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FINAL REPORT

Screening of Priority Chemicals for Potential Reproductive Hazard

NIOSH Contract No. 200-82-2542
Hazleton Study Nos. 6125-101 through 6125-110

for

Centers for Disease Control
Atlanta, Georgia

by

Hazleton Laboratories America, Inc.
Chemical & BioMedical Sciences Division
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As the chemical burden to which human beings are exposed increases, there is a need to identify, evaluate, and regulate compounds harmful to the human population. An increase in the incidence of occupationally-related reproductive dysfunctions (e.g., damaged germ cells, infertility, impotence) has produced a heightened awareness of the need to screen selected industrial chemicals and to develop priorities for more detailed traditional reproductive hazard testing. This testing program was conducted to determine the effects of 15 chemicals on reproductive performance and to evaluate their fetotoxicity when administered orally to pregnant mice on Days 7 through 14 of gestation.

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QUALITY ASSURANCE FINAL REPORT STATEMENT

Screening of Priority Chemicals
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HLA Study Nos. 6125-101 through 6125-110

The final report as herein attached for the above-mentioned study has been reviewed by the assigned Quality Assurance Unit of Hazleton Laboratories America, Inc. in accordance with the Good Laboratory Practice Regulations as set forth in 21 CFR 58.35 (b) (6) (7). It has been found to accurately identify and/or describe the authorized methods and standard operating procedures followed in the conduct of the study and that the reported data accurately reflect the raw data of the laboratory study. Furthermore, the Quality Assurance Unit has conducted the following inspections of the testing facilities utilized in the conduct of this study and has submitted written reports of said inspections to the study director and/or management.

Susan Glad Anderson

Susan Glad Anderson
Manager, Quality Assurance Unit

DATE 12/6/83

QUALITY ASSURANCE INSPECTIONS

NIOSH Contract No. 200-82-2542

HLA Study Nos. 6125-101 through 6125-110

Date of Inspection	Type of Inspection	Date Issued to Management
Experimental Block A		
12/02/82	Body Weights Animal Observations	12/02/82
1/13/83	Test Article Preparation	1/13/83
1/17/83	Body Weights Test Article Administration Animal/Cage Identification	1/17/83
2/03/83	Study Termination Data Review	2/03/83
Experimental Block B		
12/17/82	Test Article Preparation	12/17/82
2/16/83	Test Article Preparation	2/16/83
2/21/83	Animal/Cage Identification Body Weights Test Article Administration	2/21/83
3/04/83	Data Review	3/04/83
Experimental Block C		
1/19/83	Test Article Preparation	1/19/83
1/24/83	Test Article Administration Animal/Cage Identification	1/24/83
2/02/83	Data Review	2/02/83
3/11/83	Test Article Preparation	3/11/83
3/28/83	Data Review	3/28/83

QUALITY ASSURANCE INSPECTIONS

NIOSH Contract No. 200-82-2542

HLA Study Nos. 6125-101 through 6125-110

Experimental Block D		
3/07/83	Test Article Preparation	3/07/83
3/08/83	Test Article Administration	3/08/83
3/22/83	Data Review	3/22/83
4/28/83	Test Article Preparation	4/28/83
5/02/83	Test Article Administration	5/02/83
5/18/83	Study Termination	5/18/83
5/18-19/83	Data Review	5/19/83
Experimental Block E		
4/25/83	Test Article Preparation	4/25/83
4/28/83	Test Article Administration	4/28/83
5/11/83	Data Review	5/11/83
6/23/83	Test Article Preparation	6/23/83
6/27/83	Test Article Administration Animal/Cage Identification	6/27/83
7/07/83	Data Review	7/07/83
7/13/83	Study Termination Body Weights	7/13/83
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ABSTRACT

As the chemical burden to which human beings are exposed increases, there is a need to identify, evaluate, and regulate compounds harmful to the human population. An increase in the incidence of occupationally-related reproductive dysfunctions (e.g., damaged germ cells, infertility, impotence) has produced a heightened awareness of the need to screen selected industrial chemicals and to develop priorities for more detailed traditional reproductive hazard testing.

This testing program was conducted to determine the effects of 15 chemicals on reproductive performance and to evaluate their fetotoxicity when administered orally to pregnant mice on Days 7 through 14 of gestation.

The test system used was a modification of the method developed by Chernoff and Kavlock ("A Potential In Vivo Screen for the Determination of Teratogenic Effects in Mammals.," Teratology 21, (2): 33A, 1980.) as a postnatal screening test.

The results indicate that di(n-butyl)phthalate, di(isobutyl)phthalate, N-isopropyl-N'-phenyl-p-phenylenediamine, di(2-ethylhexyl)phthalate, 2-ethyl-1-hexanol, di(n-hexyl)phthalate, and mono(2-ethylhexyl)phthalate warrant high priority consideration for more detailed reproductive toxicity testing; while diethyl phthalate, dimethyl methylphosphonate, tris(2-chloroethyl)phosphate, d-phenylalanine, probenecid, trans-cinnamaldehyde, di(isodecyl)phthalate, and di(n-octyl)phthalate warrant lower priority consideration for more detailed reproductive toxicity testing.

This report was submitted in partial fulfillment of Contract No. 200-82-2542 by the Chemical & BioMedical Sciences Division of Hazleton Laboratories America, Inc., Madison, Wisconsin, under the sponsorship of the Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health, and the Centers for Disease Control.

INTRODUCTION

The purpose of the studies conducted in this testing program was to determine the effects of test materials on reproductive function and to evaluate their fetotoxicity when administered orally to pregnant mice on Days 7-14 of gestation. All aspects of work performed in this program were conducted in accordance with the U.S. Food and Drug Administration's Good Laboratory Practice Regulations for Nonclinical Laboratory Studies.[1]

In this program, 15 chemicals were screened in groups of three. Each group of three chemicals was tested against a concurrent vehicle control, and was termed an experimental block. Each block consisted of a test, hereinafter referred to as the MED Study, designed to determine for each chemical the minimum effective dose (MED) for adult female mice, followed by the screening test proper, hereinafter referred to as the Reproduction Study, which utilized, for each chemical, the MED previously determined.

This program was carried out at Hazleton Laboratories America, Inc. (HLA), Chemical & BioMedical Sciences Division in Madison, Wisconsin from November 9, 1982 through July 13, 1983.

TEST MATERIALS

Identification

A list of chemicals to be tested was supplied by NIOSH; these chemicals were purchased by HLA or supplied by NIOSH. The following information (when available) was compiled for each chemical.

- Physical/chemical properties
- Composition
- Toxicity
- Stability
- Safety (safety data sheet)
- Storage conditions

For each chemical, the identity was confirmed and the purity was determined by HLA.

Chemicals, the doses at which they were tested, and study dates are included in Appendix A.

Disposal

Any remaining dosing solutions, all animal wastes, bedding, and carcasses resulting from these studies were disposed of in a high-temperature incinerator (U.S. Smelting Furnace Company, Belleville, Illinois).

TEST SYSTEM

Test Animal

Charles River specific pathogen-free (SPF) CD-1 female mice (6 to 8 weeks old) were purchased from the Kingston, New York facility of Charles River Breeding Laboratories, Wilmington, Massachusetts, with the exception of mice for the Reproduction Study in Experimental Block A that were purchased from the St. Constant, Canada facility of Charles River Breeding Laboratories because of the unavailability of mice from the Kingston, New York facility. Virgin females were used for the MED studies, and timed-pregnant females were used for the reproduction studies.

Acclimation

Upon receipt, the animals were taken to a designated animal room where they were acclimated for 4 to 5 days before being placed on test. During acclimation, the animals were examined for clinical abnormalities indicative of health problems (e.g., diarrhea, ectoparasites, rough hair coat, nasal or ocular discharge, evidence of injury, etc.). Any animals regarded as unsuitable for the study because of improper size, poor physical condition, or other reasons were discarded, and the reason(s) were documented.

Identification

Prior to being placed on test, each animal was assigned a permanent identification number and was identified by an ear-punch/toe clip method. All data collected from an animal were recorded and filed under its identification number.

Housing and Maintenance

One animal room was assigned solely to each study. The following environmental conditions were maintained.

- Temperature: 72°F ±3°
- Relative humidity: 50% ±20%
- Air change: at least 10 changes an hour of filtered 100% outside air
- Light cycle: 12 hours light/12 hours dark

Temperature and humidity were monitored continuously by a JC/80 computer system (Johnson Controls, Madison, Wisconsin). There were no variations from prescribed environmental conditions.

Animal husbandry and housing at HLA comply with standards outlined in the "Guide for the Care and Use of Laboratory Animals."[2] Care was taken to ensure that the animals were not disturbed for reasons other than treatment, data collection, and routine maintenance. During MED studies, animals were housed five per cage (one dose level per cage) in plastic shoe box cages;

during reproduction studies, animals were housed individually in plastic cages. A bedding material consisting of heat-treated hardwood chips covered the bottom of the cages. Bedding was changed at least twice weekly during MED studies, and at least once weekly until Day 18 of gestation during reproduction studies.

Feed used for these studies was the pellet form of Purina Certified Rodent Chow® #5002 and was provided ad libitum. This feed was analyzed by the manufacturer for nutritional components and environmental contaminants; no contaminants were known to be in this feed that were expected to interfere with the studies. The following lots of feed were used for these studies:

0909822 M	1207822 C	0331831 A
0928822 C	0113832 A	0406831 D
1020821 G	0308832 H	0413832 J

Tap water was provided ad libitum fresh daily in glass water bottles fitted with stainless steel sipper tubes. HLA analyzes samples of the tap water quarterly for total dissolved solids, conductivity, microbiological content, selected elements, heavy metals, organophosphates, and chlorinated hydrocarbons. Results of these analyses are on file at HLA.

Experimental Design

A standard screening protocol was employed in which the test materials and vehicle were administered by oral gavage. The vehicle was corn oil [USP equivalent, Sigma Chemical Company, St. Louis, Missouri (MED Study - Experimental Block A); or Mazola®, Corn Products Company, Englewood Cliffs, New Jersey (all other studies)]; dose volumes were 10 mL/kg body weight/day and were administered to the nearest 0.01 mL in all studies except the MED Study for Experimental Block A when doses were administered to the nearest 0.1 mL. Animals were treated between 9 a.m. and noon, once daily for 8 consecutive days [Days 7 through 14 of gestation for reproduction studies (Day 1 of gestation was the day on which a copulatory plug was observed by the animal supplier.)].

MED Studies. For each chemical, dose levels were specified by NIOSH (Appendix A) and administered to groups of 10 nonpregnant animals. A concurrent vehicle control group of 50 animals was maintained with each block of three chemicals. The animal weight on the first day of dosing determined the dose volume administered over the entire treatment period.

All animals were observed for clinical signs of toxicity during the hour following dosing and again at least 5 hours after the first observations, unless toxic signs dictated more frequent observations. Animals were observed at least once daily after the eighth day of dosing until they were sacrificed on the eighth day following the final dose. Clinical observations were recorded using the terms given in Appendix B. Animals that died were subjected to a gross necropsy to determine whether dosing error (e.g., instillation into the lungs, perforation of the esophagus, etc.) was the cause of death.

Body weight was recorded for each animal at the time of randomization, on the first day of dosing (Day 1, used to determine each animal's daily dosage), the last day of dosing (Day 8), and 4 and 8 days after the final dose (Days 12 and 16, respectively). Body weights were recorded to the nearest 0.1 g, except in the MED Study for Experimental Block A, in which body weights were recorded to the nearest 1 g.

Mean body weight per treatment group for each day of weighing and mean body weight change (from Day 1) per treatment group were compared with the concurrent control group. On the basis of these results and of the clinical observation and mortality data, the MED was estimated. The MED was defined as the highest dose that resulted in a small number of mortalities and/or significant weight loss when compared with control data.

Reproduction Studies. In these studies, the MED for each chemical was administered to a group of 50 timed-pregnant mice. A concurrent vehicle control group of 50 timed-pregnant mice was maintained for each experimental block. All animals were observed at least once daily for clinical signs of toxicity.

Clinical observations were recorded using the terms given in Appendix B. Animals that died (and animals that were sacrificed in a moribund condition - Experimental Block A only) were subjected to a gross necropsy to determine whether dosing error (e.g., instillation into the lungs, perforation of the esophagus, etc.) was the cause of death.

Body weight was recorded at the time of randomization, on Days 7 through 14 of gestation (immediately prior to dosing - this weight was used to determine each animal's dosage for that day), on Day 18 of gestation, and on Day 3 post-partum. Body weights were recorded to the nearest 0.1 g.

Mean body weight for pregnant animals per treatment group on Days 7, 14, and 18 of gestation and on Day 3 post-partum, as well as mean body weight change for pregnant animals during treatment (Days 7 through 14 of gestation) and during pregnancy (Days 7 through 18 of gestation) were calculated and compared with those of the concurrent vehicle control group. Data from animals that died were excluded from calculations.

Animals were allowed to deliver and care for their own young with a minimum of disturbance. The first day an entire litter was observed was considered Day 1 of lactation for that litter. The number of live pups, dead pups, and total litter weight (of live pups) were recorded for each litter on Days 1 and 3.

The percent of live pups and dead pups, mean pup weight change from lactation Days 1 to 3, mean percent pup weight change from lactation Days 1 to 3, and mean pup viability per litter from lactation Days 1 to 3 [(number of pups alive at Day 3/number of pups alive at Day 1) x 100] were calculated for each treatment group and compared with those of controls.

On Day 3 of lactation, dams and litters were sacrificed and discarded. All animals that failed to deliver by Day 23 of gestation were sacrificed and the status of pregnancy was determined by opening all nongravid uteri and placing them in a 10% solution of ammonium sulfide. The reproductive index [(number

of females that had a viable litter/number of surviving females that were proven pregnant) x 100] was determined for each treatment group and compared with that of controls. A viable litter was defined as a litter that had at least one live pup on Day 1. Females were considered pregnant if they had litters or if their uteri stained positive with 10% ammonium sulfide.

