dose groups were randomized by body weight. Eighteen inseminated does were assigned to each dose group.

Dose groups received 0, 30, 200, or 600 mg/kg body weight by gavage on days 7-19 of gestation in a volume of 2 ml/kg body weight 0.5% methyl cellulose suspension in distilled water.

OBSERVATIONS:

Body weights of the does were taken upon arrival, before breeding, and on the mornings of Days 0, 6, 7, 10, 13, 16, 19, 20, 24, and 29 of gestation. Individual does were observed for clinical signs upon arrival, twice during quarantine, each morning on gestation days 0 through 29, and each afternoon during the dosing period. Food consumption was determined visually, and a doe was considered anorectic when she ate less than 1/4 of her daily ration.

After sacrifice by cervical dislocation each doe was examined grossly. The liver was removed and weighed, and the corpora lutea were counted under magnification.

The number and position of all live, dead, and resorbed fetuses were recorded. The uterus of each apparently non-pregnant doe was stained with ammonium sulfide to determine very early resorptions.

All fetuses were weighed and live fetuses were examined for external alterations & visceral alterations, and sex of each fetus was determined. "Hydrocephaly was detected by making a transverse section between parietal and frontal bones through the unfixed fetal head. . . . eyes were examined visually to detect microphthalmia," and if bilateral microphthalmia was suspected, measurements were made for comparison.

"After evisceration, all fetuses were fixed in 70% ethanol, macerated in 1% aqueous potassium hydroxide solution, and stained with alizarin red S to examine skeletons for alterations. Data from fetuses classified as dead were excluded from statistical analyses and data summaries." Appropriate statistical analyses of the data were conducted.

RESULTS: See table on next page.

CONCENTRATION OF TEST SUSPENSIONS: Nominal dose levels were 0, 30, 200, and 600 mg terbacil/kg body weight. Actual mean dose levels administered were 0, 33, 208, and 680 mg terbacil/kg body weight.

MATERNAL MORTALITY: One control doe and 5 high dose does died during the study. An additional 2 high dose does were sacrificed in extremis.

CLINICAL SIGNS:

Besides maternal mortality, the chief highly significant dose related clinical sign was anorexia. In addition, a discharge on the cageboard was seen in the high dose group.

MATERNAL BODY WEIGHT:

Anorexia in the high dose does was reflected in body weight loss, particularly during the treatment period.

POST MORTEM FINDINGS IN DOES:

Hairballs completely filled the stomachs of all does on the high dose. A lower incidence of hairballs was found in the controls and other dose groups, but these were not dose related. No other significant maternal post mortem findings were noted.

REPRODUCTION:

There were no significant differences between groups in corpora lutea, implantations, resorptions, or live and dead fetuses. The mean live fetal weight was significantly less in the high dose group.

FETAL MALFORMATIONS:

The average percent of malformed fetuses per litter was higher in the high dose (600 mg) group than in the controls or other treatment groups, but the difference is not statistically significant or dose related. (5.8%, 9.0%, 6.0%, and 16.5% for the controls, low, mid, and high dose groups, respectively).

In general, there was no significant increase in malformed fetuses between the control and treatment groups.

FETAL VARIATIONS:

At the high dose, there was a significant increase in the incidence of extra ribs, partially or unossified phalanges, and partially or unossified pubes. These all occurred in only 3 litters, and cannot be considered a significant dose-related effect. Because they occurred at a dose that was overtly toxic to the dams, they may be considered to result from maternal toxicity.

	DOSE (mg/kg/day)			
	0	30	200	600
Pregnancies	14/18 77.8%	17/18 94.4%	14/18 77.8%	15/18 83.3%
Abortions	3/14 21.4%	0 0	0 0	4/15 26.7%
Deaths	1	0	0	5 + 2
Fetal Visceral Malformations	1/1	4/2	1/1	2/1
Hypoplastic thyroid	1/1			
Hypoplastic pancreas		3/1		
Cleft palate				1/1
Microphthalmia		3/2		
Hydrocephaly		1/1	1/1	2/1
Distended sinus		1/1		
Skeletal Malformations	4/3	11/8	2/2	5/3
Malformed vertebra	1/1	1/1	1/1	1/1
Absent vertebra		2/1		1/1
Angular hyoid cornu	1/1	2/1	1/1	
Fused sternebra	2/2	5/5		3/2
Fused ribs		1/1		2/2
Total Malformed (fetuses/litters)	4/3	12/8	3/3	7/3
(Fetuses/total fetuses)	4/70	12/136	3/80	7/29
Visceral Variations	3/2	4/3	4/3	1/1
Bifurcated gall bladder		1/1		
Left carotid artery branches off innominate artery	3/2	3/2	4/3	1/1
Skeletal Variations	56/10	100/17	53/12	23/3
Skull: hole in parietal bone			1/1	
Misaligned sternebra	2/2	3/2	2/2	
Bipartite sternebra	1/1			1/1
Ribs: extra ossification center	41/9	74/15	38/11	16/3
rudimentary ribs	21/10	27/13	17/9	4/2
extra ribs	17/9	43/13	24/9	18/3
thickened ribs			1/1	
Total with skeletal variations due to retarded development	40/10	88/17	47/11	18/3
Average % fetuses with variations due to retarded development	53.5%	66.1%	54.8%	69.0%
Total fetuses with variations	64/10	123/17	73/13	23/3
Average % fetuses with variations	90.6%	90.4%	87.4%	87.2%

