

OFFICE OF TOXIC SUBSTANCES
CODING FORM FOR GLOBAL INDEXING

REV. 7/27/82

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Chemical Name (300 per name)	25	CAS No. (10)	24	
ETHYL TOLUENES		999999994		
P-ETHYL TOLUENE		622-98-8		

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Mobil Oil Corporation

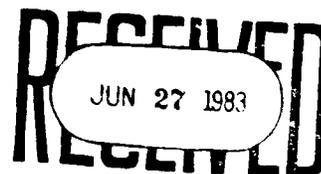
ENVIRONMENTAL AFFAIRS
AND HEALTH, SAFETY DEPARTMENT

1000 ROCKVILLE PIKE
NEW YORK, NEW YORK

CERTIFIED MAIL
RETURN RECEIPT REQUESTED

June 27, 1983

U.S. Environmental Protection Agency
TSCA - 8D1
P.O. Box 2060
Rockville, Maryland 20852



Dear Sirs:

In accordance with the requirements of TSCA Section 8(d) as published under 40 CFR, Part 716, Subpart A, we hereby submit the attached Health and Safety studies and lists of studies.

In terms of reportable substances our submission is organized and may be summarized as follows:

<u>Reportable Substances</u>	<u>No. of Studies</u>	
	<u>Listed</u>	<u>Submitted</u>
Chlorinated Paraffin*	1	1
p-Ethyltoluene	4	4
Tris (2-chloroethyl) phosphite	7	7
Benzyl butyl phthalate	1	1
1,2,4-Trimethylbenzene	1	1

The four p-ethyltoluene studies underway will be reported when complete.

You will notice that the studies we have submitted, some of which are extensive, generally showed little or no adverse effects.

* Reported as incomplete study 462-82 in earlier submission.

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We hope that the absence of adverse effects in spite of rigorous testing will be helpful to EPA in determining whether additional testing should be conducted.

Very truly yours,



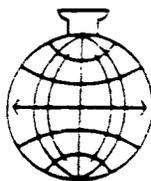
C. C. Fisher
Manager, Product Safety Coordination
(212) 883-5592

CCF/jw
Attachments

1D

878213626

RECEIVED
JUL 16 1981



International Research
and Development Corporation

MATTAWAN, MICHIGAN, U.S.A. 49671 TELEPHONE (616) 888-1144

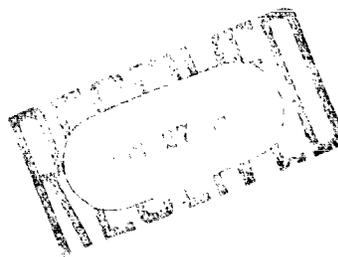
UNAUDITED DRAFT

SPONSOR: Mobil Oil Corporation

TEST ARTICLE: Sample 01038003

SUBJECT: Pilot Teratology Study in Rabbits
(MCTR-312-79)

DATE OF SUBMISSION:



450-030a

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"credence through research"

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International Research and Development Corporation

UNAUDITED DRAFT

I. QUALITY ASSURANCE STATEMENT

Study Title: Pilot Teratology Study in Rabbits (MCTR-312-79)

Test Article: Sample 01038003

Approved And
Submitted By:

Barry W. Benson, B.S.
Director of Quality Assurance

Date

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"credence through research"

*International Research & Development Corporation***UNAUDITED DRAFT****II. SYNOPSIS**

Twenty pregnant Dutch Belted rabbits, randomly assigned to one control group and three treatment groups of five rabbits each, were used in this pilot study to determine dosage levels of Sample 01038103 for a teratology study. Dose levels of 0, 25, 50 and 100 mg/kg/day were administered orally by gavage as a single daily dose on days 6 through 27 of gestation at a constant volume of 0.5 ml/kg. The control group received the vehicle only, Mazola[®] corn oil, on a comparable regimen at a constant volume of 0.5 ml/kg. Uterine examinations were performed on all surviving females on gestation day 28.

Survival was 100% in the 25 and 50 mg/kg/day groups. Five rabbits died during the treatment period: two in the control and three in the 100 mg/kg/day groups; one dam aborted prior to death at the 100 mg/kg/day level. Upon necropsy examination, an intubation error was determined as the cause of death for a 100 mg/kg/day group female; the cause of death for the remaining rabbits could not be determined.

There were no biologically meaningful differences in mean maternal body weight gain or mean uterine examination observations in the 25 or 50 mg/kg/day groups when compared to the control group. A decrease in the number of total implantations and an increase in the number of postimplantation losses with a corresponding decrease in the number of viable fetuses was observed at the 100 mg/kg/day level. Although these values were slightly outside the range of the historical control, this was probably due to one female (of the two examined) having only two implantations and are therefore not considered to be biologically significant. The remaining parameters evaluated at uterine examination in this group and mean maternal body weight gain were comparable to the control group.

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UNAUDITED DRAFT

Because it appeared that neither maternal or embryotoxicity was achieved at these dose levels, doses for the definitive study were selected above the range of doses used in this preliminary study.

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UNAUDITED DRAFT

III. INTRODUCTION

A. OBJECTIVE

The purpose of this pilot study was to establish dosage levels of the test article for a teratology study.

B. STUDY DESIGN

This study was conducted in two segments, Experiment I (450-030) and Experiment II (450-030a). In Experiment I, five treated groups and one control group of five rabbits each were placed on study. The test article was administered undiluted at dose levels of 0, 100, 300, 750, 1500 and 3000 mg/kg/day. Due to the severe toxicity observed in the groups receiving 100 mg/kg/day and higher, the study was repeated using a corn oil vehicle at dose levels of 0, 25, 50 and 100 mg/kg/day and designated as Experiment II. This report presented the methodology and results of Experiment II (designated 450-030a throughout the report). Experiment I (designated 450-030) is presented in Appendix IV.

C. TEST ARTICLE

The test article was received from Mobil Oil Corporation, Princeton, New Jersey on January 28, 1980 as indicated below:

<u>Label</u>	<u>Description</u>
01038003 MJ NORVELL Second label: CAUTION: COMBUSTIBLE KEEP AWAY FROM HEAT AND OPEN FLAME USE WITH ADEQUATE VENTILATION KEEP CONTAINERS CLOSED FOR INDUSTRIAL USE ONLY PET-D579-282-13 (2 bottles)	clear liquid

D. CONTROL ARTICLE IDENTIFICATION

The control article used was Mazola[®] corn oil.

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IV. METHODS AND PROCEDURES

A. EXPERIMENTAL DESIGN

1. Animals

Twenty sexually mature, virgin female Dutch Belted rabbits (Langshaw Farms, Augusta, Michigan) were used to determine dose levels of Sample 01038003 for a teratology study. These rabbits were approximately seven months of age at the time of insemination and had been acclimated in this laboratory for 90 days prior to study initiation. Each rabbit was assigned a unique number and ear-tagged for identification when placed on study. All rabbits were individually housed in suspended wire cages and maintained in a temperature-, humidity- and light-controlled (12 hour light/dark cycle) environment. During the treatment period, all rabbits were housed in a specially ventilated room and maintained under identical conditions. Purina® Certified Rabbit Chow® #5322 and tap water were available ad libitum; each diet-lot used was recorded.

During acclimation, stool samples were collected and analyzed for parasitic content and found to be positive for coccidiosis. Therefore, all rabbits received Purina® Sulfa^a in their drinking water for 15 consecutive days during the acclimation period. The concentration of Purina® Sulfa was 30 ml/gallon of tap water for the entire 15 days. Sulfa treatment was terminated approximately 10 weeks prior to study initiation and only rabbits screened negative for coccidiosis were placed on study.

Insemination was initiated on July 10, 1980 and the last uterine examination was performed on August 7, 1980.

^aPurina® Sulfa, a 12.5% water soluble solution of sodium sulfamethazine, is a product of the Ralston Purina Co., St. Louis, Missouri.