Statistical Analysis

Differences between groups were considered statistically significant at $p < 0.05$. Body weights, body weight changes, and litter weights were analyzed using analysis of variance (ANOVA).[3] If the group means differed significantly by ANOVA, then Dunnett's t-test was used to compare the means of the treated groups with the mean of the control group.[3]

Litter size, percent of live and dead pups, and percent weight change were analyzed using the Kruskal-Wallis (KW) test.[4] If the group means differed significantly by the KW test, then Dunn's test was used to compare the means of the treated groups with the mean of the control group.[5]

Reproductive and viability indices were analyzed by contingency table techniques.[6]

RESULTS

VEHICLE CONTROL INTERBLOCK COMPARISONS

Summary tables for the interblock comparisons of vehicle control groups are presented immediately following the text.

MED Studies

All control animals for MED studies received 10 mL/kg body weight/day of corn oil.

The only deaths in control groups occurred in Experimental Blocks A and D, where one animal in each block died because of dosing error. No deaths were recorded for control groups in Experimental Blocks B, C, or E.

Clinical signs of toxicity for controls in Experimental Blocks A and D are presented in Tables 1 and 2, respectively. These incidences of clinical signs were negligible; there were no clinical signs of toxicity recorded for controls in Experimental Blocks B, C, or E.

A summary of mean body weight data for vehicle control animals is presented in Table 3. These data were consistent among the five MED Study control groups; the maximum variance of body weights was ± 4 g, and the maximum variance of body weight changes was ± 1 g.

Reproduction Studies

All control animals for reproduction studies received 10 mL/kg body weight/day of corn oil.

There were no deaths in the control group of any experimental block. Clinical signs of toxicity in control animals were recorded only for Experimental Blocks A and C (Tables 4 and 5), and, as in the MED studies, were negligible.

A summary of mean pregnancy and female body weight data for vehicle control animals is presented in Table 6. The reproductive index, as well as body weight data for pregnant animals were consistent among the five experimental blocks. The maximum variance of body weights was ± 5 g; the maximum variance of body weight changes was ± 2 g.

A summary of mean litter and viability data for vehicle control animals is presented in Table 7. Although variations among experimental blocks were seen in total litter weights at Days 1 and 3 post-partum, mean pup weights per litter were consistent among experimental blocks.

There were four females in Experimental Block C and one female in Experimental Block E whose pups were dead on Day 1 post-partum; however, pup viability from Days 1 to 3 post-partum were consistent among experimental blocks.

Study No. 6125-101

Table 1

MED Study: Block A
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	1
Prostrate	
Ataxia	
Hunched	
Tremors	
Head tilt	
Thin	1
Wheezing	
Dyspnea	
Urine stains	
Alopecia	
Rough hair coat	
Sores	
Piloerection	

Study No. 6125-107

Table 2

MED Study: Block D
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	1[a]
Prostrate	
Ataxia	
Hunched	1[a]
Tremors	
Head tilt	
Thin	
Wheezing	
Dyspnea	
Urine stains	
Alopecia	1
Rough hair coat	1[a]
Sores	
Piloerection	

[a]Animal died because of a dosing error.

Table 3

MED Studies
Vehicle Control Interblock Comparison of Body Weight Data

	Vehicle Control Group (Experimental Block)				
	A	B	C	D	E
Weight Day 1					
N	50	50	50	50	50
Mean	26[a]	24.5	28.4	25.1	26.8
S[b]	1.3	1.16	1.34	1.00	1.03
Weight Day 8					
N	50	50	50	50	50
Mean	26	24.7	28.3	25.2	27.0
S	2.0	1.74	1.35	1.45	1.14
Weight Day 12					
N	49[c]	50	50	49[c]	50
Mean	27	25.8	29.5	26.6	28.0
S	2.1	1.76	1.60	1.22	1.22
Weight Day 16					
N	49[c]	50	50	49[c]	50
Mean	27	25.9	30.1	27.1	28.8
S	2.0	1.93	1.60	1.31	1.46
Weight Change Days 1-8					
N	50	50	50	50	50
Mean	0	0.2	-0.1	0.0	0.3
S	1.7	1.14	0.65	1.40	0.96
Weight Change Days 1-12					
N	49[c]	50	50	49[c]	50
Mean	1	1.3	1.1	1.5	1.3
S	1.9	1.26	0.84	1.36	0.92
Weight Change Days 1-16					
N	49[c]	50	50	49[c]	50
Mean	1	1.4	1.7	2.0	2.1
S	1.8	1.41	1.15	1.42	1.20

[a]Body weights for the MED Study, Experimental Block A recorded to nearest 1 g.
[b]S: Standard deviation.
[c]One death due to dosing error.

Study No. 6125-102

Table 4

Reproduction Study: Block A
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

	Number of Animals Showing Sign		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Purulent - uterine horns and cervix			1

Study No. 6125-106

Table 5

Reproduction Study: Block C
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

	Number of Animals Showing Sign		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Scab formation involving hair	1	1	1
Red exudate (vagina)	1		

Table 6

Reproduction Studies
Vehicle Control Interblock Comparison of
Pregnancy and Female Body Weight Data

	Vehicle Control Group (Experimental Block)				
	A	B	C	D	E
Number of females on test	50	50	50	50	50
Number of females found dead (excluded from results)	0	0	0	0	0
Number of females that survived	50	50	50	50	50
Number of females that produced viable litters[a]	32	37	39	33	34
Number of females with resorbed or nonviable litters	1	2	7	1	4
Number of proven pregnant females	33	39	46	34	38
Reproductive index					
[(Number of females that produced viable litters/number of proven pregnant females) x 100]	97	95	85	97	89
Mean Body Weights (g) for Pregnant Females					
Gestation Day 7					
N	33	39	46	34	38
Mean	27.3	26.2	28.0	29.9	28.8
S[b]	1.41	1.63	2.65	2.35	1.92
Gestation Day 14					
N	33	39	46	34	38
Mean	36.2	33.4	35.4	37.1	38.2
S	3.04	2.33	3.75	2.89	3.63
Gestation Day 18					
N	33	39	46	34	38
Mean	46.8	44.1	45.9	47.9	48.8
S	5.29	5.00	6.15	5.01	6.88

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.

Table 6 (Continued)

Reproduction Studies
Vehicle Control Interblock Comparison of
Pregnancy and Female Body Weight Data

	Vehicle Control Group (Experimental Block)				
	A	B	C	D	E
Post-partum Day 3					
N	32	37	39	33	34
Mean	34.7	34.4	35.8	36.8	39.3
S[a]	3.67	2.38	3.17	2.68	2.90
Mean Body Weight Changes (g) for Pregnant Females					
During Treatment (Days 7-14 of gestation)					
N	33	39	46	34	38
Mean	9.0	7.2	7.4	7.2	9.4
S	2.83	2.04	2.36	2.72	3.17
During Pregnancy (Days 7-18 of gestation)					
N	33	39	46	34	38
Mean	19.5	17.9	18.0	18.0	20.0
S	5.09	5.05	5.30	5.18	6.64

[a]S: Standard deviation.

Table 7

Reproduction Studies
Vehicle Control Interblock Comparison of
Group Litter and Viability Data

	Vehicle Control Group (Experimental Block)				
	A	B	C	D	E
Number of females that produced viable litters[a]	32	37	39	33	34
Post-partum Day 1					
Number of live pups/litter					
N	32	37	39	33	34
Mean	9.5	9.0	9.5	9.9	11.5
S[b]	2.03	3.11	2.60	2.36	1.73
Number of dead pups/litter					
N	32	37	39	33	34
Mean	0.0	0.6	0.4	0.1	0.0
S	0.00	1.83	1.27	0.29	0.00
Percent of live pups/litter					
N	32	37	39	33	34
Mean	100.0	93.6	95.1	99.2	100.0
S	0.00	18.48	14.38	2.56	0.00
Percent of dead pups/litter					
N	32	37	39	33	34
Mean	0.0	6.4	4.9	0.8	0.0
S	0.00	18.48	14.38	2.56	0.00
Litter weight (g)					
N	32	37	39	33	34
Mean	15.6	14.4	14.7	16.0	18.6
S	2.89	4.56	3.60	3.30	2.24
Mean pup weight/litter (g)					
N	32	37	39	33	34
Mean	1.7	1.6	1.6	1.6	1.6
S	0.13	0.17	0.13	0.13	0.12

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.

Table 7 (Continued)

Reproduction Studies

Vehicle Control Interblock Comparison of
Group Litter and Viability Data

	Vehicle Control Group (Experimental Block)				
	A	B	C	D	E
Post-partum Day 3					
Number of live pups/litter					
N	32	37	39[a]	33	34
Mean	9.4	8.9	9.4	9.8	11.5
S[b]	2.03	3.16	2.82	2.46	1.71
Number of dead pups/litter					
N	32	37	39[a]	33	34
Mean	0.1	0.1	0.1	0.1	0.0
S	0.25	0.39	0.41	0.24	0.17
Percent of live pups/litter					
N	32	37	39[a]	33	34
Mean	99.4	98.5	96.4	98.2	99.8
S	2.46	6.78	16.30	8.80	1.37
Percent of dead pups/litter					
N	32	37	39[a]	33	34
Mean	0.6	1.5	3.6	1.8	0.2
S	2.46	6.78	16.30	8.80	1.37
Mean pup viability/litter (Days 1 to 3)					
N	32	37	39[a]	33	34
Mean	99.4	98.4	96.4	98.2	99.8
S	2.46	6.80	16.30	8.80	1.32
Litter weight (g)					
N	32	37	38	33	34
Mean	19.9	18.4	18.8	21.6	25.8
S	3.62	5.81	4.27	4.75	3.24
Mean pup weight/litter (g)					
N	32	37	38	33	34
Mean	2.1	2.1	2.0	2.2	2.3
S	0.30	0.33	0.20	0.19	0.20
Mean percent pup weight change (Days 1 to 3)					
N	32	37	38	33	34
Mean	29.4	30.0	26.7	35.3	39.0
S	12.19	9.79	7.29	6.28	6.07

[a]Includes one female whose pups were dead on Day 3.
[b]S: Standard deviation.

EXPERIMENTAL BLOCK A

Data referred to in the following sections are tabulated and discussed by experimental block. Tables for MED studies can be found immediately following the text associated with each chemical; tables for reproduction studies can be found immediately following the text associated with each experimental block.

MED Study No. 6125-101.
Diethyl phthalate (DEP). (Tables 8-11)

Dose levels used:
- Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
- Group 2 - 1,000 mg DEP/kg/day
- Group 3 - 1,830 mg DEP/kg/day
- Group 4 - 3,345 mg DEP/kg/day
- Group 5 - 6,120 mg DEP/kg/day
- Group 6 - 11,200 mg DEP/kg/day (undiluted)

One animal in the control group died 2 days after the end of treatment; this death was due to a dosing error. All of the mice treated with 11,200 mg DEP/kg were dead by the second day of treatment and one-half the animals in the 6,120-mg/kg group were dead by the third day (Table 8). No dosing errors were evident. No deaths occurred at lower doses. The most frequent clinical observation in these animals was languidity which was seen on the first day of treatment in the animals that died on the second day of treatment (Tables 9 and 10). There were no significant differences in body weights or body weight changes for mice treated with DEP (Table 11).

Based on these data, a dose level of 4,500 mg DEP/kg/day was used as the MED in the reproduction study.

Table 8

Survival of Mice in the MED Study
for Experimental Block A

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days								Mice Alive on Day 8		Mice Alive on Day 16	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	49[a]	98
Diethyl phthalate													
1,000	10	0	0	0	0	0	0	0	0	10	100	10	100
1,830	10	0	0	0	0	0	0	0	0	10	100	10	100
3,345	10	0	0	0	0	0	0	0	0	10	100	10	100
6,120	10	1	3	1	0	0	0	0	0	5	50	5	50
11,200†	10	3	7	-	-	-	-	-	-	0	0	0	0
Di(n-butyl)phthalate													
1,000	10	0	0	0	0	0	0	0	0	10	100	10	100
1,800	10	0	0	0	0	0	0	0	0	10	100	10	100
3,240	10	0	0	0	1	0	1	0	0	8	80	8	80
5,035	10	0	2	4	2	0	0	0	0	2	20	1	10
10,500†	10	1	0	3	0	2	0	0	0	4	40	4	40
Di(isobutyl)phthalate													
1,000	10	0	0	0	0	0	0	0	0	10	100	10	100
1,795	10	0	0	0	0	0	0	0	0	10	100	10	100
3,225	10	0	0	0	0	0	0	0	0	10	100	9[a]	90
5,790	10	1	0	2	2	0	0	0	0	5	50	5	50
10,400†	10	0	0	0	0	0	0	0	2[b]	8[b]	80	8[b]	80

†Undiluted.
[a]One death due to dosing error.
[b]Two deaths due to dosing error.