It is noted that fertility of the controls was 14/18; fertility of the high dose (600 mg/kg) rabbits was 15/18. The proposed guidelines suggest 12 (pregnant) rabbits per group. Of the 14 pregnant does in the control group, 3 aborted and 1 died. These 4 does all had hair balls in their stomachs.

In this study and in other rabbit studies where dosing is by gavage I have noted a high incidence of trichobezoars, or gastric "hair balls", with resulting anorexia, abortions, and death. Apparently the hair consumption is a sequel to the stresses of dosing and handling procedure or chemical toxicity. In one laboratory where very few trichobezoars were encountered I noted that a very large breed of rabbits were used with apparently less physical resistance of the dosing procedure.

So in this study, while there were only 10 litters from 18 control does, there were 14/18 pregnancies. At the low and mid dose levels there was good fertility (17/18 and 14/18) with no abortions or deaths, and the mid-dose level (200 mg/kg) can be considered a NOEL for maternal and embryotoxicity, so in a sense the low and mid dose groups can be considered with the controls. Obvious toxicity is seen at the 600 mg/kg (high dose) level.

The treatment levels are relatively high. So even though there are only few litters from the high dose (600 mg/kg) to evaluate because of maternal toxicity, the relatively high NOEL level (200 mg/kg) indicates a large margin of safety.

CONCLUSIONS:

1. Terbacil is not teratogenic in the rabbit.
2. The NOEL for maternal toxicity and embryotoxicity was 200 mg/kg.
3. The data meet Core Minimum requirements.

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Study 1A: Unscheduled DNA Synthesis/Rat Hepatocytes in Vitro with Terbacil (technical 3-tert-butyl-5-chloro-6-methyluracil, 96.1% purity). Haskell Laboratory Report No. 379-84. July 20, 1984. Accession Number 255763.

PROCEDURE:

1. Indicator Cells

The freshly isolated hepatocytes were obtained from the livers of 8-week old, male Charles River/Sprague Dawley rats (number of rats not stated).

2. Cytotoxicity Determination

The levels of lactate dehydrogenase activity in the treatment medium were used to assess cytotoxicity (Sigma Technical Bulletin No. 226-UV; 8 82).

3. UDS ASSAY METHOD

Five x 10^5 viable cells were seeded onto 25 mm round plastic coverslips in 35mm 6-well tissue culture dishes. Each well contained 2 ml of WMES. The cells were incubated at 5% CO_2, 90% humidity incubator at 37° C. to allow the cells to attach to the coverslips for 2 hours prior to testing. The freshly isolated liver cells attached on coverslips were washed, refed with 2 ml WMEG containing 5 uCi/ml thymidine and the test compound (0.1, 0.033, 0.10, 0.33, 1.0, 2.5, 5.0, 7.5, and 10.0 mM), and incubated for 18 hours. After incubation, the coverslips with attached cells were washed again with WME, swelled with 1% sodium citrate solution and fixed with ethanol-glacial acetic acid (3:1). The coverslips were mounted on glass slides with permamount, dipped into Kodak nuclear track emulsion (Type NTB 2) and dried. The coated coverslips were stored in a light-tight box for 3 - 5 days at 4° C. The emulsions were developed in Kodak D-19 developer, fixed in Kodak fixer, and stained with hemotoxylin.

Incorporation of thymidine (methyl - 3H) into the DNA of the hepatocytes was detected as silver grains in the developed emulsion layer over the nuclei. Grains were quantitated with an MPV3 Microscope Photometer (Leitz) by measuring the intensity of light reflected by the silver grains.

RESULTS:

The submitted results of this study are incomplete. Results of the first trial for Terbacil in the UDS assay are missing in the report.