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2. Insemination Procedures

Three proven male rabbits of the same strain and source were selected to serve as semen donors. Semen was collected using an artificial vagina^b and the gelatinous plug was removed from the ejaculate. The semen was immediately evaluated for motility and was used for insemination only if the motility was 55% or greater. The ejaculate was diluted with 4 ml of 0.9% Sodium Chloride for Injection, U.S.P.^c at 35°C and 1/4 to 1/2 ml of this dilute semen was introduced into the anterior vagina of the female using an insemination pipette^d. Immediately after insemination, ovulation was induced by an injection of 100 units of A.P.L.^d into the marginal ear vein of the female. Semen from each male was used to inseminate an equal number of females in each group. Insemination procedures were performed on one day with the day of insemination designated as day 0 of gestation.

3. Organization of Test Groups

Prior to insemination, females were randomly assigned according to body weights by a computer-generated program to one vehicle control group and three treatment groups consisting of five rabbits each.

B. TEST ARTICLE ADMINISTRATION

The appropriate amount of Sample 01038003 was admixed with the vehicle, Mazola[®] corn oil, and shaken by hand to ensure proper mixture. The test article was prepared and dispensed fresh daily

^bArtificial vagina and insemination pipette for rabbits obtained from Holborn Surgical Instrument Co., Ltd., London, England.

^cSodium Chloride Injection, U.S.P. obtained from Cutter Laboratories, Inc., Berkeley, California.

^dA.P.L.[®] (Chorionic Gonadotropin for Injection, U.S.P.) is a registered trademark of Ayerst Laboratories, Inc., New York, New York.

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at concentrations to permit administration at dosage levels of 25, 50 and 100 mg/kg/day at a constant volume of 0.5 ml/kg. The test article was administered orally by gavage as a single daily dose on days 6 through 27 of gestation. The control group received the vehicle only on a comparable regimen at a constant volume of 0.5 ml/kg. Individual dosages were determined from individual body weights recorded on gestation day 6.

C. MATERNAL OBSERVATIONS

1. Appearance and Behavior

Prior to treatment, the dams were observed daily for mortality and overt changes in appearance and behavior. They were observed daily for mortality and clinical signs of toxicity on days 6 through 28 of gestation. Females not surviving to the scheduled sacrifice day were necropsied in an attempt to determine the cause of death. One dam aborted prior to death and was necropsied and examined for grossly evident morphological changes; the aborted tissue was discarded. Maternal tissues were preserved in 10% neutral buffered formalin for histopathological examination only as deemed necessary by the gross findings.

2. Body Weights

Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24 and 28.

3. Uterine Examination Observations

On gestation day 28, all surviving females were sacrificed by an overdose of sodium pentobarbital via a marginal ear vein. Immediately following sacrifice, the uterus and ovaries were exposed by an abdominal incision and the number and location of viable and nonviable fetuses, early and late resorptions and the number of total

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implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly evident morphological changes and the carcasses discarded. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy.

D. PROTOCOL AND PROTOCOL ADDENDA

Copies of the original protocol and all protocol addenda for the -50-030 and -50-030a studies are presented in Appendix II. These studies were conducted in accordance with these guidelines.

E. DATA RETENTION

All preservable specimens, raw data, a sample of the test article and copies of the final report are retained in the International Research and Development Corporation Archives in Mattawan, Michigan.

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V. RESULTS

A. MATERNAL OBSERVATIONS

1. Appearance and Behavior

Survival was 100% in the 25 and 50 mg/kg/day groups. Five rabbits died during the treatment period: two in the control group, one each on gestation days 22 and 27 (#9614 and #9613) and three in the 100 mg/kg/day group, one each on gestation days 14, 21 and 25 (#9707, #9605 and #9606, respectively). Female #9606 aborted prior to death. At necropsy examination, an intubation error was determined the cause of death for a 100 mg/kg/day group female (#9707). The cause of death for the remaining females could not be determined.

A summary of individual necropsy findings is presented in Table 1. Pitted kidneys and/or creamy material in the intestine were observed in one dam dying in the control (#9613), two females at the 25 mg/kg/day level (#9595 and #9596) and in the three rabbits dying in the 100 mg/kg/day group (#9605, #9606 and #9607). Histopathologic examination revealed slight pericholangitis and diffuse cytoplasmic vacuolation of the hepatocytes (probably lipid) in the liver of one of these females (#9606). Erosions of the stomach mucosa were also observed in two dams at the 100 mg/kg/day level (#9606 and #9607).

A reduction in the amount of feces observed beneath the cages was observed in a majority of the rabbits in the control and treated groups. Nasal discharge and/or hair loss (occurring primarily on the neck and thorax) occurred infrequently at all dosage levels. Matting of the anogenital haircoat was observed in one control animal and two animals at the 100 mg/kg/day level for one or two days during the treatment period.

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2. Body Weights

A summary of group mean maternal body weights and body weight change is presented in Table 2; individual body weights are presented in Table 3.

Slight losses in mean maternal body weight were observed in the 25, 50 and 100 mg/kg/day groups during the treatment period. However, these findings were probably comparable to the control group based on the somewhat erratic nature of maternal body weight gain in this species.

3. Uterine Examination Observations

A summary of group mean uterine examination observations is presented in Table 4; individual uterine examination observations are presented in Table 5 and a summary of mean historical control data is presented in Appendix III.

There were no biologically meaningful differences in the mean numbers of corpora lutea, total implantations, postimplantation loss, early or late resorptions or viable fetuses in the 25 or 50 mg/kg/day groups when compared to the control group. A slight increase in postimplantation loss with a corresponding decrease in the number of viable fetuses was observed in the two females examined at the 100 mg/kg/day level. A decrease in the number of total implantations was also cited in these females when compared to the control group. Although these values were slightly outside the range of the respective historical control values, this was probably due to one female having only two implantations and the finding is therefore not considered biologically significant. The number of corpora lutea per dam in the 100 mg/kg/day group were comparable to the control group.

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VI. DISCUSSION AND CONCLUSION

Survival was 100% in the 25 and 50 mg/kg/day groups. Five rabbits died during the course of this study: two in the control and three in the 100 mg/kg/day groups. One of these females (at the 100 mg/kg/day level) aborted prior to death. Upon necropsy examination, an intubation error was cited as the cause of death for a 100 mg/kg/day female; the cause of death for the remaining females could not be determined.

A decrease in the number of total implantations and an increase in the number of postimplantation losses with a corresponding decrease in the number of viable fetuses was observed at the 100 mg/kg/day level when compared to the control group. Although these values were slightly outside the range of the respective historical control values, this was probably due to one female (of the two examined) having only two implantations.

Based on these results and the apparent lack of either maternal or embryotoxicity at these dosages, doses for the definitive teratology study were selected above the range of doses used in this preliminary study.

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VII. COMPLIANCE STATEMENT

To the best of the signers' knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of the study.

E. J. F. Spicer, M.B., Ch.B., F.R.C.Path. Study Director, Director of International Coordination and Planning	Date
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Prepared By:	Shawna O. McMeekin, B.S. Report Writer, Reproduction and Teratology	Date
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Reviewed By:	James L. Schardein, M.S. Director of Reproduction and Teratology	Date
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Malcolm Blair, Ph.D. Director of Toxicology Division	Date
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TABLE Summary of Individual Maternal Observations at Necropsy