Study No. 6125-101

Table 9

MED Study: Block A
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	1
Prostrate	
Ataxia	
Hunched	
Tremors	
Head tilt	
Thin	1
Wheezing	
Dyspnea	
Urine stains	
Alopecia	
Rough hair coat	
Sores	
Piloerection	

Study No. 6125-101

Table 10

MED Study: Block A
Incidence of Clinical Signs: Diethyl phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day)				
	1,000	1,830	3,345	6,120	11,200
Languid				2	7
Prostrate					
Ataxia					
Hunched					
Tremors					
Head tilt					
Thin					
Wheezing					
Dyspnea					
Urine stains					
Alopecia					
Rough hair coat					
Sores					
Piloerection					
Opaque eyes					2

Table 11
Summary of Mean Body Weight Data (g)
Diethyl phthalate

MG/KG		0	1,000	1,830	3,345	6,120	11,200
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	26	25	26	25	26	26
	S[a]	1.3	0.9	0.9	1.3	1.0	0.9
	SE[b]	0.2	0.3	0.3	0.4	0.3	0.3
WEIGHT DAY 8							
	NO	50	10	10	10	5	0
	MEAN	26	26	27	26	27	--
	S	2.0	1.5	1.1	1.5	1.4	0.0
	SE	0.3	0.5	0.3	0.5	0.6	0.0
WEIGHT DAY 12							
	NO	49[c]	10	10	10	5	0
	MEAN	27	26	27	26	27	--
	S	2.1	1.1	1.2	1.2	1.4	0.0
	SE	0.3	0.3	0.4	0.4	0.6	0.0
WEIGHT DAY 16							
	NO	49[c]	10	10	10	5	0
	MEAN	27	27	27	27	27	--
	S	2.0	1.7	1.6	1.3	1.6	0.0
	SE	0.3	0.5	0.5	0.4	0.7	0.0
WT. CHANGE DAYS 1-8							
	NO	50	10	10	10	5	0
	MEAN	0	1	1	0	2	--
	S	1.7	1.1	1.1	1.1	1.3	0.0
	SE	0.2	0.3	0.3	0.3	0.6	0.0
WT. CHANGE DAYS 1-12							
	NO	49[c]	10	10	10	5	0
	MEAN	1	0	1	1	2	--
	S	1.9	1.1	0.8	1.3	1.3	0.0
	SE	0.3	0.3	0.2	0.4	0.6	0.0
WT. CHANGE DAYS 1-16							
	NO	49[c]	10	10	10	5	0
	MEAN	1	1	1	1	2	--
	S	1.8	1.6	1.3	1.4	1.4	0.0
	SE	0.3	0.5	0.4	0.4	0.6	0.0

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.

Di(n-butyl)phthalate (DNBP). (Tables 8, 9, 12, and 13)

Dose levels used: Group 1 - Vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,000 mg DNBP/kg/day
Group 3 - 1,800 mg DNBP/kg/day
Group 4 - 3,240 mg DNBP/kg/day
Group 5 - 5,035 mg DNBP/kg/day
Group 6 - 10,500 mg DNBP/kg/day (undiluted)

One animal in the control group died 2 days after the end of treatment; this death was due to a dosing error. Survival was 80%, 10%, and 40% in mice treated with 3,240, 5,035, and 10,500 mg DNBP/kg, respectively (Table 8). With the exception of one animal that died on Day 9, the deaths occurred during Days 1 through 6 of treatment. No dosing errors were evident. Languidity was observed in all groups except the lowest dose level (Tables 9 and 12). There were no significant differences in body weights or body weight changes for mice treated with DNBP (Table 13).

Based on these data, a dose level of 2,500 mg DNBP/kg/day was used as the MED in the reproduction study.

Study No. 6125-101

Table 12

MED Study: Block A
Incidence of Clinical Signs: Di(n-butyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day) 1,000	1,800	3,240	5,035	10,500
Languid		1	4	8	6
Prostrate			1	6	5
Ataxia					
Hunched					
Tremors					
Head tilt					
Thin					
Swollen head		1			
Wheezing					
Dyspnea					
Urine stains					
Alopecia					
Rough hair coat					
Sores					
Piloerection					

T-125018 TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK A, STUDY NO. 6125-104

Table 13
Summary of Mean Body Weight Data (g)
Di(n-butyl)phthalate

MG/KG		0	1,000	1,800	3,240	5,035	10,500
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	26	26	26	25	25	25
	S[a]	1.3	1.2	1.1	0.7	0.9	1.2
	SE[b]	0.2	0.4	0.3	0.2	0.3	0.4
WEIGHT DAY 8							
	NO	50	10	10	8	2	4
	MEAN	26	26	26	26	25	25
	S	2.0	1.2	1.0	0.7	1.4	0.0
	SE	0.3	0.4	0.3	0.3	1.0	0.0
WEIGHT DAY 12							
	NO	49[c]	10	10	8	1	4
	MEAN	27	27	26	25	25	25
	S	2.1	1.5	1.2	0.5	0.0	0.6
	SE	0.3	0.5	0.4	0.2	0.0	0.3
WEIGHT DAY 16							
	NO	49[c]	10	10	8	1	4
	MEAN	27	27	26	26	25	25
	S	2.0	1.7	1.4	0.7	0.0	1.2
	SE	0.3	0.5	0.4	0.2	0.0	0.6
WT. CHANGE DAYS 1-8							
	NO	50	10	10	8	2	4
	MEAN	0	1	0	0	0	0
	S	1.7	1.2	0.9	1.2	1.4	0.5
	SE	0.2	0.4	0.3	0.4	1.0	0.2
WT. CHANGE DAYS 1-12							
	NO	49[c]	10	10	8	1	4
	MEAN	1	1	0	0	0	0
	S	1.9	1.5	1.3	1.0	0.0	0.5
	SE	0.3	0.5	0.4	0.4	0.0	0.3
WT. CHANGE DAYS 1-16							
	NO	49[c]	10	10	8	1	4
	MEAN	1	2	0	1	0	0
	S	1.8	1.6	1.3	1.2	0.0	1.0
	SE	0.3	0.5	0.4	0.4	0.0	0.5

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.

Di(isobutyl)phthalate (DIBP). (Tables 8, 9, 14, and 15)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,000 mg DIBP/kg/day
Group 3 - 1,795 mg DIBP/kg/day
Group 4 - 3,225 mg DIBP/kg/day
Group 5 - 5,790 mg DIBP/kg/day
Group 6 - 10,400 mg DIBP/kg/day (undiluted)

The only treatment-related death associated with this compound occurred between Days 1 and 4 of treatment in mice which were given 5,790 mg DIBP/kg; 50% of these animals died (Table 8). The deaths of one animal each in the control group and in the group treated with 3,225 mg DIBP/kg, and of two animals in the 10,400-mg/kg group were due to dosing errors. Languidity was observed for a brief period (1 to 3 days between Days 1 and 4 of treatment) in mice treated with 3,225 and 5,790 mg DIBP/kg (Tables 9 and 14). Animals in the highest dose group had urine stains on the abdomen and in the anal region at various times during the dosing interval and had rough hair coat during Days 6 through 8 or 11 of treatment. There were no significant differences in body weights or body weight changes for mice treated with DIBP (Table 15).

Based on these data, a dose level of 4,000 mg DIBP/kg/day was used as the MED in the reproduction study.

Study No. 6125-101

Table 14

MED Study: Block A
Incidence of Clinical Signs: Di(isobutyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day) 1,000	1,795	3,225	5,790	10,400
Languid			2	5	
Prostrate				4	
Ataxia					
Hunched					
Tremors					
Head tilt					
Thin					
Wheezing					
Dyspnea					
Urine stains					10[a]
Alopecia					
Rough hair coat					10[a]
Sores					
Piloerection					
Opaque eyes				1	
Discoloration (hair yellow)				1	

[a]Includes two animals that died because of a dosing error.

Table 15
Summary of Mean Body Weight Data (g)
Di(isobutyl)phthalate

MG/KG		0	1,000	1,795	3,225	5,790	10,400
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	26	26	26	26	25	25
	S[a]	1.3	1.3	1.4	1.4	0.5	1.2
	SE[b]	0.2	0.4	0.5	0.4	0.2	0.4
WEIGHT DAY 8							
	NO	50	10	10	10	5	10
	MEAN	26	26	27	26	26	26
	S	2.0	1.3	1.4	2.0	0.5	1.9
	SE	0.3	0.4	0.5	0.6	0.2	0.6
WEIGHT DAY 12							
	NO	49[c]	10	10	9[c]	5	8[d]
	MEAN	27	26	26	26	27	26
	S	2.1	1.4	1.1	1.7	1.1	1.6
	SE	0.3	0.5	0.4	0.6	0.5	0.5
WEIGHT DAY 16							
	NO	49[c]	10	10	9[c]	5	8[d]
	MEAN	27	27	27	26	26	26
	S	2.0	1.6	1.5	1.4	1.3	1.8
	SE	0.3	0.5	0.5	0.5	0.6	0.7
WT. CHANGE DAYS 1-8							
	NO	50	10	10	10	5	10
	MEAN	0	0	1	0	1	1
	S	1.7	0.8	0.9	1.3	0.8	1.3
	SE	0.2	0.3	0.3	0.4	0.4	0.4
WT. CHANGE DAYS 1-12							
	NO	49[c]	10	10	9[c]	5	8[d]
	MEAN	1	1	1	0	1	1
	S	1.9	0.8	0.8	0.7	1.1	0.9
	SE	0.3	0.3	0.3	0.2	0.5	0.3
WT. CHANGE DAYS 1-16							
	NO	49[c]	10	10	9[c]	5	8[d]
	MEAN	1	1	1	1	1	1
	S	1.8	1.2	0.9	0.9	1.2	1.2
	SE	0.3	0.4	0.3	0.3	0.5	0.4

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.
d Two deaths due to dosing errors.

Reproduction Study No. 6125-102
Diethyl phthalate (DEP). (Tables 16-18, 21, and 22)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 4,500 mg DEP/kg/day

No deaths were recorded for the vehicle control group (Table 16); however, one female had purulent uterine horns and cervix when sacrificed after 23 days of gestation (Table 17).

One animal treated with DEP died on Day 9 of gestation (i.e., Day 3 of treatment); on Day 8 of gestation (Day 2 of treatment), one animal was sacrificed in a moribund condition (languidity and dyspnea were reported for this animal); and one animal aborted on Day 3 of gestation (Tables 16 and 18). No evidence of dosing error was found in these animals. No other clinical signs of toxicity were recorded.

Group pregnancy and female body weight data are presented in Table 21. No significant differences from control data were seen in the parameters examined.

Group litter and viability data are presented in Table 22. No significant differences from control data were seen in the parameters examined.

Di(n-butyl)phthalate (DNBP). (Tables 16, 17, 19, 21, and 22)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 2,500 mg DNBP/kg/day

No deaths were recorded for the vehicle control group (Table 16); however, one female had purulent uterine horns and cervix when sacrificed after 23 days of gestation (Table 17).

On Day 8 of gestation (i.e., Day 2 of treatment), one animal treated with DNBP died because of a dosing error, and four animals were sacrificed in a moribund condition (Table 16). Clinical signs of toxicity included languidity, prostration, dyspnea, and coldness to touch, and were reported only for those animals that died or were sacrificed (Table 19).

Group pregnancy and female body weight data are presented in Table 21. The reproductive index, body weights on Days 14 and 18 of gestation, and body weight changes during treatment and pregnancy were significantly lower than those of controls.

No viable litters were produced by females treated with 2,500 mg DNBP/kg/day (Table 22).

Di(isobutyl)phthalate (DIBP). (Tables 16, 17, and 20-22)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 4,000 mg DIBP/kg/day

No deaths were recorded for the vehicle control group (Table 16); however, one female had purulent uterine horns and cervix when sacrificed after 23 days of gestation (Table 17).

Eleven animals treated with DIBP died [three each on Days 8, 9, and 10 of gestation (i.e., Days 2, 3, and 4 of treatment, respectively), and two on Day 12 of gestation (Day 6 of treatment)]; sixteen animals were sacrificed in a moribund condition [one on Day 7 of gestation, twelve on Day 8 of gestation, and three on Day 9 of gestation (i.e., Days 1, 2, and 3 of treatment, respectively)]. No dosing errors were evident (Table 16). Clinical signs of toxicity included languidity, prostration, dyspnea, and coldness to touch, and with the exception of one animal, were reported only for those animals that died or were sacrificed (Table 20).

Group pregnancy and female body weight data are presented in Table 21. The reproductive index, body weights on Days 14 and 18 of gestation, and body weight changes during treatment and pregnancy were significantly lower than those of controls.

No viable litters were produced by females treated with 4,000 mg DIBP/kg/day (Table 22).

Table 16

Survival of Mice in the Reproduction Study
for Experimental Block A

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days[a] 1	2	3	4	5	6	7	8	Mice Alive on Day 8 Number	Percent	Mice Alive at Study End Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
Diethyl phthalate													
4,500	50	0	1	1	0	0	0	0	0	48	96	48	96
Di(n-butyl)phthalate													
2,500	50	0	5[b]	0	0	0	0	0	0	45[b]	90	44[b]	88
Di(isobutyl)phthalate													
4,000	50	1	15	6	3	0	2	0	0	23	46	23	46

[a]Treatment Days 1-8 = Gestation Days 7-14.
[b]One death due to dosing error.

Study No. 6125-102

Table 17

Reproduction Study: Block A
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

	Number of Animals Showing Sign		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Purulent - uterine horns and cervix			1

Study No. 6125-102

Table 18

Reproduction Study: Block A
Incidence of Clinical Signs: Diethyl phthalate

	Dose Level: 4,500 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	1		
Prostrate			
Ataxia			
Moribund	1		
Aborted	1		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea	1		
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Study No. 6125-102

Table 19

Reproduction Study: Block A
Incidence of Clinical Signs: Di(n-butyl)phthalate

	Dose Level: 2,500 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	3[a]		
Prostrate	2		
Ataxia			
Moribund	4		
Cold to touch	3[a]		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea	3[a]		
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

[a]Includes one animal that died because of a dosing error.

Study No. 6125-102

Table 20

Reproduction Study: Block A
Incidence of Clinical Signs: Di(isobutyl)phthalate

	Dose Level: 4,000 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	17		
Prostrate	14		
Ataxia			
Moribund	16		
Cold to touch	17		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea	10		
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Table 21

Group Pregnancy and Female Body Weight Data
for Experimental Block A

	Treatment			
	Vehicle Control (10 mL/kg)	Diethyl phthalate (4,500 mg/kg)	Di(n-butyl) phthalate (2,500 mg/kg)	Di(isobutyl) phthalate (4,000 mg/kg)
Number of females on test	50	50	50	50
Number of females found dead[a] (excluded from results)	0	2	6[b]	27
Number of females that survived	50	48	44	23
Number of females that produced viable litters[c]	32	31	0	0
Number of females with resorbed or nonviable litters	1	2	26	17
Number of proven pregnant females	33	33	26	17
Reproductive index				
[(Number of females that produced viable litters/number of proven pregnant females) x 100]	97	94	0*	0*

[a]Includes animals that were sacrificed in a moribund condition.
[b]One death due to dosing error.
[c]A viable litter was defined as a litter that had at least one live pup on Day 1.
*$p < 0.05$.