EVALUATION:

Several other pages of the report for this study also are missing: Page 2 - Procedure; Pages 4 & 6, Results and Discussion; Page ?, Table II; Pages 10 - ?. The evaluation of this study cannot be made until the complete report of the study are resubmitted.

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Study 1B: Terbacil (IND-732-53): CHO/HGPRT Assay for Gene Mutation. Haskell Laboratory Report No. 87-84. January 31, 1984. Accession Number 255763.

EVALUATION:

The submitted report of this study is incomplete. The following pages of the report are missing: Pages 2, 3, & 4 - Procedures; Page 6, Results; Page 8, Table I; Page 10, Table III; Page 12, Table V. The evaluation of mutagenic activity of the test compound in the CHO/HGPRT mutation assay cannot be made until the complete report of this study is resubmitted.

Study 1C: Terbacil: In Vivo Assay for Chromosome Aberrations in Rat Bone Marrow Cells. Hazleton Biotechnologies Corporation HLA Project No. 201-723. October 24, 1984. By Thomas Cortina and Hewsed Padilla-Nash.

MATERIAL TESTED: Terbacil. (#H 14,673). LH Number 21,046B. Purity known by sponsor, but not stated to testing laboratory.

POSITIVE CONTROLS: Cyclophosphamide 40 mg/kg in corn oil by oral gavage.

VEHICLE CONTROL: Corn oil.

ANIMALS: Male and female albino rats, Sprague-Dawley CD strain, 40 - 51 days old obtained from Charles River Breeding Laboratories; Kingston, N.Y.

DOSE: Dose volume = 15 ml/kg for all groups. Dose levels based on LD_{50} determination performed prior to the study. LD_{50} males = 1225 mg/kg; females = 934 mg/kg. Doses were 20, 100, or 500 mg/kg for low, mid, and high dose groups, administered once by oral gavage.

PROCEDURE: Fifteen males and 15 females were dosed with single doses of the chemical at 20, 100, or 500 mg/kg. Five males and 5 females on corn oil and on each terbacil level were killed at 6, 24, and 48 hours after treatment. Five males and 5 females receiving cyclophosphamide were killed 24 hours after administration.

At about 4, 22, and 46 hours after gavage, the appropriate rats received a single injection of colchicine (2.0 mg/kg body weight, 5 ml/kg) "to inhibit mitosis and arrest cells in metaphase." Two hours after the colchicine injection the rats were killed by CO_2.

The bone marrow cells from both femurs of each animal were then aspirated into Hank's Balanced Salt Solution, warmed to 37° C, centrifuged for 5 minutes, the supernatant decanted, and 5.0 ml of warmed 0.075M KCl added to each tube. After 25 minutes, 5 drops of methanol:acetic acid (3:1) fixative were added to each tube. The tubes were capped, inverted to mix the contents, and centrifuged for 5 minutes. The supernate was decanted and 5 ml fixative aded slowly down the sides of each tube. The cells were resuspended and recentrifuged for 5 minutes. The supernate was discarded, and the procedure was repeated. Drops of the final cell suspension were dispersed onto slides and air dried. Two to 4 slides were made for each animal and stained with Giemsa stain.

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Cells in metaphase were examined for cytogenetic abnormalities: chromosomal aberrations, mitotic index, chromosome number for each metaphase, and vernier location of each metaphase containing damage.

RESULTS:

TOTAL NUMBERS OF STRUCTURAL ABERRATIONS OBSERVED (500 cells/animal examined)

Dose	No. Animals/ Group	6 hr	%	24 hr	%	48 hr	%
corn oil vehicle	10	0	0	1	.20	0	0
cyclophosphamide	10	—	—	102	25.0	—	—
20 mg/kg	10	0	0	0	0	0	0
100 mg/kg	10	1	.21	0	0	1	.21
500 mg/kg	10	0	0	1	.20	0	0

The above table shows that no increase in the frequency of chromosomal aberrations was seen in any groups treated with terbacil. However, a significant increase was seen in the cyclophosphamide positive control.

The data also show no effect on mean chromosome numbers or mean mitotic index.

EVALUATION:

Under the test conditions reported, the test compound (96.1% pure) appeared to be negative in clastogenetic activity from the rat bone marrow cytogenetic assay (20 through 500 mg/kg). However the evaluation of the study cannot be accomplished without the following information accompanying the test report:

1. Toxicological information for repeated exposure of the test compound should be provided.

2. No subchronic study of the test compound for the rat bone marrow cytogenetic analysis was given in the report. The subchronic study which generally involves only one sampling time after 5 repeated consecutive doses is required to provide more definite results. Therefore, the study is judged unacceptable in the present form.

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END