Test Article, Dosage Level Dam Number	Gestation Day of Sacrifice	Death	Necropsy Observations
<u>0 mg/kg/day (Control):</u>			
9610	28		No gross lesions
9611	28		No gross lesions
9612	28		Hydroceles, right oviduct
9613		27	Intestines contain tan mucoid material; lungs, congested, severe; cause of death undetermined
9614		22	Umbilical hernia, 2 cm x 2 cm; cause of death undetermined
<u>Sample 01038003, 25 mg/kg/day:</u>			
9595	28		Kidney, pitted, slight, bilateral; hydrocele, right oviduct
9596	28		Kidney, pitted, moderate, bilateral
9597	28		No gross lesions
9598	28		No gross lesions
9599	28		No gross lesions
<u>Sample 01038003, 30 mg/kg/day:</u>			
9600	28		Lungs, congested, moderate, all lobes; hydrocele, right oviduct
9601	28		No gross lesions
9602	28		No gross lesions
9603	28		No gross lesions
9604	28		Hydrocele, right oviduct
<u>Sample 01038003, 100 mg/kg/day:</u>			
9605		21	Kidney, pitted, slight, bilateral; intestine, contains tan creamy material; cause of death undetermined
9606		25	Aborted prior to death; kidney, pitted, slight, bilateral; small intestine con- tains tan creamy material; descending colon, contains formed fecal material; stomach, multiple pinpoint erosions on mucosa, emitted strong chemical odor; liver, pale in color, all lobes; histo- pathology, diffuse cytoplasmic vacuolation of the hepatocytes, slight pericholangitis; cause of death undetermined
9607		14	Kidney, pitted, moderate, bilateral; stomach, several 1 mm erosions on mucosa; small intestine, contains yellow creamy material; cause of death, intubation error
9608	28		Lungs, congested, moderate, all lobes
9609	28		No gross lesions

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TABLE 2. Summary of Group mean Maternal Body Weights and Body Weight Changes

Day of Gestation	Group Mean Maternal Body Weights (grams)		Sample 01038003 (mg/kg/day)		100 Mean
	0 (Control)		25		
	Mean	Mean	Mean	Mean	
0	2719	2890	2830	2953	
6	2799	2962	2807	2921	
12	2793	2933	2627	2850	
18	2783	2979	2556	2850	
24	2751	2957	2551	2800	
28	2794	2880	2781	2926	
Group Mean Maternal Body Weight Change (grams)					
0 to 6	80	72	-23	-32	
6 to 12	-6	-29	-180	-71	
12 to 18	-10	46	-71	0	
18 to 24	-32	-22	-5	-50	
24 to 28	63	-77	230	126	
6 to 28	-5	-82	-26	5	
0 to 28	75	-10	-49	-27	

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TABLE 3. Individual and Group Mean Maternal Body Weights (grams)

Test Article, Dosage Level, Dam Number	Day of Gestation					
	0	6	12	18	24	28
<u>0 mg/kg/day (Control):</u>						
9610	3420	3418	3376	3452	3430	3286
9611	2814	2816	2752	2734	2726	2799
9612	2516	2580	2547	2613	2654	2692
9613	3186	2947	2766	2592	2390	Died gestation day 27
9614	2827	2844	2809	2860	Died gestation day 22	
Mean	2953	2921	2850	2850	2800	2926
<u>Sample 01038003, 25 mg/kg/day:</u>						
9595	2611	2714	2709	2778	2806	2850
9596	2554	2593	2609	2629	2543	2641
9597	2515	2620	2699	2756	2884	2972
9598	3556	3616	3490	3246	3056	2930
9599	2360	2451	2457	2507	2467	2577
Mean	2719	2799	2793	2783	2751	2794
<u>Sample 01038003, 50 mg/kg/day:</u>						
9600	2816	2884	2902	3042	3006	2866
9601	2683	2787	2780	2892	2873	2855
9602	3502	3666	3495	3368	3218	3060
9603	2469	2481	2491	2561	2565	2626
9604	2979	2993	2996	3032	3122	2992
Mean	2890	2962	2933	2979	2957	2880
<u>Sample 01038003, 100 mg/kg/day:</u>						
9605	3248	3218	2851	2576	Died gestation day 21	
9606 ^a	2915	2799	2523	2334	2212	Died gestation day 25
9607	2603	2615	2424	Died gestation day 14		
9608	2825	2818	2765	2646	2718	2761
9609	2557	2584	2571	2668	2722	2801
Mean	2830	2807	2627	2556	2551	2781

^aDam aborted prior to death

TABLE 4. Summary of Group Mean Maternal Observations at Uterine Examination

	Sample 01038003 (mg/kg/day)		
	0 (Control)	25	50
Animals on study:	5	5	5
Animals that were gravid:	5	5	5
Animals that died:	2	0	0
Nongravid:	0	0	0
Gravid:	2	0	0
Animals that aborted:	0	0	0
Animals examined at uterine examination:	3	5	5
Nongravid:	0	0	0
Gravid:	3	5	5
Dams with resorptions only:	0	0	0
Dams with viable fetuses:	3	5	5
Viable fetuses/dam:	5.3	6.8	6.0
Postimplantation loss/dam:	0.3	0.0	1.5
Total implantations/dam:	5.7	6.8	4.0
Corpora lutea:	8.3	9.2	9.0
Group mean preimplantation loss (%): ^b	32.0	26.1	11.1
Group mean postimplantation loss (%): ^c	5.9	0.0	37.5

^aTotal includes one dam that aborted prior to death

^bTotal No. Corpora Lutea - Total No. Implantations x 100 = Group Mean Preimplantation Loss (%)

^cTotal No. Implantations - Total No. Viable Fetuses x 100 = Group Mean Postimplantation Loss (%)

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TABLE 5. Individual and Group Mean Maternal Observations at Uterine Examination

Test Article, Dosage Level, Dam Number	Viable Fetuses	Nonviable Fetuses	Late Resorptions	Early Resorptions	Post- implantation Loss	Total Implantations	Corpora Lutea
<u>0 mg/kg/day (Control):</u>							
9610	6	0	0	0	0	6	8
9611	7	0	0	0	0	7	9
9612	3	0	0	1	1	4	8
9613	Died - gravid						
9614	Died - gravid						
Total	16	0	0	1	1	17	25
Mean	5.3	0.0	0.0	0.3	0.3	5.7	8.3
<u>Sample 01038003, 25 mg/kg/day:</u>							
9595	4	0	0	0	0	4	9
9596	7	0	0	0	0	7	7
9597	8	0	0	0	0	8	11
9598	8	0	0	0	0	8	9
9599	7	0	0	0	0	7	10
Total	36	0	0	0	0	34	46
Mean	6.8	0.0	0.0	0.0	0.0	6.8	9.2
<u>Sample 01038003, 50 mg/kg/day:</u>							
9600	7	0	0	0	0	7	8
9601	3	0	0	0	0	3	5
9602	8	0	0	0	0	8	8
9603	7	0	0	0	0	7	11
9604	5	0	0	1	1	6	9
Total	30	0	0	1	1	31	41
Mean	6.0	0.0	0.0	0.2	0.2	6.2	8.2

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TABLE 5. Cont. Individual and Group Mean Maternal Observations at Uterine Examination

Test Article, Dosage Level, Dam Number	Viable Fetuses	Nonviable Fetuses	Late Resorptions	Early Resorptions	Post- implantation Loss	Total Implantations	Corpora Lutea
Sample 01038003, 100 mg/kg/day:							
9605		Died - gravid					
9606a		Died - gravid					
9607		Died - gravid					
9608	0	0	0	2	2	2	Regressing
9609	5	0	0	1	1	6	9
Total	5	0	0	3	3	8	9
Mean	2.5	0.0	0.0	1.5	1.5	4.0	9.0

^aDam aborted prior to death

APPENDIX I
Quality Assurance Inspections

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APPENDIX II
Protocol and Protocol Addenda

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INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 1 Study No. 450-030

Title: PILOT TERATOLOGY STUDY IN RABBITS

Justification:

Conduct study in accordance with the attached protocol.

Study Director Mr. Rodwell
[Signature] 12/31/78
Signature Date

1. TITLE
Teratology Study in Rabbits

2. PURPOSE OF THE STUDY

To establish dosage levels of the test article for a teratology study.

3. STUDY NUMBER

Mobil Oil No.: MCTR-312-79
IRDC No.: 450-030

4. TESTING FACILITY

International Research and Development Corporation
Mattawan, Michigan 49071

5. SPONSOR

Mobil Oil Corporation
Toxicology Division
P. O. Box 1026
Environmental Affairs and
Toxicology Department
Princeton, New Jersey 08540

6. SPONSOR'S REPRESENTATIVE

Michael Norvell, Ph.D.
Senior Toxicologist

7. IRDC PERSONNEL RESPONSIBILITIES

Study Director:

Dean E. Rodwell, M.S.
Director of Teratology

Director of General Toxicology
Division (Acting):

Edwin I. Goldenthal, Ph.D.