Table 21 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block A

	Treatment			
	Vehicle Control (10 mL/kg)	Diethyl phthalate (4,500 mg/kg)	Di(n-butyl) phthalate (2,500 mg/kg)	Di(isobutyl) phthalate (4,000 mg/kg)
Mean Body Weights (g) for Pregnant Females				
Gestation Day 7				
N	33	33	26	17
Mean	27.3	27.2	27.0	26.7
S[a]	1.41	1.51	1.07	1.66
Gestation Day 14				
N	33	33	26	17
Mean	36.2	36.3	29.2*	29.1*
S	3.04	3.24	2.24	2.51
Gestation Day 18				
N	33	33	26	17
Mean	46.8	46.1	28.1*	28.0*
S	5.29	6.20	1.67	2.34
Post-partum Day 3				
N	32	31	0[b]	0[b]
Mean	34.7	34.4	-	-
S	3.67	2.58	-	-

[a]S: Standard deviation.
[b]No litters produced.
*$p < 0.05$.

Table 21 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block A

	Treatment			
	Vehicle Control (10 mL/kg)	Diethyl phthalate (4,500 mg/kg)	Di(n-butyl) phthalate (2,500 mg/kg)	Di(isobutyl) phthalate (4,000 mg/kg)
Mean Body Weight Changes (g) for Pregnant Females				
During Treatment (Days 7-14 of gestation)				
N	33	33	26	17
Mean	9.0	9.1	2.2*	2.5*
S[a]	2.83	2.96	2.08	1.91
During Pregnancy (Days 7-18 of gestation)				
N	33	33	26	17
Mean	19.5	18.8	1.1*	1.4*
S	5.09	5.76	1.05	1.56

[a]S: Standard deviation.
*$p < 0.05$.

Table 22

Group Litter and Viability Data
for Experimental Block A

	Treatment			
	Vehicle Control (10 mL/kg)	Diethyl phthalate (4,500 mg/kg)	Di(n-butyl) phthalate (2,500 mg/kg)	Di(isobutyl) phthalate (4,000 mg/kg)
Number of females that produced viable litters[a]	32	31	0	0
Post-partum Day 1				
Number of live pups/litter				
N	32	31	0	0
Mean	9.5	9.5	-	-
S[b]	2.03	2.51	-	-
Number of dead pups/litter				
N	32	31	0	0
Mean	0.0	0.2	-	-
S	0.00	0.60	-	-
Percent of live pups/litter				
N	32	31	0	0
Mean	100.0	97.6	-	-
S	0.00	7.19	-	-
Percent of dead pups/litter				
N	32	31	0	0
Mean	0.0	2.4	-	-
S	0.00	7.19	-	-
Litter weight (g)				
N	32	31	0	0
Mean	15.6	15.2	-	-
S	2.89	3.51	-	-
Mean pup weight/litter (g)				
N	32	31	0	0
Mean	1.7	1.6	-	-
S	0.13	0.17	-	-

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.

Table 22 (Continued)

Group Litter and Viability Data
for Experimental Block A

	Treatment			
	Vehicle Control (10 mL/kg)	Diethyl phthalate (4,500 mg/kg)	Di(n-butyl) phthalate (2,500 mg/kg)	Di(isobutyl) phthalate (4,000 mg/kg)
Post-partum Day 3				
Number of live pups/litter				
N	32	31	0	0
Mean	9.4	9.1	-	-
S[a]	2.03	2.92	-	-
Number of dead pups/litter				
N	32	31	0	0
Mean	0.1	0.5	-	-
S	0.25	1.80	-	-
Percent of live pups/litter				
N	32	31	0	0
Mean	99.4	95.7	-	-
S	2.46	16.50	-	-
Percent of dead pups/litter				
N	32	31	0	0
Mean	0.6	4.3	-	-
S	2.46	16.50	-	-
Mean pup viability/litter (Days 1 to 3)				
N	32	31	0	0
Mean	99.4	95.7	-	-
S	2.46	16.49	-	-
Litter weight (g)				
N	32	31	0	0
Mean	19.9	18.0	-	-
S	3.62	5.06	-	-
Mean pup weight/litter (g)				
N	32	31	0	0
Mean	2.1	2.0	-	-
S	0.30	0.31	-	-
Mean percent pup weight change (Days 1 to 3)				
N	32	31	0	0
Mean	29.4	25.0	-	-
S	12.19	11.62	-	-

[a]S: Standard deviation.

EXPERIMENTAL BLOCK B

MED Study No. 6125-103
Dimethyl methylphosphonate (DMMP). (Tables 23-26)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg/body weight/day)
Group 2 - 1,000 mg DMMP/kg/day
Group 3 - 1,750 mg DMMP/kg/day
Group 4 - 3,150 mg DMMP/kg/day
Group 5 - 5,600 mg DMMP/kg/day
Group 6 - 10,000 mg DMMP/kg/day

With this compound, deaths occurred only at the two highest dose levels tested (Table 23). Four mice in the 5,600-mg/kg group were dead after 6 days of treatment and nine of the animals treated with 10,000 mg DMMP/kg were dead after the second dosing interval. No dosing errors were evident. No deaths and no clinical observations were reported at lower doses (Tables 23, 24, and 25). The most common clinical signs in the groups of animals in which deaths occurred were languidity, ataxia, and dyspnea (Table 25). There were no biologically significant differences in body weights or body weight changes (Table 26).

Based on these data, a dose level of 4,175 mg DMMP/kg/day was used as the MED in the reproduction study.

Table 23

Survival of Mice in the MED Study
for Experimental Block B

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days								Mice Alive on Day 8		Mice Alive on Day 16	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
Dimethyl methylphosphonate													
1,000	10	0	0	0	0	0	0	0	0	10	100	10	100
1,750	10	0	0	0	0	0	0	0	0	10	100	10	100
3,150	10	0	0	0	0	0	0	0	0	10	100	10	100
5,600	10	0	1	1	1	0	1	0	0	6	60	6	60
10,000	10	2	7	0	0	0	0	0	0	1	10	1	10
tris(2-Chloroethyl)phosphate													
1,000	10	0	1	1	0	0	0	0	0	8	80	8	80
1,315	10	0	2[a]	1	0	0	0	0	0	7[a]	70	7[a]	70
1,730	10	2	3	1	0	1	0	0	0	3	30	3	30
2,280	10	3	2	2	1	0	1	0	0	1	10	1	10
3,000	10	6	4	-	-	-	-	-	-	0	0	0	0
N-isopropyl-N'-phenyl-p-phenylenediamine													
100	10	0	0	0	0	0	0	0	0	10	100	10	100
175	10	0	0	0	0	0	0	0	0	10	100	10	100
315	10	0	0	1[b]	0	0	0	0	0	9[b]	90	9[b]	90
560	10	0	0	0	0	0	0	0	0	10	100	10	100
1,000	10	0	1	0	1	0	0	0	1	7	70	7	70

[a]Two deaths due to dosing error.
[b]One death due to dosing error.

Study No. 6125-103

Table 24

MED Study: Block B
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	
Prostrate	
Ataxia	
Hunched	
Tremors	
Head tilt	
Thin	
Wheezing	
Dyspnea	
Urine stains	
Alopecia	
Rough hair coat	
Sores	
Piloerection	

Study No. 6125-103

Table 25

MED Study: Block B
Incidence of Clinical Signs: Dimethyl methylphosphonate

Clinical Sign of Toxicity	Dose Level (mg/kg/day) 1,000	1,750	3,150	5,600	10,000
Languid				10	10
Prostrate				4	6
Ataxia				5	1
Hunched				4	1
Tremors					
Head tilt					
Thin				1	
Cold to touch				3	6
Wheezing					
Dyspnea				4	8
Urine stains					
Alopecia					
Rough hair coat					
Sores					
Piloerection					

T-12503A TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK B, STUDY NO. 6425-103

Table 26
Summary of Mean Body Weight Data (g)
Dimethyl methylphosphonate

MG/KG		0	1,000	1,750	3,150	5,600	10,000
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	24.5	24.7	25.0	25.4	24.0	25.9 *
	S [a]	1.16	1.16	1.11	1.42	1.38	1.11
	SE [b]	0.16	0.37	0.35	0.45	0.43	0.35
WEIGHT DAY 8							
	NO	50	10	10	10	6	1
	MEAN	24.7	25.4	25.8	25.4	22.8*	24.4
	S	1.74	1.29	1.88	1.47	2.49	0.0
	SE	0.25	0.41	0.59	0.46	1.02	0.0
WEIGHT DAY 12							
	NO	50	10	10	10	6	1
	MEAN	25.8	26.1	26.6	26.2	24.7	27.0
	S	1.76	1.49	2.09	1.37	2.24	0.0
	SE	0.25	0.47	0.66	0.43	0.91	0.0
WEIGHT DAY 14							
	NO	50	10	10	10	6	1
	MEAN	25.9	26.1	26.2	26.3	25.0	27.6
	S	1.93	1.12	2.11	1.26	1.11	0.0
	SE	0.27	0.36	0.67	0.40	0.45	0.0
WT. CHANGE DAYS 1-8							
	NO	50	10	10	10	6	1
	MEAN	0.2	0.7	0.8	-0.0	-1.4*	-2.7
	S	1.14	0.70	1.24	1.47	2.36	0.0
	SE	0.16	0.22	0.39	0.46	0.96	0.0
WT. CHANGE DAYS 1-12							
	NO	50	10	10	10	6	1
	MEAN	1.3	1.4	1.6	0.8	0.4	-0.1
	S	1.26	0.94	1.33	1.06	2.12	0.0
	SE	0.18	0.30	0.42	0.33	0.86	0.0
WT. CHANGE DAYS 1-14							
	NO	50	10	10	10	6	1
	MEAN	1.4	1.4	1.3	0.9	0.7	0.5
	S	1.41	1.01	1.26	1.24	0.82	0.0
	SE	0.20	0.32	0.40	0.39	0.34	0.0

a S: Standard deviation.
b SE: Standard error.
* $p < 0.05$.

tris(2-Chloroethyl)phosphate (TCP). (Tables 23, 24, 27, and 28)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,000 mg TCP/kg/day
Group 3 - 1,315 mg TCP/kg/day
Group 4 - 1,730 mg TCP/kg/day
Group 5 - 2,280 mg TCP/kg/day
Group 6 - 3,000 mg TCP/kg/day

Survival appeared to be dose related for mice treated with this compound (Table 23), ranging from 0% at the highest dose level tested (3,000 mg TCP/kg) to 80% at the lowest dose level (1,000 mg TCP/kg). Most of the deaths occurred before the fourth dosing interval. Two animals in the 1,315-mg/kg group died because of dosing errors. Clinical signs were reported for all animals on test, the most common being periodic occurrences of ataxia and tremors shortly after dosing (Tables 24 and 27). There were no statistically significant differences in body weights or body weight gains at any of the doses tested (Table 28).

Based on these data, a dose level of 940 mg TCP/kg/day was used as the MED in the reproduction study.

Study No. 6125-103

Table 27

MED Study: Block B
Incidence of Clinical Signs: tris(2-Chloroethyl)phosphate

Clinical Sign of Toxicity	Dose Level (mg/kg/day)				
	1,000	1,315	1,730	2,280	3,000
Languid		1	3	3	4
Prostrate			2	1	4
Ataxia	10	10[a]	10	10	10
Toxic convulsions			1		
Hunched					
Tremors	10	10[a]	10	10	10
Head tilt					
Thin					
Wheezing					
Dyspnea			1	1	
Urine stains					
Alopecia					
Rough hair coat					
Sores					
Piloerection					

[a]Includes two animals that died because of dosing errors.

T-12503B TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK B, STUDY NO. 6125-103

Table 28
Summary of Mean Body Weight Data (g)
tris(2-Chloroethyl)phosphate

MG/KG		0	1,000	1,315	1,730	2,280	3,000
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	24.5	24.9	24.5	24.8	24.2	25.5
	S [a]	1.16	0.95	1.47	1.19	1.35	1.41
	SE [b]	0.16	0.30	0.46	0.38	0.43	0.45
WEIGHT DAY 8							
	NO	50	8	7 [c]	3	1	0
	MEAN	24.7	25.6	25.7	26.4	22.4	--
	S	1.74	1.58	0.71	1.19	0.0	0.0
	SE	0.25	0.56	0.27	0.69	0.0	0.0
WEIGHT DAY 12							
	NO	50	8	7 [c]	3	1	0
	MEAN	25.8	26.2	26.5	27.5	24.4	--
	S	1.76	1.56	0.97	1.13	0.0	0.0
	SE	0.25	0.55	0.37	0.65	0.0	0.0
WEIGHT DAY 16							
	NO	50	8	7 [c]	3	1	0
	MEAN	25.9	26.0	26.4	26.8	24.8	--
	S	1.93	1.25	1.28	0.70	0.0	0.0
	SE	0.27	0.44	0.49	0.40	0.0	0.0
WT. CHANGE DAYS 1-8							
	NO	50	8	7 [c]	3	1	0
	MEAN	0.2	0.8	0.9	1.1	-1.1	--
	S	1.14	0.80	1.10	0.52	0.0	0.0
	SE	0.16	0.28	0.42	0.30	0.0	0.0
WT. CHANGE DAYS 1-12							
	NO	50	8	7 [c]	3	1	0
	MEAN	1.3	1.4	1.7	2.2	0.9	--
	S	1.26	0.80	0.85	0.59	0.0	0.0
	SE	0.18	0.28	0.32	0.34	0.0	0.0
WT. CHANGE DAYS 1-16							
	NO	50	8	7 [c]	3	1	0
	MEAN	1.4	1.1	1.6	1.5	1.3	--
	S	1.41	1.08	0.84	0.40	0.0	0.0
	SE	0.20	0.38	0.32	0.23	0.0	0.0

a S: Standard deviation.
b SE: Standard error.
c Two deaths due to dosing errors.

N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD). (Tables 23, 24, 29, and 30)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 100 mg IPPD/kg/day
Group 3 - 175 mg IPPD/kg/day
Group 4 - 315 mg IPPD/kg/day
Group 5 - 560 mg IPPD/kg/day
Group 6 - 1,000 mg IPPD/kg/day

One animal in the 315-mg/kg group died because of a dosing error. The only treatment-related deaths with this compound were in the highest dose group (three animals at 1,000 mg IPPD/kg) (Table 23). There were no clinical signs of toxicity (Tables 24 and 29), and no biologically significant differences in body weights or body weight changes (Table 30).

Based on these data, a dose level of 800 mg IPPD/kg/day was used as the MED in the reproduction study.

Study No. 6125-103

Table 29

MED Study: Block B
Incidence of Clinical Signs: N-isopropyl-N'-phenyl-p-phenylenediamine

Clinical Sign of Toxicity	Dose Level (mg/kg/day)				
	100	175	315	560	1,000
Languid					
Prostrate					
Ataxia					
Hunched					
Tremors					
Head tilt					
Thin					
Wheezing					
Dyspnea					
Urine stains					
Alopecia					
Rough hair coat					
Sores					
Piloerection					
Brown stains					1
Swollen cheeks and neck					1

Table 30
Summary of Mean Body Weight Data (g)
N-isopropyl-N'-phenyl-p-phenylenediamine

MG/KG		0	100	175	345	560	1,000
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	24.5	24.5	24.3	24.2	24.0	23.8
	S[a]	1.16	1.14	0.85	1.06	1.39	1.04
	SE[b]	0.16	0.36	0.27	0.33	0.44	0.33
WEIGHT DAY 8							
	NO	50	10	10	9[c]	10	8
	MEAN	24.7	26.2	25.6	26.7 *	25.6	25.9
	S	1.74	1.73	0.85	1.45	1.66	1.15
	SE	0.25	0.55	0.27	0.48	0.53	0.41
WEIGHT DAY 12							
	NO	50	10	10	9[c]	10	7
	MEAN	25.8	27.2	26.1	26.6	25.7	26.2
	S	1.76	2.34	1.01	1.12	1.97	0.91
	SE	0.25	0.74	0.32	0.37	0.62	0.34
WEIGHT DAY 16							
	NO	50	10	10	9[c]	10	7
	MEAN	25.9	26.9	26.9	26.9	26.1	26.0
	S	1.93	2.06	0.85	1.68	1.80	0.98
	SE	0.27	0.65	0.27	0.56	0.57	0.37
WT. CHANGE DAYS 1-8							
	NO	50	10	10	9[c]	10	8
	MEAN	0.2	1.7 *	1.3	2.4*	1.6 *	2.1*
	S	1.14	1.04	0.87	1.24	1.14	1.48
	SE	0.16	0.33	0.27	0.41	0.36	0.52
WT. CHANGE DAYS 1-12							
	NO	50	10	10	9[c]	10	7
	MEAN	1.3	2.7 *	1.8	2.3	1.7	2.5*
	S	1.26	1.50	0.83	0.91	0.79	0.82
	SE	0.18	0.47	0.26	0.30	0.25	0.31
WT. CHANGE DAYS 1-16							
	NO	50	10	10	9[c]	10	7
	MEAN	1.4	2.4	2.6	2.6*	2.0	2.3
	S	1.41	1.38	0.93	1.24	0.76	0.34
	SE	0.20	0.44	0.29	0.41	0.24	0.43

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.
* $p < 0.05$.

Reproduction Study No. 6125-104
Dimethyl methylphosphonate (DMMP). (Tables 31-33, 36, and 37)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 4,175 mg DMMP/kg/day

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 31 and 32).

One animal treated with DMMP died on Day 9 of gestation (i.e., Day 3 of treatment); no dosing error was evident (Table 31). Instances of languidity were reported in five animals and circling behavior was reported in one animal during the dosing period (Table 33).

Group pregnancy and female body weight data are presented in Table 36. There were no significant differences from the control group in the parameters examined.

Group litter and viability data are presented in Table 37. Although mean pup weight per litter at Day 1 post-partum was significantly lower than that of controls, no significant differences from control data were seen in the other parameters examined.

tris(2-Chloroethyl)phosphate (TCP). (Tables 31, 32, 34, 36, and 37)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 940 mg TCP/kg/day

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 31 and 32).

One animal treated with TCP died because of a dosing error on Day 8 of gestation (i.e., Day 2 of treatment); no other mortalities occurred (Table 31). Instances of languidity, prostration, and jerking movements were recorded during the dosing period (Days 7 through 14 of gestation) (Table 34).

Group pregnancy and female body weight data are presented in Table 36. No significant differences from control data were seen in the parameters examined.

Group litter and viability data are presented in Table 37. No significant differences from control data were seen in the parameters examined.

N-isopropyl-N'-phenyl-p-phenylenediamine (IPPD). (Tables 31, 32, and 35-37)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 800 mg IPPD/kg/day

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 31 and 32).

Forty-eight animals treated with IPPD died [five on Day 9 of gestation, thirteen on Day 10 of gestation, eight on Day 11 of gestation, nine on Day 12 of gestation, ten on Day 13 of gestation, and three on Day 14 of gestation (i.e., Days 3 through 8 of treatment, respectively)]; all of these deaths were attributed to the chemical (Table 31). Various clinical signs of toxicity were recorded for the animals that died on test; a red vaginal exudate, languidity, coldness to touch, and a rough coat was recorded during the dosing period for one of the two animals that survived. During Days 15-18 of gestation, this same animal was cold to the touch and had a rough hair coat; only a rough hair coat was recorded from Day 19 of gestation to Day 3 post-partum (Table 35).

Group pregnancy and female body weight data are presented in Table 36. The reproductive index was significantly lower than that of controls. There were not enough surviving animals to statistically analyze other data, but body weights on Days 14 and 18 of gestation, and body weight gains during treatment and pregnancy were much lower than those of the control group.

No viable litters were produced by females treated with 800 mg IPPD/kg/day (Table 37).

Table 31

Survival of Mice in the Reproduction Study
for Experimental Block B

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days[a]								Mice Alive on Day 8		Mice Alive at Study End	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
Dimethyl methylphosphonate													
4,175	50	0	0	1	0	0	0	0	0	49	98	49	98
tris(2-Chloroethyl)phosphate													
940	50	0	1[b]	0	0	0	0	0	0	49[b]	98	49[b]	98
N-isopropyl-N'-phenyl-p-phenylenediamine													
800	50	0	0	5	13	8	9	10	3	2	4	2	4

[a]Treatment Days 1-8 = Gestation Days 7-14.
[b]One death due to dosing error.

Study No. 6125-104

Table 32

Reproduction Study: Block B
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign		
	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Study No. 6125-104

Table 33

Reproduction Study: Block B
Incidence of Clinical Signs: Dimethyl methylphosphonate

	Dose Level: 4,175 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	5		
Prostrate			
Ataxia			
Circling	1		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Study No. 6125-104

Table 34

Reproduction Study: Block B
Incidence of Clinical Signs: tris(2-Chloroethyl)phosphate

	Dose Level: 940 mg/kg day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	1		
Prostrate	2		
Ataxia			
Jerking movements	2		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Study No. 6125-104

Table 35

Reproduction Study: Block B
Incidence of Clinical Signs: N-isopropyl-N'-phenyl-p-phenylenediamine

	Dose Level: 800 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	26		
Prostrate	2		
Ataxia	8		
Cold to touch	2	1	
Hunched	3		
Tremors	2		
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat	5	1	1
Sores			
Piloerection			
Red exudate (vagina)	1		
Compound colored urogenital area	1		

Table 36

Group Pregnancy and Female Body Weight Data
for Experimental Block B

	Treatment			
	Vehicle Control (10 mL/kg)	Dimethyl methyl-phosphonate (4,175 mg/kg)	tris(2-Chloroethyl) phosphate (940 mg/kg)	N-isopropyl-N'-phenyl-p-phenylenediamine (800 mg/kg)
Number of females on test	50	50	50	50
Number of females found dead (excluded from results)	0	1	1[a]	48
Number of females that survived	50	49	49	2
Number of females that produced viable litters[b]	37	32	35	0
Number of females with resorbed or nonviable litters	2	4	2	2
Number of proven pregnant females	39	36	37	2
Reproductive index				
[(Number of females that produced viable litters/number of proven pregnant females) x 100]	95	89	95	0*

[a]One death due to dosing error.
[b]A viable litter was defined as a litter that had at least one live pup on Day 1.
*$p < 0.05$.

Table 36 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block B

	Treatment			
	Vehicle Control (10 mL/kg)	Dimethyl methyl-phosphonate (4,175 mg/kg)	tris(2-Chloroethyl) phosphate (940 mg/kg)	N-isopropyl-N'-phenyl-p-phenylenediamine (800 mg/kg)
Mean Body Weights (g) for Pregnant Females				
Gestation Day 7				
N	39	36	37	2
Mean	26.2	25.7	26.1	23.9[b]
S[a]	1.63	1.72	1.44	0.71
Gestation Day 14				
N	39	36	37	2
Mean	33.4	32.9	32.9	21.4[b]
S	2.33	3.47	2.68	0.85
Gestation Day 18				
N	39	36	37	2
Mean	44.1	41.9	43.4	20.8[b]
S	5.00	6.93	5.63	1.70
Post-partum Day 3				
N	37	32	35	0[c]
Mean	34.4	34.0	33.5	-
S	2.38	3.03	2.32	-

[a]S: Standard deviation.
[b]Too few animals to analyze statistically.
[c]No litters produced.

Table 36 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block B

	Treatment			
	Vehicle Control (10 mL/kg)	Dimethyl methyl-phosphonate (4,175 mg/kg)	tris(2-Chloroethyl) phosphate (940 mg/kg)	N-isopropyl-N'-phenyl-p-phenylenediamine (800 mg/kg)
Mean Body Weight Changes (g) for Pregnant Females				
During Treatment (Days 7-14 of gestation)				
N	39	36	37	2
Mean	7.2	7.2	6.8	-2.5[b]
S[a]	2.04	2.44	2.13	0.14
During Pregnancy (Days 7-18 of gestation)				
N	39	36	37	2
Mean	17.9	16.2	17.3	-3.1[b]
S	5.05	6.05	5.40	0.99

[a]S: Standard deviation.
[b]Too few animals to analyze statistically.

Table 37

Group Litter and Viability Data
for Experimental Block B

	Treatment			
	Vehicle Control (10 mL/kg)	Dimethyl methyl-phosphonate (4,175 mg/kg)	tris(2-Chloroethyl) phosphate (940 mg/kg)	N-isopropyl-N'-phenyl-p-phenylenediamine (800 mg/kg)
Number of females that produced viable litters[a]	37	32	35	0
Post-partum Day 1				
Number of live pups/litter				
N	37	32	35	0
Mean	9.0	9.4	10.1	-
S[b]	3.11	2.54	2.48	-
Number of dead pups/litter				
N	37	32	35	0
Mean	0.6	0.3	0.1	-
S	1.83	0.74	0.24	-
Percent of live pups/litter				
N	37	32	35	0
Mean	93.6	96.2	99.5	-
S	18.48	10.27	2.24	-
Percent of dead pups/litter				
N	37	32	35	0
Mean	6.4	3.8	0.5	-
S	18.48	10.27	2.24	-
Litter weight (g)				
N	37	32	35	0
Mean	14.4	14.3	15.9	-
S	4.56	3.48	3.24	-
Mean pup weight/litter (g)				
N	37	32	35	0
Mean	1.6	1.5*	1.6	-
S	0.17	0.14	0.14	-

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.
*$p < 0.05$.

Table 37 (Continued)

Group Litter and Viability Data
for Experimental Block B

	Treatment			
	Vehicle Control (10 mL/kg)	Dimethyl methyl-phosphonate (4,175 mg/kg)	tris(2-Chloroethyl) phosphate (940 mg/kg)	N-isopropyl-N'-phenyl-p-phenylenediamine (800 mg/kg)
Post-partum Day 3				
Number of live pups/litter				
N	37	32	35	0
Mean	8.9	9.2	9.9	-
S[a]	3.16	2.58	2.42	-
Number of dead pups/litter				
N	37	32	35	0
Mean	0.1	0.2	0.2	-
S	0.39	0.45	0.55	-
Percent of live pups/litter				
N	37	32	35	0
Mean	98.5	97.8	98.0	
S	6.78	6.78	4.96	
Percent of dead pups/litter				
N	37	32	35	0
Mean	1.5	2.2	2.0	-
S	6.78	6.78	4.96	-
Mean pup viability/litter (Days 1 to 3)				
N	37	32	35	0
Mean	98.4	97.8	98.0	-
S	6.80	6.85	4.95	-
Litter weight (g)				
N	37	32	35	0
Mean	18.4	18.8	19.3	-
S	5.81	4.81	3.79	-
Mean pup weight/litter (g)				
N	37	32	35	0
Mean	2.1	2.1	2.0	-
S	0.33	0.31	0.27	-
Mean percent pup weight change (Days 1 to 3)				
N	37	32	35	0
Mean	30.0	33.7	24.8	-
S	9.79	11.77	9.54	-

[a]S: Standard deviation.