Assistant Director of General
Toxicology Division:

Malcolm Blair, Ph.D.

Director of Special Toxicology
Division:

D. Clifford Jessup, Ph.D.

Director of Chronic Toxicity:

Norman D. Jefferson, B.A.

Assistant Director of Teratology:

Jacqueline M. Wrenn, Ph.D.

Director of Quality Assurance:

Barry W. Hanson, B.S.

Director of Laboratory Services:

Patrick E. Traster, B.S.

8. SCHEDULE

Proposed starting date of study: March, 1980

Proposed completion date of study: April, 1980

Proposed date of final report: July, 1980

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9. TEST ARTICLE DATA

- a) Identification: Sample 12177900 of a volatile Mobil test substance.
- b) Lot Number: NA
- c) Batch Number: NA
- d) Physico-Chemical Properties: Colorless liquid, insoluble in water, soluble in most organic solvents.
B.P. = 323°F, V.P. = 28 mm Hg at 150°F,
S.G. = 0.36, F.P. = 140°F.
- e) Purity: 96%
- f) Shelf Life: Indefinite
- g) Storage Conditions: Room temperature in sealed container.
- h) Safety Precautions: Avoid ingestion, dermal contact or inhaling vapors.

10. TEST DIET DATA

- a) Test Diet: Purina® Certified Rabbit Chow® #5322 (Identification of each lot used will be recorded in the raw data).
- b) Contaminant Levels: The diet used will be a certified diet with guarantee of appropriate analyses performed by the manufacturer.

11. TEST ANIMALS

- a) Species: Rabbit
- b) Strain: Dutch Belted
- c) Source: Langshaw Farms
Augusta, Michigan
- d) Age at Start of Study: 5 - 8 months old
- e) Method of Identification: Monel metal ear tags
- f) Housing: Individual suspended wire cages
- g) Quarantine: 30 days (minimum)
- h) Number on Study: 30 females
- i) Reason for Selection: The rabbit is an acceptable model for teratology studies. This laboratory has historical control data on the incidence of fetal malformations and variations in this strain from this source.

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12. STUDY DURATION

The breeding and gestation phase of the study will require approximately five (5) weeks. A study schedule will be issued that will include the following information: dates for animal arrival, artificial insemination, administration of test and control articles (initiation and termination), sacrifice and uterine examination.

13. METHOD OF ADMINISTRATION OF THE TEST ARTICLE

The test and control articles shall be administered orally by gavage.

14. EXPERIMENTAL DESIGN

Sexually mature virgin female rabbits will be placed on study following a detailed observation and a 30-day (minimum) acclimation period. During the initial phase of the acclimation, stool samples from each rabbit will be examined for ova and parasites. If any rabbit is found to be positive for ova or parasites, all animals will be treated with a standard course of suitable therapy. All animals will then be re-examined and only animals judged free of parasitic infection will be placed on study.

The rabbits will be randomly assigned by a computer-generated program to five (5) treatment groups and one (1) control group of five (5) rabbits each and properly identified by ear tag prior to artificial insemination. Female rabbits will be artificially inseminated with diluted semen from proven males of the same strain and source. Ovulation will be induced by an injection of human chorionic gonadotrophin (HCG, 100 U.S.P. Units) via the marginal ear vein within one (1) hour following insemination. The day of insemination will be designated day "0" of gestation. All females will be maintained in a temperature-, humidity- and light-controlled room (12 hour light/dark cycle). Purina® Certified Rabbit Chow® #5322 and tap water will be available ad libitum. Nesting material will not be provided since the females will be sacrificed prior to delivery.

The test article will be administered orally by gavage as a single daily dose. The dosage levels will be 0, 100, 300, 750, 1500 and 3000 mg/kg/day of undiluted test article. Test article administration will begin on day 6 and continue up to and including day 27 of gestation. The control group will receive distilled water on a comparable regimen at a rate equal to that of the highest dosage group. Individual dosages will be based on gestation day 6 body weights.

Prior to test article administration, the rabbits will be observed daily for mortality and overt changes in appearance and behavior. The females will then be observed daily for mortality and clinical signs of toxicity from gestation days 6 through 28. The dams will be weighed on gestation days 0, 6, 12, 18, 24 and 28. On the 28th day of gestation, each female will be sacrificed by an injection of sodium pentobarbital via the marginal ear vein and the uterus and ovaries exposed by a mid-abdominal

000027

13. EXPERIMENTAL DESIGN (continued)

incision. The location of viable and nonviable fetuses, early and late resorptions, and the number of total implantations and corpora lutea will be recorded. The thoracic and abdominal cavities and organs of the dams will be examined for grossly evident morphological changes and the carcasses discarded.

Uteri from females that appear nongravid will be opened and placed in a 10% ammonium sulfide solution for confirmation of pregnancy status.

Any female showing signs of abortion or premature delivery will remain on study until the schedule sacrifice date to determine any effect of the test article. The aborted tissue will be discarded.

A gross necropsy will be performed on all rabbits not surviving to the scheduled sacrifice in an attempt to determine the cause of death. Maternal tissues will be preserved for microscopic examination in 10% neutral buffered formalin only as deemed necessary by the gross findings.

15. REPORT

A comprehensive report will be prepared upon completion of the study. The report will include mean maternal body weights of pregnant animals, mean numbers of viable and nonviable fetuses, early and late resorptions, postimplantation losses, total implantations and corpora lutea per dam.

a) Tables

Maternal body weights (individual)
Uterine examination data (individual)

b) Appendices

Protocol and protocol addenda
Test article data
Dates of Quality Assurance inspections and reports of significant deviations from protocol.

16. PERSONNEL HEALTH AND SAFETY

Normal safety precautions will be employed in the handling of the test article.

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17. DATA RETENTION

All data, including reports from this study, will be retained for at least ten (10) years after completion of the study in the IRDC Archives and will be made available for inspection upon request by authorized personnel of the Sponsor. An appropriate sample of the test article will be retained for ten (10) years following completion of the study.

18. QUALITY ASSURANCE

The final report will be reviewed by IRDC's Quality Assurance Department in accordance with IRDC's Standard Operating Procedures.

19. GOOD LABORATORY PRACTICES

This non-clinical laboratory study will be conducted in accordance with the Good Laboratory Practice regulations.

20. ALTERATION OF DESIGN

Alterations of this protocol may be made as the study progresses. No changes in the protocol will be made without the specific written request or consent of the Sponsor. In the event that the Sponsor authorizes a protocol change verbally, such change will be honored by IRDC. However, it then becomes the responsibility of the Sponsor to follow such verbal change with a written verification. All protocol modifications will be signed by the Study Director.

Approved by Sponsor

MOBIL OIL CORPORATION

By: Michael Norvell
Michael Norvell, Ph.D.

Title: Senior Toxicologist

Date: December 14, 1979

Issued by

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

By: Dean E. Rodwell
Dean E. Rodwell, M.S.

Title: Director of Teratology

Date: November 29, 1979

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INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL DISTRIBUTION SHEET

Protocol Sheet No. 2

Study No. 450-030

TITLE: PILOT TERATOLOGY STUDY IN RABBITS

Dr. Wazeter	<u>Z</u>	Dr. Spicer	<u> </u>
Dr. Goldenthal	<u>X</u>	Dr. Jessup	<u> </u>
Quality Assurance	<u>X</u>	Dr. Blair	<u> </u>
Accounting	<u>X</u>	Dr. Laveglia	<u> </u>
Report Processing	<u>X</u>	Mr. Rodwell	<u> X </u>
Statistics and Computer	<u>X</u>	Dr. Lang	<u> </u>
Report Status	<u>X</u>	Pathology	<u> </u>
Sponsor	<u>X</u>	Inhalation	<u> </u>
		Chronic Toxicity	<u> X </u>
		Teratology ✓	<u> X </u>
		Acute Toxicology and Special Studies	<u> </u>
		Clinical Pathology	<u> </u>
		Laboratory Animal Medicine	<u> </u>
		Test Material Control	<u> X </u>
		Analytical Chemistry	<u> </u>

<u>Dr. Michael Norvell</u> Sponsor Representative (name)	<u>Dean Rodwell</u> Study Director
<u>1/20/80</u> Date of Approval	<u>Dean G. Rodwell</u> <u>2/1/80</u> Signature Date

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 2

Study No. 450-030

Title: PILOT TERATOLOGY STUDY IN RABBITS

Justification:

Compound Identification: Sample 12177903
IRDC No.: 6945 & 6945B

At the Sponsor's request, the Sample 12177903 as stated on page 2 of 5 under Section 9, Test Article Data, a) Identification should read: Sample 01038003.

e) Purity: 97% (do not adjust for purity).