EXPERIMENTAL BLOCK C

MED Study No. 6125-105
D-phenylalanine (DPA). (Tables 38-41)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg/body weight/day)
Group 2 - 500 mg DPA/kg/day
Group 3 - 890 mg DPA/kg/day
Group 4 - 1,580 mg DPA/kg/day
Group 5 - 2,810 mg DPA/kg/day
Group 6 - 5,000 mg DPA/kg/day

The only death that occurred in mice treated with this compound was in the highest dose group (5000 mg DPA/kg) and was compound related (Table 38). The animal was languid after treatment on Day 3 and was found dead prior to treatment on Day 4. No other clinical signs of toxicity were recorded (Tables 39 and 40), and there were no biologically significant differences from controls in body weights or body weight changes (Table 41).

Based on these data, a dose level of 5,000 mg DPA/kg/day was used as the MED in the reproduction study.

Table 38

Survival of Mice in the MED Study
for Experimental Block C

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days								Mice Alive on Day 8		Mice Alive on Day 16	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
						D-phenylalanine							
500	10	0	0	0	0	0	0	0	0	10	100	10	100
890	10	0	0	0	0	0	0	0	0	10	100	10	100
1,580	10	0	0	0	0	0	0	0	0	10	100	10	100
2,810	10	0	0	0	0	0	0	0	0	10	100	10	100
5,000	10	0	0	0	1	0	0	0	0	9	90	9	90
						Probenecid							
200	10	0	0	0	0	0	0	0	0	10	100	10	100
355	10	0	0	0	0	0	0	0	0	10	100	10	100
630	10	0	0	0	0	0	0	0	0	10	100	10	100
1,125	10	0	0	0	0	0	0	0	0	10	100	10	100
2,000	10	0	3	4	1	1	0	0	0	1	10	1	10
						trans-Cinnamaldehyde							
250	10	0	0	0	0	0	0	0	0	10	100	10	100
445	10	0	0	0	0	0	0	0	0	10	100	10	100
790	10	0	0	0	0	0	0	0	0	10	100	10	100
1,405	10	0	0	0	3	0	0	0	0	7	70	7	70
2,500	10	5	4	1	-	-	-	-	-	0	0	0	0

Study No. 6125-105

Table 39

MED Study: Block C
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight /day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	
Prostrate	
Ataxia	
Hunched	
Tremors	
Head tilt	
Thin	
Wheezing	
Dyspnea	
Urine stains	
Alopecia	
Rough hair coat	
Sores	
Piloerection	

Study No. 6125-105

Table 40

MED Study: Block C
Incidence of Clinical Signs: D-phenylalanine

Clinical Sign of Toxicity	Dose Level (mg/kg/day)				
	500	890	1,580	2,810	5,000
Languid					1
Prostrate					
Ataxia					
Hunched					
Tremors					
Head tilt					
Thin					
Wheezing					
Dyspnea					
Urine stains					
Alopecia					
Rough hair coat					
Sores					
Piloerection					

Table 41
Summary of Mean Body Weight Data (g)

D-phenylalanine

MG/KG		0	500	890	1,580	2,810	5,000
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	28.4	27.8	28.2	28.8	27.8	27.9
	S[a]	1.34	1.40	1.31	1.16	1.51	1.52
	SE[b]	0.19	0.44	0.41	0.37	0.48	0.48
WEIGHT DAY 8							
	NO	50	10	10	10	10	9
	MEAN	28.3	27.6	28.7	29.3	28.6	28.4
	S	1.35	1.39	1.44	1.11	1.45	1.38
	SE	0.19	0.44	0.45	0.35	0.46	0.46
WEIGHT DAY 12							
	NO	50	10	10	10	10	9
	MEAN	29.5	28.3	30.0	30.0	29.5	29.2
	S	1.60	1.53	1.48	0.94	1.75	1.51
	SE	0.23	0.48	0.47	0.30	0.55	0.50
WEIGHT DAY 16							
	NO	50	10	10	10	10	9
	MEAN	30.1	28.8	30.3	31.1	29.8	29.2
	S	1.60	1.68	1.67	1.69	1.84	1.38
	SE	0.23	0.53	0.53	0.54	0.58	0.46
WT. CHANGE DAYS 1-8							
	NO	50	10	10	10	10	9
	MEAN	-0.1	-0.3	0.6	0.5	0.7 *	0.4
	S	0.65	0.76	1.16	0.85	1.15	0.75
	SE	0.09	0.24	0.36	0.27	0.36	0.25
WT. CHANGE DAYS 1-12							
	NO	50	10	10	10	10	9
	MEAN	1.1	0.5	1.8	1.2	1.7	1.3
	S	0.84	0.82	1.41	1.15	1.41	0.87
	SE	0.12	0.26	0.45	0.36	0.45	0.29
WT. CHANGE DAYS 1-16							
	NO	50	10	10	10	10	9
	MEAN	1.7	1.0	2.1	2.3	1.9	1.3
	S	1.15	0.95	1.61	1.67	1.53	0.69
	SE	0.16	0.30	0.51	0.53	0.48	0.23

a S: Standard deviation.
b SE: Standard error.
* $p < 0,05$.

Probenecid (PBN). (Tables 38, 39, 42, and 43)

Dose levels used: Group 1 - vehicle control (10-mL corn oil/kg body weight/day)
Group 2 - 200 mg PBN/kg/day
Group 3 - 355 mg PBN/kg/day
Group 4 - 630 mg PBN/kg/day
Group 5 - 1,125 mg PBN/kg/day
Group 6 - 2,000 mg PBN/kg/day

Deaths with this compound occurred only in the highest dose group (nine animals at 2,000 mg PBN/kg), and were compound related (Table 38). Clinical signs were reported for animals in the three highest dose groups (630, 1,125, and 2,000 mg PBN/kg), the most common being languidity, jerking movements, and dyspnea after dosing (Tables 39 and 42). There were no biologically significant differences in body weights or body weight changes; however, there were too few animals in the 2000-mg/kg group to analyze the data statistically (Table 43).

Based on these data, a dose level of 1,200 mg PBN/kg/day was used as the MED in the reproduction study.

Study No. 6125-105

Table 42

MED Study: Block C
Incidence of Clinical Signs: Probenecid

Clinical Sign of Toxicity	Dose Level (mg/kg/day)				
	200	355	630	1,125	2,000
Languid				2	4
Prostrate					3
Ataxia					1
Cold to touch				1	3
Jerking movements					2
Hunched					2
Tremors					1
Head tilt					
Thin					
Wheezing					
Dyspnea					3
Urine stains					
Alopecia					
Rough hair coat					3
Sores					
Piloerection					
Soft feces			1		

T-12505B TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK C, STUDY NO. 6426-105

Table 43
Summary of Mean Body Weight Data (g)

		Probenecid					
MG/KG		0	200	355	630	1,425	2,000
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	28.4	28.0	28.6	27.2	28.0	27.9
	S [a]	1.34	1.56	1.37	1.11	1.59	1.26
	SE [b]	0.19	0.49	0.43	0.35	0.50	0.40
WEIGHT DAY 8							
	NO	50	10	10	10	10	1 [c]
	MEAN	28.3	28.3	29.6	29.2	30.2*	26.7
	S	1.35	1.51	1.30	1.57	2.47	0.0
	SE	0.19	0.48	0.41	0.50	0.69	0.0
WEIGHT DAY 12							
	NO	50	10	10	10	10	1 [c]
	MEAN	29.5	29.3	30.2	29.9	29.7	28.3
	S	1.60	1.60	1.47	1.89	2.22	0.0
	SE	0.23	0.51	0.46	0.60	0.70	0.0
WEIGHT DAY 16							
	NO	50	10	10	10	10	1 [c]
	MEAN	30.1	29.7	30.6	29.8	30.1	29.3
	S	1.60	1.58	1.35	1.89	1.93	0.0
	SE	0.23	0.50	0.43	0.60	0.61	0.0
WT. CHANGE DAYS 1-8							
	NO	50	10	10	10	10	1 [c]
	MEAN	-0.1	0.3	1.0*	2.0*	2.3*	-1.1
	S	0.65	0.72	1.02	0.70	0.93	0.0
	SE	0.09	0.23	0.32	0.22	0.29	0.0
WT. CHANGE DAYS 1-12							
	NO	50	10	10	10	10	1 [c]
	MEAN	1.1	1.3	1.6	2.7*	1.8	0.5
	S	0.84	0.73	1.02	0.98	0.91	0.0
	SE	0.12	0.23	0.32	0.31	0.29	0.0
WT. CHANGE DAYS 1-16							
	NO	50	10	10	10	10	1 [c]
	MEAN	1.7	1.7	2.0	2.6	2.2	1.5
	S	1.15	0.77	0.92	1.28	0.69	0.0
	SE	0.16	0.24	0.29	0.41	0.22	0.0

a S: Standard deviation.
b SE: Standard error.
c Too few animals to analyze statistically.
* $p < 0.05$.

trans-Cinnamaldehyde (TCA). (Tables 38, 39, 44, and 45)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 250 mg TCA/kg/day
Group 3 - 445 mg TCA/kg/day
Group 4 - 790 mg TCA/kg/day
Group 5 - 1,405 mg TCA/kg/day
Group 6 - 2,500 mg TCA/kg/day

With this compound, deaths occurred at the two highest dose levels tested (Table 38). Three mice in the 1,405-mg/kg group were dead on Day 4 of treatment, and all of the animals treated with 2,500 mg TCA/kg (10) were dead after the third dosing interval. No dosing errors were evident, and no deaths were reported at lower doses (Table 38). One animal in the 790-mg/kg group was dyspneic and convulsed after dosing on Day 1 of treatment; this animal was normal on Day 2 and remained on test until terminal sacrifice (Tables 39 and 44). The most common clinical signs in the groups of animals in which deaths occurred were languidity, dyspnea, and prostration after dosing. The only biologically and statistically significant difference in body weight and body weight change was for weight change from Days 1-12 of treatment in the 1,405-mg/kg group; the mean change for controls was +1.1 g, while animals in the 1,405-mg/kg group had a mean change of -0.3 g (Table 45).

Based on these data, a dose level of 1,200 mg TCA/kg/day was used as the MED in the reproduction study.

Study No. 6125-105

Table 44

MED Study: Block C
Incidence of Clinical Signs: trans-Cinnamaldehyde

Clinical Sign of Toxicity	Dose Level (mg/kg/day)				
	250	445	790	1,405	2,500
Languid				4	2
Prostrate				3	2
Ataxia					
Convulsions			1		
Cold to touch					2
Hunched				3	2
Tremors					
Head tilt					
Thin					
Wheezing					
Dyspnea			1	4	
Urine stains					
Alopecia					
Rough hair coat				1	
Sores					
Piloerection					
Wet stains				1	

T-12505C TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK C, STUDY NO. 6125-105

Table 45
Summary of Mean Body Weight Data (g)

trans-Cinnamaldehyde

MG/KG		0	250	445	790	1,405	2,500
WEIGHT DAY 1							
	NO	50	10	10	10	10	10
	MEAN	28.4	27.6	27.9	27.7	27.9	27.6
	S [a]	1.34	1.23	1.32	1.18	1.16	1.21
	SE [b]	0.19	0.39	0.42	0.37	0.37	0.38
WEIGHT DAY 8							
	NO	50	10	10	10	7	0
	MEAN	28.3	28.3	29.2	29.3	28.1	--
	S	1.35	1.34	0.80	1.11	2.01	0.0
	SE	0.19	0.42	0.25	0.35	0.76	0.0
WEIGHT DAY 12							
	NO	50	10	10	10	7	0
	MEAN	29.5	29.5	29.6	29.2	27.8	--
	S	1.60	1.46	1.19	1.06	2.71	0.0
	SE	0.23	0.46	0.38	0.34	1.02	0.0
WEIGHT DAY 16							
	NO	50	10	10	10	7	0
	MEAN	30.1	30.3	30.2	29.6	28.4	--
	S	1.60	1.99	1.30	1.21	4.88	0.0
	SE	0.23	0.63	0.41	0.38	1.85	0.0
WT. CHANGE DAYS 1-8							
	NO	50	10	10	10	7	0
	MEAN	-0.1	0.7*	1.3*	1.6*	0.0	--
	S	0.65	0.59	0.98	0.59	1.67	0.0
	SE	0.09	0.19	0.31	0.19	0.63	0.0
WT. CHANGE DAYS 1-12							
	NO	50	10	10	10	7	0
	MEAN	1.1	1.9	1.7	1.4	-0.3*	--
	S	0.84	0.96	1.10	1.01	2.54	0.0
	SE	0.12	0.30	0.35	0.32	0.96	0.0
WT. CHANGE DAYS 1-16							
	NO	50	10	10	10	7	0
	MEAN	1.7	2.7	2.4	1.9	0.3	--
	S	1.15	1.43	1.19	0.93	4.41	0.0
	SE	0.16	0.45	0.37	0.29	1.67	0.0

a S: Standard deviation.
b SE: Standard error.
* $p < 0,05$.

Reproduction Study No. 6125-106
D-phenylalanine (DPA). (Tables 46-48, 51 and 52)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 5,000 mg DPA/kg/day

No deaths were recorded for the vehicle control group (Table 46). One animal had a red vaginal exudate during the dosing period (Days 7 through 14 of gestation), and one animal had a scab formation throughout the study period (Table 47).

One animal treated with DPA died on Day 21 of gestation; no dosing error was evident (Table 46). One animal had a scab formation throughout the study period, and two animals had a red vaginal exudate during the dosing period (Table 48).

Group pregnancy and female body weight data are presented in Table 51; no significant differences from the control group were seen in the parameters examined.

Group litter and viability data are presented in Table 52; no significant differences from the control group were seen in the parameters examined.

Probenecid (PBN). (Tables 46, 47, 49, 51, and 52)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,200 mg PBN/kg/day

No deaths were recorded for the vehicle control group (Table 46). One animal had a red vaginal exudate during the dosing period (Days 7 through 14 of gestation), and one animal had a scab formation throughout the study period (Table 47).