Study Director Dean Rodwell //

Dean E. Rodwell
Signature

2/1/87
Date

IR90-49-4

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INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION

PROTOCOL DISTRIBUTION SHEET

Protocol Sheet No. 3

Study No. 450-030

TITLE: PILOT TERATOLOGY STUDY IN RABBITS

Dr. Wazeter	<u>X</u>	Sponsor	<u>X</u>
Dr. Goldenthal	<u>X</u>	Dr. Spicer	<u> </u>
Quality Assurance	<u>X</u>	Dr. Jessup	<u> </u>
Accounting	<u>X</u>	Dr. Blair	<u> </u>
Report Processing	<u>X</u>	Dr. Laveglia	<u> </u>
Statistics and Computer	<u>X</u>	Mr. Rodwell	<u>X</u>
Report Status	<u>X</u>	Dr. Lang	<u> </u>
Pathology	<u> </u>	Dr. Wrenn	<u> </u>
Inhalation	<u> </u>		
Chronic Toxicity	<u>X</u>		
Teratology ✓	<u>X</u>		
Acute Toxicology and Special Studies	<u> </u>		
Clinical Pathology	<u> </u>		
Laboratory Animal Medicine	<u> </u>		
Test Material Control	<u>X</u>		
Analytical Chemistry	<u> </u>		

Dr. Michael Norvell
Sponsor Representative (name)

6/5/80
Date of Approval

Study Director Dean E. Rodwell

Dean E. Rodwell
Signature

6/6/80
Date

IR90-2-14

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INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 3

Study No. 450-030

Title: PILOT TERATOLOGY STUDY IN RABBITS

Justification:

Due to excessive toxicity at the lowest dosage level, three additional treatment groups and one control group will be conducted for this study. The dosage levels will be 0, 25, 50 and 100 mg/kg/day administered with a constant volume of 0.5 ml/kg of Mazola® corn oil.

Study Director: Dean E. Rodwell

Signature

5/6/80
Date

IR90-49-4

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INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL DISTRIBUTION SHEET

Protocol Sheet No. 4 Study No. 450-030

TITLE: PILOT TERATOLOGY STUDY IN RABBITS

Dr. Wazeter	<u>X</u>	Sponsor	<u>X</u>
Dr. Goldenthal	<u>X</u>	Dr. Spicer	<u>X</u>
Quality Assurance	<u>X</u>	Dr. Jessup	_____
Accounting	<u>X</u>	Dr. Blair	_____
Report Processing	<u>X</u>	Dr. Johnson	_____
Statistics and Computer	<u>X</u>	Dr. Phillips	_____
Report Status	<u>X</u>	Dr. Laveglia	_____
Pathology	_____	Mr. Rodwell	<u>X</u>
Inhalation	_____	Dr. Lang	_____
Chronic Toxicity	<u>X</u>	Dr. McGe	_____
Reproduction and Teratology ✓	<u>X</u>	Dr. Klonne	_____
Acute Toxicology and Special Studies	_____	OTHER:	_____
Clinical Pathology	_____	_____	_____
Laboratory Animal Medicine	_____	_____	_____
Test Material Control	<u>X</u>	_____	_____
Analytical Chemistry	_____	_____	_____

N/A
Sponsor Representative (name)

Study Director Dr. Goldenthal

Date of Approval

[Signature] 1/26/81
Signature Date

IR90-2-17

000034

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 4

Study No. 450-030

Title: PILOT TERATOLOGY STUDY IN RABBITS

ITEM

JUSTIFICATION

1

Due to the resignation of Mr. Rodwell,
a new Study Director is being assigned.

ITEM

PROTOCOL REVISION OR CLARIFICATION

1

Effective February 1, 1981 the Study
Director will be Dr. Eric Spicer.

Study Director Dr. Goldenthal

Signature

Goldenthal 1/26/81

Date

IR90-49-5

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INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL DISTRIBUTION SHEET

Protocol Sheet No. 5 Study No. 450-030
TITLE: PILOT TERATOLOGY STUDY IN RABBITS

Dr. Wazeter	<u>X</u>	Sponsor	<u>X</u>
Dr. Goldenthal	<u>X</u>	Dr. Spicer	<u>X</u>
Quality Assurance	<u>X</u>	Dr. Jessup	_____
Accounting	<u>X</u>	Dr. Blair	_____
Report Processing	<u>X</u>	Dr. Johnson	_____
Statistics and Computer	<u>X</u>	Dr. Phillips	_____
Report Status	<u>X</u>	Dr. Laveglia	_____
Pathology	<u>X</u>	Mr. Schardein	<u>X</u>
Inhalation	_____	Dr. Lang	_____
Chronic Toxicity	_____	Dr. McGee	<u>X</u>
Reproduction and Teratology ✓	<u>X</u>	Dr. Klonne	_____
Acute Toxicology and Special Studies	_____	OTHER:	_____
Clinical Pathology	_____	_____	_____
Laboratory Animal Medicine	_____	_____	_____
Test Material Control	<u>X</u>	_____	_____
Analytical Chemistry	_____		

N/A
Sponsor Representative (name)

Study Director Dr. Spicer

_____ Date of Approval

 Signature 3/25/80 Date/

INTERNATIONAL RESEARCH AND DEVELOPMENT CORPORATION
PROTOCOL REVISION OR CLARIFICATION

Protocol Sheet No. 5

Study No. 450-030

Title: PILOT TERATOLOGY STUDY IN RABBITS

ITEM

JUSTIFICATION

1

The following personnel are currently responsible for this study. The changes are required due to reorganization, personnel promotion and/or resignation.

ITEM

PROTOCOL REVISION OR CLARIFICATION

1

Study Director:

Eric J. F. Spicer, M.B., Ch.B.,
F.R.C.Path.

Director of Toxicology Division:
Associate Director of Toxicology
Division:

Malcolm Blair, Ph.D.

Director of Reproduction and
Teratology:

Dale E. Johnson, Pharm.D.; Ph.D.

Director of Quality Assurance:

James L. Schardein, M.S.

Director of Laboratory Services:

Barry W. Benson, B.S.

Patrick E. Traster, B.S.

Study Director Dr. Spicer

Signature [Signature]

Date 3/25/51

IR90-49-5

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APPENDIX III
Historical Control Data

450-030a

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IRDC Historical Control Data - Dutch Belted Rabbits

Summary of Maternal and Fetal Observations at Cesarean Section

No. of animals in the historical control:	185
No. of animals that died:	3
No. of animals that aborted:	9
No. of animals examined at Cesarean section:	173
No. nongravid:	21
No. gravid:	152
No. of dams with resorptions only:	3
No. of dams with live fetuses:	149
No. of live fetuses/dam:	6.3 [4.9- 7.6]
No. of postimplantation losses/dam:	0.6 [0.3- 1.2]
No. of implantations/dam:	6.8 [5.6- 7.8]
No. of corpora lutea/dam:	9.7 [8.0-12.2]
Fetal sex ratio: Male:Female	468:482
Mean fetal body weight (g):	32.5 [27.7-36.2]

[] - Range of means

APPENDIX IV
Personnel Involved in the Study

450-030a

000040

Pilot Teratology Study in Rabbits

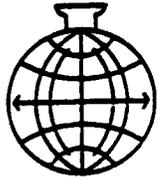
The following list of people were responsible for the supervision of various phases of this study:

Stephen Allen, B.S.	Unit Supervisor, Test Material Control
Carol Bajo	Group Supervisor, Reproduction and Teratology
Daniel Black, B.S.	Group Supervisor, Reproduction and Teratology
Karen Jo Bowman, B.S.	Unit Supervisor, Reproduction and Teratology
Shawna O. McMeekin, B.S.	Report Writer, Reproduction and Teratology
Mark D. Nemec, B.S.	Assistant to the Director, Reproduction and Teratology
Thomas Platte, B.S.	Unit Supervisor, Reproduction and Teratology
Dean E. Rodwell, M.S.	Director of Reproduction and Teratology
Colleen A. Schwartz	Manager, Reproduction and Teratology
E. J. F. Spicer, M.B., Ch.B., F.R.C.Path.	Study Director and Director of International Coordination and Planning
Elaine J. Tasker, B.S.	Group Supervisor, Report Preparation, Reproduction and Teratology
Patrick E. Traster, B.S.	Director, Laboratory Services

APPENDIX V
Pilot Teratology Study in Rabbits (MCTR-312-79)

450-030a

000042



International Research
and Development Corporation

MATTAWAN, MICHIGAN, U.S.A. 49071 TELEPHONE (616) 668-3336

SPONSOR: Mobil Oil Corporation

TEST ARTICLE: Sample 01038003

SUBJECT: Pilot Teratology Study in Rabbits
(MCTR-312-79)

DATE OF SUBMISSION:

450-030

"credence through research"

000043

International Research and Development Corporation

I. QUALITY ASSURANCE STATEMENT

Study Title: Pilot Teratology Study in Rabbits (MCTR-312-79)

Test Article: Sample 01038003

Approved And
Submitted By:

Barry W. Benson, B.S.
Director of Quality Assurance

Date

450-030

"credence through research"

000044

International Research & Development Corporation

II. SYNOPSIS

Thirty pregnant Dutch Belted rabbits, randomly assigned to one control group and five treatment groups of five rabbits each, were used in this pilot study to determine dosage levels of Sample 01038003 for a teratology study. Dose levels of 0, 100, 300, 750, 1500 and 3000 mg/kg/day were administered orally undiluted by gavage as a single daily dose on days 6 through 27 of gestation at total dosage volumes of 3.488, 0.116, 0.349, 0.872, 1.744 and 3.488 ml/kg, respectively. The control group received distilled water only, on a comparable regimen. Uterine examinations were performed on all surviving females on gestation day 28.

Survival was 100% in the control group only. Four rabbits at the 100 mg/kg/day level and all rabbits in the 300, 750; 1500 and 3000 mg/kg/day groups died prior to the scheduled sacrifice; the surviving females at the 100 mg/kg/day level aborted. An intubation error was determined the cause of death for three dams; two in the 300 and one in the 1500 mg/kg/day groups. Pneumonia was cited as the possible cause of death for another 300 mg/kg/day group female. Necropsy examinations did not establish the cause of death for the remaining animals that died. Losses in maternal body weight were observed prior to death in all females dying in the 100 mg/kg/day group and in a majority of the females dying in the 300 mg/kg/day group. A loss in maternal body weight was also noted in the surviving 100 mg/kg/day group female during the treatment period. Due to the excessive mortality observed in the 750, 1500 and 3000 mg/kg/day groups, meaningful comparisons could not be made with respect to maternal body weights or to uterine examination observations.

450-030

000045

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Due to inconclusive results at these dosage levels, this study was repeated and designated as 450-030a. Dosage levels selected were 0, 25, 50 and 100 mg/kg/day and were administered with a constant volume of 0.5 ml/kg in a Mazola[®] corn oil vehicle.

450-030

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III. INTRODUCTION

A. OBJECTIVE

The purpose of this pilot study was to establish dosage levels of the test article for a teratology study.

B. TEST ARTICLE

The test article was received in two shipments from Mobil Oil Corporation, Princeton, New Jersey on January 10 and January 28, 1981 as indicated below:

<u>Date</u>	<u>Label</u>	<u>Description</u>
January 10, 1980	01038003 Second label: CAUTION: COMBUSTIBLE KEEP AWAY FROM HEAT AND OPEN FLAME USE WITH ADEQUATE VENTILATION KEEP CONTAINER CLOSED FOR INDUSTRIAL USE ONLY PET-D579-282-13 (2 bottles)	clear liquid

<u>Date</u>	<u>Label</u>	<u>Description</u>
January 28, 1980	01038003 MJ NORVELL Second label: CAUTION: COMBUSTIBLE KEEP AWAY FROM HEAT AND OPEN FLAME USE WITH ADEQUATE VENTILATION KEEP CONTAINERS CLOSED FOR INDUSTRIAL USE ONLY PET-D579-282-13 (2 bottles)	clear liquid

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IV. METHODS AND PROCEDURES

A. EXPERIMENTAL DESIGN

1. Animals

Thirty sexually mature, virgin female Dutch Belted rabbits (Langshaw Farms, Augusta, Michigan) were used to determine dose level of Sample 01038003 for a teratology study. These rabbits were approximately six months of age at the time of insemination and had been acclimated in this laboratory for 46 days prior to study initiation. Each rabbit was assigned a unique number and ear-tagged for identification when placed on study. All rabbits were individually housed in suspended wire cages and maintained in a temperature-, humidity- and light-controlled (12 hour light/dark cycle) environment. During the treatment period, all rabbits were housed in a specially ventilated room and maintained under identical conditions. Purina® Certified Rabbit Chow® #5322 and tap water were available ad libitum; each diet-lot used was recorded.

During acclimation, stool samples were collected and analyzed for parasitic content and found to be positive for coccidiosis. Therefore, all rabbits received Purina® Sulfa^a in their drinking water for 14 consecutive days during the acclimation period. The concentration of Purina® Sulfa was 30 ml/gallon of tap water for the entire 14 days. Sulfa treatment was terminated approximately four weeks prior to study initiation and only rabbits screened negative for coccidiosis were placed on study.

Insemination was initiated on March 25, 1980 and the last uterine examination was performed on April 22, 1980.

^aPurina® Sulfa, a 12.5% water soluble solution of sodium sulfamethazine, is a product of the Ralston Purina Co., St. Louis, Missouri.

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2. Insemination Procedures

Three proven male rabbits of the same strain and source were selected to serve as semen donors. Semen was collected using an artificial vagina^b and the gelatinous plug was removed from the ejaculate. The semen was immediately evaluated for motility and was used for insemination only if the motility was 55% or greater. The ejaculate was diluted with 4 ml of 0.9% Sodium Chloride for Injection, U.S.P.^c at 35°C and 1/4 to 1/2 ml of this dilute semen was introduced into the anterior vagina of the female using an insemination pipette^b. Immediately after insemination, ovulation was induced by an injection of 100 units of A.P.L.^d into the marginal ear vein of the female. Semen from each male was used to inseminate an equal number of females in each group. Insemination procedures were performed on one day with the day of insemination designated as day 0 of gestation.

3. Organization of Test Groups

Prior to insemination, females were randomly assigned according to body weight by a computer-generated program to one control group and five treatment groups consisting of five rabbits each.

B. TEST ARTICLE ADMINISTRATION

The appropriate amount of Sample 01038003 (specific gravity 0.86 g/ml) was dispensed and administered undiluted at dosage levels of 100, 300, 750, 1500 and 3000 mg/kg/day at total dosage volumes of

^bArtificial vagina and insemination pipette for rabbits obtained from Holborn Surgical Instrument Co., Ltd., London, England.
^cSodium Chloride Injection, U.S.P. obtained from Cutter Laboratories, Inc., Berkeley, California.
^dA.P.L.[®] (Chorionic Gonadotropin for Injection, U.S.P.) is a registered trademark of Ayerst Laboratories, Inc., New York, New York.

International Research & Development Corporation

0.116, 0.349, 0.872, 1.744 and 3.488 ml/kg, respectively. The test article was administered orally by gavage as a single daily dose on days 6 through 27 of gestation. The control group received distilled water only on a comparable regimen at a volume equal to that of the highest dosage group (3.488 ml/kg). Individual dosages were determined from individual body weights recorded on gestation day 5.