No deaths occurred that could be attributed to the chemical, but three animals treated with PBN died because of dosing errors [one on Day 9 of gestation (Day 3 of treatment) and two on Day 10 of gestation (Day 4 of treatment)] (Table 46). The only clinical signs of toxicity that were reported for animals that did not die because of dosing error were in one animal that had a scab formation and a hard movable nodule at the scab site throughout the test period (Table 49).

Group pregnancy and female body weight data are presented in Table 51; no significant differences from the control group were seen in the parameters examined.

Group litter and viability data are presented in Table 52; no significant differences from the control group were seen in the parameters examined.

trans-Cinnamaldehyde (TCA). (Tables 46, 47, and 50-52)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,200 mg TCA/kg/day

No deaths were recorded for the vehicle control group (Table 46). One animal had a red vaginal exudate during the dosing period (Days 7 through 14 of gestation), and one animal had a scab formation throughout the study period (Table 47).

No deaths occurred that could be attributed to treatment; but one animal treated with TCA died because of a dosing error on Day 8 of gestation (i.e., Day 2 of treatment) (Table 46). The only clinical signs of toxicity recorded were alopecia and a scab formation in one animal (Table 50).

Group pregnancy and female body weight data are presented in Table 51; no significant differences from the control group were seen in the parameters examined.

Group litter and viability data are presented in Table 52; no significant differences from the control group were seen in the parameters examined.

Table 46

Survival of Mice in the Reproduction Study
for Experimental Block C

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days[a]								Mice Alive on Day 8		Mice Alive at Study End	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
D-phenylalanine													
5,000	50	0	0	0	0	0	0	0	0	50	100	49	98
Probenecid													
1,200	50	0	0	1[b]	2[c]	0	0	0	0	47[d]	94	47[d]	94
trans-Cinnamaldehyde													
1,200	50	0	1[b]	0	0	0	0	0	0	49[b]	98	49[b]	98

[a]Treatment Days 1-8 = Gestation Days 7-14.
[b]One death due to dosing error.
[c]Two deaths due to dosing error.
[d]Three deaths due to dosing error.

Study No. 6125-106

Table 47

Reproduction Study: Block C
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

	Number of Animals Showing Sign		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Scab formation involving hair	1	1	1
Red exudate (vagina)	1		

Study No. 6125-106

Table 48

Reproduction Study: Block C
Incidence of Clinical Signs: D-phenylalanine

Clinical Sign of Toxicity	Dose Level: 5,000 mg/kg/day		
	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Scab formation involving hair	1	1	1
Dark red exudate (vagina)	2		

Study No. 6125-106

Table 49

Reproduction Study: Block C
Incidence of Clinical Signs: Probenecid

	Dose Level: 1,200 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	2[a]		
Prostrate	1[a]		
Ataxia			
Cold to touch	1[a]		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Scab formation involving hair	1	1	
Dark red exudate (vagina)	1[a]		
Hard movable nodule at scab site	1	1	1

[a]Animals died because of dosing errors.

Study No. 6125-106

Table 50

Reproductive Study: Block C
Incidence of Clinical Signs: trans-Cinnamaldehyde

	Dose Level: 1,200 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			1
Rough hair coat			
Sores			
Piloerection			
Scab formation involving hair	1	1	1

Table 51

Group Pregnancy and Female Body Weight Data
for Experimental Block C

	Treatment			
	Vehicle Control (10 mL/kg)	D-phenylalanine (5,000 mg/kg)	Probenecid (1,200 mg/kg)	trans-Cinnamaldehyde (1,200 mg/kg)
Number of females on test	50	50	50	50
Number of females found dead (excluded from results)	0	1	3[a]	1[b]
Number of females that survived	50	49	47	49
Number of females that produced viable litters[c]	39	34	33	34
Number of females with resorbed or nonviable litters	7	5	6	0
Number of proven pregnant females	46	39	39	34
Reproductive index				
[(Number of females that produced viable litters/number of proven pregnant females) x 100]	85	87	85	100

[a]Three deaths due to dosing error.
[b]One death due to dosing error.
[c]A viable litter was defined as a litter that had at least one live pup on Day 1.

Table 51 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block C

	Treatment			
	Vehicle Control (10 mL/kg)	D-phenylalanine (5,000 mg/kg)	Probenecid (1,200 mg/kg)	trans-Cinnamaldehyde (1,200 mg/kg)
Mean Body Weights (g) for Pregnant Females				
Gestation Day 7				
N	46	39	39	34
Mean	28.0	27.6	27.8	26.7
S[a]	2.65	3.19	2.91	2.61
Gestation Day 14				
N	46	39	39	34
Mean	35.4	34.5	35.2	34.4
S	3.75	4.41	3.93	3.35
Gestation Day 18				
N	46	39	39	34
Mean	45.9	44.4	45.1	44.7
S	6.15	7.82	7.19	4.58
Post-partum Day 3				
N	39	34	33	34
Mean	35.8	35.1	35.0	34.2
S	3.17	2.75	3.15	3.15

[a]S: Standard deviation.

Table 51 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block C

	Treatment			
	Vehicle Control (10 mL/kg)	D-phenylalanine (5,000 mg/kg)	Probenecid (1,200 mg/kg)	trans-Cinnamaldehyde (1,200 mg/kg)
Mean Body Weight Changes (g) for Pregnant Females				
During Treatment (Days 7-14 of gestation)				
N	46	39	39	34
Mean	7.4	6.9	7.4	7.7
S[a]	2.36	2.49	2.66	1.51
During Pregnancy (Days 7-18 of gestation)				
N	46	39	39	34
Mean	18.0	16.8	17.3	18.0
S	5.30	6.52	6.47	3.08

[a]S: Standard deviation.

Table 52

Group Litter and Viability Data
for Experimental Block C

	Treatment			
	Vehicle Control (10 mL/kg)	D-phenylalanine (5,000 mg/kg)	Probenecid (1,200 mg/kg)	trans-Cinnamaldehyde (1,200 mg/kg)
Number of females that produced viable litters[a]	39	34	33	34
Post-partum Day 1				
Number of live pups/litter				
N	39	34	33	34
Mean	9.5	9.9	10.1	9.4
S[a]	2.60	2.85	2.45	2.50
Number of dead pups/litter				
N	39	34	33	34
Mean	0.4	0.1	0.5	0.2
S	1.27	0.24	1.94	0.52
Percent of live pups/litter				
N	39	34	33	34
Mean	95.1	99.5	95.3	97.5
S	14.38	2.12	16.30	8.20
Percent of dead pups/litter				
N	39	34	33	34
Mean	4.9	0.5	4.7	2.5
S	14.38	2.12	16.30	8.20
Litter weight (g)				
N	39	34	33	34
Mean	14.7	15.0	15.5	14.7
S	3.60	3.59	3.67	3.62
Mean pup weight/litter (g)				
N	39	34	33	34
Mean	1.6	1.6	1.6	1.6
S	0.13	0.17	0.11	0.12

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.

Table 52 (Continued)

Group Litter and Viability Data
for Experimental Block C

	Treatment			
	Vehicle Control (10 mL/kg)	D-phenylalanine (5,000 mg/kg)	Probenecid (1,200 mg/kg)	trans-Cinnamaldehyde (1,200 mg/kg)
Post-partum Day 3				
Number of live pups/litter				
N	39[a]	34	33	34
Mean	9.4	9.7	9.5	9.1
S[b]	2.82	2.83	3.08	2.69
Number of dead pups/litter				
N	39[a]	34	33	34
Mean	0.1	0.2	0.6	0.3
S	0.41	0.73	1.48	0.52
Percent of live pups/litter				
N	39[a]	34	33	34
Mean	96.4	98.2	90.7	95.9
S	16.30	6.14	23.73	8.30
Percent of dead pups/litter				
N	39[a]	34	33	34
Mean	3.6	1.8	9.3	4.1
S	16.30	6.14	23.73	8.30
Mean pup viability/litter (Days 1 to 3)				
N	39[a]	34	33	34
Mean	96.4	98.2	90.7	95.9
S	16.30	6.21	23.70	8.32
Litter weight (g)				
N	38	34	33	34
Mean	18.8	18.4	18.3	17.7
S	4.27	4.87	4.99	4.87
Mean pup weight/litter (g)				
N	38	34	33	34
Mean	2.0	1.9	1.9	2.0
S	0.20	0.24	0.23	0.27
Mean percent pup weight change (Days 1 to 3)				
N	38	34	33	34
Mean	26.7	23.7	21.6	25.4
S	7.29	8.64	11.06	11.82

[a]Includes one female whose pups were dead on Day 3.
[b]S: Standard deviation.

EXPERIMENTAL BLOCK D

MED Study No. 6125-107
Di(2-ethylhexyl)phthalate (DEHP). (Tables 53-56)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg/body weight/day)
Group 2 - 6,000 mg DEHP/kg/day
Group 3 - 7,690 mg DEHP/kg/day
Group 4 - 9,860 mg DEHP/kg/day (undiluted)

One animal in the control group died because of a dosing error. No deaths occurred in mice treated with DEHP at any of the doses tested (Table 53). Clinical signs of toxicity were noted in all animals at all doses (Tables 54 and 55); these included wet stains (appeared to be urine) in the abdominal and genital areas after dosing each day and the appearance of an oily coat. Animals appeared normal by the third day after the dosing interval. There were no significant differences from the control group in body weights or body weight changes (Table 56).

Based on these data, a dose level of 9,860 mg DEHP/kg/day (i.e., undiluted DEHP) was used as the MED in the reproduction study.

Table 53

Survival of Mice in the MED Study
for Experimental Block D

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days								Mice Alive on Day 8		Mice Alive on Day 16	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	49[a]	98
Di(2-ethylhexyl)phthalate													
6,000	10	0	0	0	0	0	0	0	0	10	100	10	100
7,690	10	0	0	0	0	0	0	0	0	10	100	10	100
9,860†	10	0	0	0	0	0	0	0	0	10	100	10	100
Di(isodecyl)phthalate													
1,000	10	0	0	0	0	0	0	0	0	10	100	10	100
1,575	10	0	0	0	0	0	0	0	0	10	100	10	100
2,475	10	0	1[a]	0	0	0	0	0	0	9[a]	90	9[a]	90
3,900	10	0	0	0	0	0	0	0	0	10	100	10	100
6,130	10	0	0	0	0	0	0	0	0	10	100	10	100
9,650†	10	0	0	0	0	0	0	0	0	10	100	10	100
2-Ethyl-1-hexanol													
1,000	10	0	1[a]	0	0	0	0	0	0	9[a]	90	9[a]	90
1,525	10	0	1	0	0	0	0	0	0	9	90	9	90
2,330	10	2	3	4	1	–	–	–	–	0	0	0	0
3,560	10	2	8	–	–	–	–	–	–	0	0	0	0
5,435	10	3	7	–	–	–	–	–	–	0	0	0	0
8,300†	10	8	2	–	–	–	–	–	–	0	0	0	0

†Undiluted.
[a]One death due to dosing error.

Study No. 6125-107

Table 54

MED Study: Block D
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	1[a]
Prostrate	
Ataxia	
Hunched	1[a]
Tremors	
Head tilt	
Thin	
Wheezing	
Dyspnea	
Urine stains	
Alopecia	1
Rough hair coat	1[a]
Sores	
Piloerection	

[a]Animal died because of a dosing error.

Study No. 6125-107

Table 55

MED Study: Block D
Incidence of Clinical Signs: Di(2-ethylhexyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day) 6,000	7,690	9,860
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Wet stains	10	10	10
Oily coat	10	10	10

T-12507A TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK D, STUDY NO. 6125-107

Table 56
Summary of Mean Body Weight Data (g)
Di(2-ethylhexyl)phthalate

MG/KG		0	6,000	7,690	9,860
WEIGHT DAY 1					
	NO	50	10	10	10
	MEAN	25.1	25.3	25.4	24.7
	S [a]	1.00	1.12	1.07	0.69
	SE [b]	0.14	0.35	0.34	0.22
WEIGHT DAY 8					
	NO	50	10	10	10
	MEAN	25.2	25.2	24.7	24.8
	S	1.45	1.49	1.70	1.37
	SE	0.20	0.47	0.54	0.43
WEIGHT DAY 12					
	NO	49 [c]	10	10	10
	MEAN	26.6	26.1	26.1	26.5
	S	1.22	1.15	1.62	1.19
	SE	0.17	0.36	0.48	0.38
WEIGHT DAY 16					
	NO	49 [c]	10	10	10
	MEAN	27.1	26.7	26.6	27.3
	S	1.31	1.28	1.40	1.68
	SE	0.19	0.41	0.44	0.53
WT. CHANGE DAYS 1-8					
	NO	50	10	10	10
	MEAN	0.0	-0.1	-0.8	0.0
	S	1.40	1.66	1.09	1.20
	SE	0.20	0.53	0.35	0.38
WT. CHANGE DAYS 1-12					
	NO	49 [c]	10	10	10
	MEAN	1.5	0.8	0.6	1.8
	S	1.36	1.18	0.84	1.10
	SE	0.19	0.37	0.26	0.35
WT. CHANGE DAYS 1-16					
	NO	49 [c]	10	10	10
	MEAN	2.0	1.4	1.2	2.6
	S	1.42	1.19	0.90	1.50
	SE	0.20	0.38	0.28	0.47

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.