C. MATERNAL OBSERVATIONS

1. Appearance and Behavior

Prior to treatment, the dams were observed daily for mortality and overt changes in appearance and behavior. They were observed daily for mortality and clinical signs of toxicity on days 6 through 28 of gestation. Dams showing signs of abortion remained on study and were sacrificed and examined for grossly evident morphological changes on the day of scheduled uterine examination. All aborted tissue was examined and discarded. Females not surviving to the scheduled sacrifice day were necropsied in an attempt to determine the cause of death.

2. Body Weights

Individual maternal body weights were recorded on gestation days 0, 6, 12, 18, 24 and 28.

3. Uterine Examination Observations

On gestation day 28, all surviving females were sacrificed by an overdose of sodium pentobarbital via a marginal ear vein. Immediately following sacrifice, the uterus and ovaries were exposed by an abdominal incision and the number and location of viable and nonviable fetuses, early and late resorptions and the number of total implantations and corpora lutea were recorded. The abdominal and thoracic cavities and organs of the dams were examined for grossly

International Research & Development Corporation

evident morphological changes and the carcasses discarded. Maternal tissues were preserved in 10% neutral buffered formalin for histopathological examination only as deemed necessary by the gross findings. Uteri from females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for confirmation of pregnancy.

D. DATA RETENTION

All preservable specimens, raw data, a sample of the test article and copies of the final report are retained in the International Research and Development Corporation Archives in Mattawan, Michigan.

International Research & Development Corporation

V. RESULTS

A. MATERNAL OBSERVATIONS

1. Appearance and Behavior

Survival was 100% in the control group only. Four rabbits in the 100 mg/kg/day group died, one each on gestation days 19, 20, 24 and 28 (#8197, #8198, #8195 and #8196, respectively); one female aborted on gestation day 22 (#8199). All rabbits in the 300, 750, 1500 and 3000 mg/kg/day groups died between gestation days 7 and 21. An intubation error was determined the cause of death for three rabbits, two in the 300 mg/kg/day group (#8200 and #8204) and one in the 1500 mg/kg/day group (#8213). Pneumonia was determined the probable cause of death for another 300 mg/kg/day group female (#8203). The cause of death for the remaining rabbits could not be determined.

A summary of individual necropsy findings are presented in Table 1. At the 100 mg/kg/day level, nodules on the lung (determined to be abscesses at histopathological examination) were cited in one dam (#8197). Multiple pinpoint erosions on the stomach lining or tan mucoid material in the intestine were observed in two other females (#8195 and #8198, respectively). One dam in the 750 mg/kg/day group was observed to have a small pitted spleen while a female in the 1500 mg/kg/day group had accentuated lobulation of all lobes of the liver (#8209 and #8213, respectively). Additional postmortem findings included occasional instances of congested lungs, foci on the lungs and/or hydroceles on the oviducts in the 100, 1500 and 3000 mg/kg/day groups as well as the control group.

Nasal discharge and/or a reduction in the amount of feces observed beneath the cages were cited in several rabbits in the control, 100 and 300 mg/kg/day groups, at various intervals during the gestation period.

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2. Body Weights

A summary of group mean maternal body weights and body weight change is presented in Table 2; individual body weights are presented in Table 3.

Losses in maternal body weight were observed prior to death in the four rabbits dying at the 100 mg/kg/day level and in three out of the five females dying at the 300 mg/kg/day level. The one female surviving to uterine examination in the 100 mg/kg/day group exhibited a maternal body weight loss during gestation days 6 to 18 (prior to abortion). Because of the high mortality observed during the treatment period, meaningful comparisons could not be made at the 750, 1500 or 3000 mg/kg/day levels.

3. Uterine Examination Observations

A summary of group mean uterine examination observations is presented in Table 4; individual uterine examination observations are presented in Table 5.

Due to the excessive mortality observed in all treatment groups, meaningful comparisons with respect to uterine examination observations were not obtainable.

VI. DISCUSSION AND CONCLUSION

Survival was 100% in the control group only. Four rabbits in the 100 mg/kg/day group and all females in the 300, 750, 1500 and 3000 mg/kg/day groups died. In addition, one female in the 100 mg/kg/day group aborted. An intubation error was determined to be the cause of death for three rabbits, two in the 300 and one in the 1500 mg/kg/day groups; pneumonia was cited as the cause of death for another 300 mg/kg/day group female. A necropsy examination could not establish the cause of death for the remaining rabbits. A loss in maternal body weight was observed in the one surviving female at the 100 mg/kg/day level during the treatment period. Maternal body weight losses were observed prior to death in all females dying in the 100 mg/kg/day group and in a majority of the females dying in the 300 mg/kg/day group. Due to the excessive mortality observed in the 750, 1500 and 3000 mg/kg/day groups, meaningful comparisons could not be made with respect to maternal body weights and in all of the Sample 01038003 treated groups with respect to uterine examination observations.

Due to inconclusive results at these dosage levels, this study was repeated and designated as 450-030a. Dosage levels selected were 0, 25, 50 and 100 mg/kg/day and were administered with a constant volume of 0.5 ml/kg in a Mazola[®] corn oil vehicle.

International Research and Development Corporation

VII. COMPLIANCE STATEMENT

To the best of the signers' knowledge, there were no significant deviations from the Good Laboratory Practice Regulations which affected the quality or integrity of the study. This study was conducted in conformance with the Good Laboratory Practice Regulations. This report accurately reflects the raw data obtained during the performance of the study.

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Date

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TABLE 1.

Summary of Individual Maternal Observations at Necropsy

Test Article, Dosage Level, Dam Number	Gestation Day of Sacrifice	Death	Necropsy Observations
<u>0 mg/kg/day (Control):</u>			
8190	28		Lungs, multiple pinpoint to 2 mm foci, all lobes
8191	28		No gross lesions
8192	28		No gross lesions
8193	28		Lungs, multiple pinpoint to 2 mm foci, all lobes
8194	28		Lungs, multiple pinpoint to 1 mm foci, all lobes
<u>Sample 01038003, 100 mg/kg/day:</u>			
8195		27	Stomach, multiple pinpoint to 4 mm erosions on lining of stomach; cause of death undetermined
8196		28	Trachea, contained white cheesy material; cause of death undetermined
8197		19	Lungs, hard, yellow nodule 0.5 cm to 0.5 cm, left lobe; several smaller nodules on apical and cardiac lobes on right side 1 mm to 3 mm; histopathology, abscesses; cause of death undetermined
8198		20	Small intestine, contains tan mucoid material; cause of death undetermined
8199	28		(Aborted gestation day 22) lungs, emphysematous, all lobes, severe; hydrocele, right oviduct
<u>Sample 01038003, 300 mg/kg/day:</u>			
8200		10	Esophagus, 2 mm hole in apex; cause of death intubation error
8201		17	No gross lesions; cause of death undetermined
8202		7	Cervix, enlarged, inner vagina near cervix hemorrhagic; cause of death undetermined
8203		21	Lungs, consolidated, abscessed; probable cause of death pneumonia
8204		17	Trachea, 1 mm x 2 mm hole near lungs; lungs have slight chemical odor; cause of death intubation error
<u>Sample 01038003, 750 mg/kg/day:</u>			
8205		8	No gross lesions; cause of death undetermined
8206		7	No gross lesions; cause of death undetermined
8207		8	No gross lesions; cause of death undetermined
8208		10	No gross lesions; cause of death undetermined
8209		7	Spleen, small in size, pitted, slight; cause of death undetermined

TABLE 1. Cont.

Summary of Individual Maternal Observations at Necropsy

Test Article, Dosage Level, Dam Number	Gestation Day of Sacrifice	Death	Necropsy Observations
Sample 01038003, <u>1500 mg/kg/day:</u>			
8210		7	No gross lesions; cause of death undetermined
8211		7	Hydrocele, left oviduct; cause of death undetermined
8212		7	No gross lesions; cause of death undetermined
8213		7	Liver, accentuated lobulation, all lobes; congested, moderate, pinpoint dark red foci, all lobes; esophagus, contains small hole; trachea, lining reddened, slight; cause of death intubation error
8214		7	No gross lesions; cause of death undetermined
Sample 01038003, <u>3000 mg/kg/day:</u>			
8215		8	No gross lesions; cause of death undetermined
8216		7	Kidney, pitted, moderate, bilateral; cause of death undetermined
8217		7	Hydrocele, oviduct, bilateral; cause of death undetermined
8218		7	Thoracic cavity, two abscesses; right dorsal, area attached to connective tissue, red in color and high vascularized; cause of death undetermined
8219		8	No gross lesions; cause of death undetermined

TABLE 2. Summary of Group Mean Maternal Body Weights and Body Weight Changes

Day of Gestation	Group Mean Maternal Body Weights (grams)									
	Sample 01038003 (mg/kg/day)									
	0 (Control)	100	300	750	1500	3000	Mean	Mean	Mean	Mean
0	2841	2820	2972	2903	2731	2762				
6	2965	2956	3121	3062	2860	2847				
12	2951	2778	2835	-	-	-				
18	2994	2555	2562	-	-	-				
24	3003	2814	-	-	-	-				
28	2991	2830	-	-	-	-				
Group Mean Maternal Body Weight Change (grams)										
0 to 6	124	136	149	159	129	85				
6 to 12	-14	-178	-286	-	-	-				
12 to 18	63	-223	-	-	-	-				
18 to 24	9	259	-	-	-	-				
24 to 28	-12	16	-	-	-	-				
6 to 28	26	-126	-	-	-	-				
0 to 28	150	10	-	-	-	-				

- Not applicable

TABLE 3. Individual and Group Mean Maternal Body Weights (grams)

Test Article, Dosage Level, Dam Number	Day of Gestation					
	0	6	12	18	24	28
<u>0 mg/kg/day (Control):</u>						
8190	3198	3392	3476	3616	3672	3668
8191	3062	3140	3148	2947	2774	2650
8192	2583	2655	2519	2732	2765	2812
8193	2340	2472	2376	2375	2505	2570
8194	3020	3164	3234	3298	3300	3256
Mean	2841	2965	2951	2994	3003	2991
<u>Sample 01038003, 100 mg/kg/day:</u>						
8195	3060	3276	3220	2830	2588	Died gesta- tion day 24
8196 ^b	2954	3068	3090	3096	3040	2830
8197	2813	2887	2600	2294	Died gestation day 19	
8198 ^a	2565	2734	2403	2219	Died gestation day 20	
8199 ^c	2454	2593	2203	1998	2185	2228
Mean	2820	2956	2778	2555	2814	2830
<u>Sample 01038003, 300 mg/kg/day:</u>						
8200 ^a	2586	2781	Died gestation day 10			
8201	2968	3154	2661	Died gestation day 17		
8202	2798	2858	Died gestation day 7			
8203 ^a	3152	3290	3104	2562	Died gestation day 21	
8204	3150	3352	3008	Died gestation day 17		
Mean	2972	3121	2835	-	-	-
<u>Sample 01038003, 750 mg/kg/day:</u>						
8205	2991	3208	Died gestation day 8			
8206 ^a	2921	2936	Died gestation day 7			
8207	2848	3022	Died gestation day 8			
8208 ^a	2455	2587	Died gestation day 10			
8209	2871	2956	Died gestation day 7			
Mean	2903	3062	-	-	-	-

^aNongravid, not included in calculation of mean

^bDam died gestation day 28, prior to uterine examination

^cDam aborted gestation day 22, not included in calculation of mean after gestation day 18

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- Not applicable

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TABLE 3. Cont. Individual and Group Mean Maternal Body Weights (grams)

Test Article, Dosage Level, Dam Number	Day of Gestation					
	0	6	12	18	24	28
<u>Sample 01038003, 1500 mg/kg/day:</u>						
8210	3128	3276	Died gestation day 7			
8211	2568	2595	Died gestation day 7			
8212	2353	2483	Died gestation day 7			
8213	2492	2641	Died gestation day 7			
8214	3114	3306	Died gestation day 7			
Mean	2731	2860	-	-	-	-
<u>Sample 01038003, 3000 mg/kg/day:</u>						
8215 ^a	2860	3014	Died gestation day 8			
8216 ^a	2508	2532	Died gestation day 7			
8217	2851	2910	Died gestation day 7			
8218	2550	2593	Died gestation day 7			
8219	2884	3038	Died gestation day 8			
Mean	2762	2847	-	-	-	-

^aNongravid, not included in calculation of mean
 - Not applicable

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TABLE 4. Summary of Group Mean Maternal Observations at Uterine Examination

	0 (Control)	Sample 01038003 (mg/kg/day)			
		100	300	750	1500
Animals on study:	5	5	5	5	5
Animals that were gravid:	5	4	3	3	3
Animals that died:	0	4	5	5	5
Nongravid:	0	1	2	2	0
Gravid:	0	3	3	3	5
Animals that aborted:	0	1	0	0	0
Animals examined at uterine examination:	5	0	0	0	0
Nongravid:	0	-	-	-	-
Gravid:	5	-	-	-	-
Dams with resorptions only:	0	-	-	-	-
Dams with viable fetuses:	5	-	-	-	-
Viable fetuses/dam:	7.2	-	-	-	-
Postimplantation loss/dam:	0.4	-	-	-	-
Total implantations/dam:	7.6	-	-	-	-
Corpora lutea/dam:	10.4	-	-	-	-
Group mean preimplantation loss (%): ^a	26.9	-	-	-	-
Group mean postimplantation loss (%): ^b	5.3	-	-	-	-

^aTotal No. Corpora Lutea - Total No. Implantations x 100 = Group Mean Preimplantation Loss (%)

Total No. Corpora Lutea

^bTotal No. Implantations - Total No. Viable Fetuses x 100 = Group Mean Postimplantation Loss (%)

Total No. Implantations

- Not applicable

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TABLE 5. Individual and Group Mean Maternal Observations at Uterine Examination

Test Article, Dosage Level, Dam Number	Viable Fetuses	Nonviable Fetuses	Late Resorptions	Early Resorptions	Post- implantation Loss	Total Implantations	Corpora Lutea
<u>0 mg/kg/day (Control):</u>							
8190	8	0	1	0	1	9	10
8191	7	0	0	0	0	7	9
8192	3	0	0	1	1	4	12
8193	7	0	0	0	0	7	9
8194	11	0	0	0	0	11	12
Total	36	0	1	1	2	38	52
Mean	7.2	0.0	0.2	0.2	0.4	7.6	10.4
<u>Sample 01038003, 100 mg/kg/day:</u>							
8195		Died - gravid					
8196		Died - gravid					
8197		Died - gravid					
8198		Died - nongravid					
8199		Aborted					
<u>Sample 01038003, 300 mg/kg/day:</u>							
8200		Died - nongravid					
8201		Died - gravid					
8202		Died - gravid					
8203		Died - nongravid					
8204		Died - gravid					

TABLE 5. Cont. Individual and Group Mean Maternal Observations at Uterine Examination

Test Article, Dosage Level, Dam Number	Viable Fetuses	Nonviable Fetuses	Late Resorptions	Early Resorptions	Post- implantation Loss	Total Implantations	Corpora Lutea
Sample 01038003,							
750 mg/kg/day:							
8205	Died - gravid						
8206	Died - nongravid						
8207	Died - gravid						
8208	Died - nongravid						
8209	Died - gravid						
Sample 01038003,							
1500 mg/kg/day:							
8210	Died - gravid						
8211	Died - gravid						
8212	Died - gravid						
8213	Died - gravid						
8214	Died - gravid						
Sample 01038003,							
3000 mg/kg/day:							
8215	Died - nongravid						
8216	Died - nongravid						
8217	Died - gravid						
8218	Died - gravid						
	Died - gravid						

APPENDIX I
Quality Assurance Inspections

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APPENDIX II
Personnel Involved in the Study

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Pilot Teratology Study in Rabbits

The following list of people were responsible for the supervision of various phases of this study:

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