Di(isodecyl)phthalate (DIDP). (Tables 53, 54, 57, and 58)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,000 mg DIDP/kg/day
Group 3 - 1,575 mg DIDP/kg/day
Group 4 - 2,475 mg DIDP/kg/day
Group 5 - 3,900 mg DIDP/kg/day
Group 6 - 6,130 mg DIDP/kg/day
Group 7 - 9,650 mg DIDP/kg/day (undiluted)

One animal in the control group and one animal in the 2,475-mg/kg group died because of dosing errors; no compound-related deaths occurred in mice treated with DIDP at any of the doses tested (Table 53). Clinical signs of toxicity were reported for animals in the four highest dose groups (2,475, 3,900, 6,130, and 9,650 mg DIDP/kg); wet urogenital areas were seen in 20%, 40%, 100%, and 100%, respectively, of animals receiving these dosage levels. All (10) of the animals in the 9,650-mg/kg group also had oily-appearing coats (Tables 54 and 57). The only differences in body weight data that were statistically significant were lower body weight and body weight gain at Day 12 in the 9,650-mg/kg group when compared with those of controls (Table 58).

Based on these data, a dose level of 9,650 mg DIDP/kg/day (i.e., undiluted DIDP) was used as the MED in the reproduction study.

Study No. 6125-107

Table 57

MED Study: Block D
Incidence of Clinical Signs: Di(isodecyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day)					
	1,000	1,575	2,475	3,900	6,130	9,650
Languid						
Prostrate						
Ataxia						
Hunched						
Tremors						
Head tilt						
Thin						
Wheezing						
Dyspnea						
Urine stains						
Alopecia						
Rough hair coat						
Sores						
Piloerection						3
Wet and discolored coat			1	2	1	
Wet stains			1	2	10	10
Oily coat						10

T-12507B TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK D. STUDY NO. 6425-107

Table 58
Summary of Mean Body Weight Data (g)
Di(isodecyl)phthalate

MG/KG		0	1,000	1,575	2,475	3,900	6,130	9,650
WEIGHT DAY 1								
	NO	50	10	10	10	10	10	10
	MEAN	25.1	25.4	25.3	25.5	25.0	24.9	25.0
	S [a]	1.00	0.94	1.12	1.03	0.95	1.40	0.63
	SE [b]	0.14	0.30	0.35	0.33	0.30	0.44	0.20
WEIGHT DAY 8								
	NO	50	10	10	9 [c]	10	10	10
	MEAN	25.2	25.7	25.6	25.4	25.2	25.1	25.3
	S	1.45	0.87	1.23	1.31	1.05	1.24	0.94
	SE	0.20	0.28	0.39	0.44	0.33	0.39	0.30
WEIGHT DAY 12								
	NO	49 [c]	10	10	9 [c]	10	10	10
	MEAN	26.6	26.9	26.8	26.4	26.2	26.6	25.0 *
	S	1.22	1.19	1.91	1.49	0.94	1.25	1.67
	SE	0.17	0.38	0.60	0.50	0.30	0.40	0.53
WEIGHT DAY 16								
	NO	49 [c]	10	10	9 [c]	10	10	10
	MEAN	27.1	27.4	27.3	27.4	27.5	27.0	27.4
	S	1.31	1.40	1.54	2.21	0.97	1.35	1.79
	SE	0.19	0.44	0.49	0.74	0.31	0.43	0.57
WT. CHANGE DAYS 1-8								
	NO	50	10	10	9 [c]	10	10	10
	MEAN	0.0	0.4	0.3	-0.0	0.3	0.3	0.3
	S	1.40	0.58	0.59	0.67	0.67	0.95	0.64
	SE	0.20	0.18	0.19	0.22	0.21	0.30	0.20
WT. CHANGE DAYS 1-12								
	NO	49 [c]	10	10	9 [c]	10	10	10
	MEAN	1.5	1.5	1.5	0.9	1.3	1.7	-0.0 *
	S	1.36	0.62	1.24	0.69	0.84	1.12	1.32
	SE	0.19	0.20	0.39	0.23	0.26	0.35	0.42
WT. CHANGE DAYS 1-16								
	NO	49 [c]	10	10	9 [c]	10	10	10
	MEAN	2.0	2.0	2.0	2.0	2.5	2.2	2.3
	S	1.42	0.88	0.79	1.48	0.83	1.40	1.45
	SE	0.20	0.28	0.25	0.49	0.26	0.44	0.46

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.
* $p < 0.05$.

2-Ethyl-1-hexanol (ETH). (Tables 53, 54, 59, and 60)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,000 mg ETH/kg/day
Group 3 - 1,525 mg ETH/kg/day
Group 4 - 2,330 mg ETH/kg/day
Group 5 - 3,560 mg ETH/kg/day
Group 6 - 5,435 mg ETH/kg/day
Group 7 - 8,300 mg ETH/kg/day (undiluted)

One animal in the control group and one animal in the 1,000-mg/kg group died because of dosing errors; compound-related mortality was 100% at the four highest dose levels tested (Table 53); all (10) animals in the 2,330-mg/kg group were dead by Day 4 of treatment; in the 3,560-, 5,435-, and 8,300-mg/kg groups, all animals were dead by Day 2 of treatment. One animal in the 1,525-mg/kg group died on Day 2 of treatment. Clinical signs of languidity, hunched posture, and prostration were present in all but the lowest dose groups (Tables 54 and 59). There were no significant differences from the controls in body weights or body weight gains (Table 60).

Based on these data, a dose level of 1,525 mg ETH/kg/day was used as the MED in the reproduction study.

Study No. 6125-107

Table 59

MED Study: Block D
Incidence of Clinical Signs: 2-Ethyl-1-hexanol

Clinical Sign of Toxicity	Dose Level (mg/kg/day)					
	1,000	1,525	2,330	3,560	5,435	8,300
Languid		2	4	1	4	2
Prostrate		1	5	4	3	
Ataxia						
Cold to touch				1		
Hunched		5	4	1	4	2
Tremors						
Head tilt						
Thin						
Wheezing						
Dyspnea						
Urine stains						
Alopecia						
Rough hair coat						
Sores						
Piloerection						

T-12507C TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK D, STUDY NO. 6125-107

Table 60
Summary of Mean Body Weight Data (g)
2-Ethyl-1-hexanol

MG/KG		0	1,000	1,525	2,330	3,560	5,435	8,300
WEIGHT DAY 1								
	NO	50	10	10	10	10	10	10
	MEAN	25.1	25.0	25.1	24.8	25.4	24.4	25.0
	S [a]	1.00	0.84	0.74	0.78	0.98	1.32	1.20
	SE [b]	0.14	0.26	0.23	0.25	0.31	0.42	0.38
WEIGHT DAY 8								
	NO	50	9 [c]	9	0	0	0	0
	MEAN	25.2	25.3	24.5	--	--	--	--
	S	1.45	0.70	0.99	0.0	0.0	0.0	0.0
	SE	0.20	0.23	0.33	0.0	0.0	0.0	0.0
WEIGHT DAY 12								
	NO	49 [c]	9 [c]	9	0	0	0	0
	MEAN	26.6	26.4	25.7	--	--	--	--
	S	1.22	0.84	1.25	0.0	0.0	0.0	0.0
	SE	0.17	0.27	0.42	0.0	0.0	0.0	0.0
WEIGHT DAY 16								
	NO	49 [c]	9 [c]	9	0	0	0	0
	MEAN	27.1	27.6	26.6	--	--	--	--
	S	1.31	1.18	1.34	0.0	0.0	0.0	0.0
	SE	0.19	0.39	0.45	0.0	0.0	0.0	0.0
WT. CHANGE DAYS 1-8								
	NO	50	9 [c]	9	0	0	0	0
	MEAN	0.0	0.2	-0.7	--	--	--	--
	S	1.40	0.83	0.38	0.0	0.0	0.0	0.0
	SE	0.20	0.28	0.13	0.0	0.0	0.0	0.0
WT. CHANGE DAYS 1-12								
	NO	49 [c]	9 [c]	9	0	0	0	0
	MEAN	1.5	1.4	0.6	--	--	--	--
	S	1.36	1.13	0.83	0.0	0.0	0.0	0.0
	SE	0.19	0.38	0.28	0.0	0.0	0.0	0.0
WT. CHANGE DAYS 1-16								
	NO	49 [c]	9 [c]	9	0	0	0	0
	MEAN	2.0	2.5	1.5	--	--	--	--
	S	1.42	1.67	1.03	0.0	0.0	0.0	0.0
	SE	0.20	0.56	0.34	0.0	0.0	0.0	0.0

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.

Reproduction Study No. 6125-108
Di(2-ethylhexyl)phthalate (DEHP). (Tables 61-63, 66, and 67)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 9,860 mg DEHP/kg/day (undiluted)

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 61 and 62).

No deaths occurred that could be attributed to the chemical, but two animals treated with DEHP died because of dosing errors on Day 12 of gestation (i.e., Day 6 of treatment) (Table 61). Clinical signs of toxicity included oily coat and wet and dry stains in the urogenital area (Table 63).

Group pregnancy and female body weight data are presented in Table 66. The reproductive index, body weights on Days 14 and 18 of gestation, and body weight changes during treatment and pregnancy were significantly lower than those of the control group.

Group litter and viability data are presented in Table 67; there were too few litters produced for the data to be analyzed statistically.

Di(isodecyl)phthalate (DIDP). (Tables 61, 62, 64, 66, and 67)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 9,650 mg DIDP/kg/day (undiluted)

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 61 and 62).

No animals treated with DIDP died during the test period (Table 61); clinical signs of toxicity included the presence of an oily coat, wet and dry stains in the urogenital region, and rough coat (Table 64).

Group pregnancy and female body weight data are presented in Table 66; no significant differences from the control group were seen in the parameters examined.

Group litter and viability data are presented in Table 67; no significant differences from the control group were seen in the parameters examined.

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2-Ethyl-1-hexanol (ETH). (Tables 61, 62, and 65-67)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 1,525 mg ETH/kg/day

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 61 and 62).

One animal treated with ETH died because of a dosing error on Day 8 of gestation (i.e., Day 2 of treatment); sixteen deaths occurred that were attributed to the chemical [nine on Day 8 of gestation, three on Day 9 of gestation, two on Day 11 of gestation, one on Day 12 of gestation, and one on Day 13 of gestation (i.e., Days 2, 3, 5, 6, and 7 of treatment, respectively)]. One chemically-related death also occurred on Day 17 of gestation (Table 61). Clinical signs of toxicity included languidity, ataxia, coldness to touch, wet stains, oily coat, and one animal that had a dark red discharge from its anus (Table 65).

Group pregnancy and female body weight data are presented in Table 66. In addition to the reproductive index, body weights on Days 14 and 18 of gestation, and on Day 3 post-partum, as well as body weight changes during treatment and pregnancy, were significantly lower than those of the control group.

Group litter and viability data are presented in Table 67. Most of the parameters examined were significantly different from those of the control group (i.e., mean number of live pups per litter, litter weights, and pup weights on Days 1 and 3, percent pup weight change from Days 1 to 3, mean pup viability per litter from Days 1 to 3, as well as percent of live pups per litter on Day 1 post-partum were significantly lower than those of controls; the mean numbers and percents of dead pups were significantly greater than those of controls at Day 1 post-partum).

Table 61

Survival of Mice in the Reproduction Study
for Experimental Block D

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days[a]								Mice Alive on Day 8		Mice Alive at Study End	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
Di(2-ethylhexyl)phthalate													
9,860†	50	0	0	0	0	0	2[b]	0	0	48[b]	96	48[b]	96
Di(isodecyl)phthalate													
9,650†	50	0	0	0	0	0	0	0	0	50	100	50	100
2-Ethyl-1-hexanol													
1,525	50	0	10[c]	3	0	2	1	1	0	33[c]	66	32[c]	64

†Undiluted.
[a]Treatment Days 1-8 = Gestation Days 7-14.
[b]Two deaths due to dosing error.
[c]One death due to dosing error.

Study No. 6125-108

Table 62

Reproduction Study: Block D
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

	Number of Animals Showing Sign		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Study No. 6125-108

Table 63

Reproduction Study: Block D
Incidence of Clinical Signs: Di(2-ethylhexyl)phthalate

	Dose Level: 9,860 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat	1		
Sores			
Piloerection			
Oily coat	35[a]	30	
Wet stains	8	2	
Dry stains	6	6	

[a]Includes two animals that died because of dosing errors.

Study No. 6125-108

Table 64

Reproduction Study: Block D
Incidence of Clinical Signs: Di(isodecyl)phthalate

	Dose Level: 9,650 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat	1		
Sores			
Piloerection			
Oily coat	16	48	
Wet stains	3		
Dry stains	5	5	

Study No. 6125-108

Table 65

Reproduction Study: Block D
Incidence of Clinical Signs: 2-Ethyl-1-hexanol

	Dose Level: 1,525 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	12	1	
Prostrate			
Ataxia	2	1	
Cold to touch	1	1	
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Wet stains	2		
Oily coat	2	1	
Dark red discharge (anus)	1		

Table 66

Group Pregnancy and Female Body Weight Data
for Experimental Block D

	Treatment			
	Vehicle Control (10 mL/kg)	Di(2-ethylhexyl) phthalate (9,860 mg/kg) (undiluted)	Di(isodecyl) phthalate (9,650 mg/kg) (undiluted)	2-Ethyl-1-hexanol (1,525 mg/kg)
Number of females on test	50	50	50	50
Number of females found dead (excluded from results)	0	2[a]	0	18[b]
Number of females that survived	50	48	50	32
Number of females that produced viable litters[c]	33	2	31	11
Number of females with resorbed or nonviable litters	1	30	3	9
Number of proven pregnant females	34	32	34	20
Reproductive index				
[(Number of females that produced viable litters/number of proven pregnant females) x 100]	97	6*	91	55*

[a]Two deaths due to dosing error.
[b]One death due to dosing error.
[c]A viable litter was defined as a litter that had at least one live pup on Day 1.
*$p < 0.05$.

Table 66 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block D

	Treatment			
	Vehicle Control (10 mL/kg)	Di(2-ethylhexyl) phthalate (9,860 mg/kg) (undiluted)	Di(isodecyl) phthalate (9,650 mg/kg) (undiluted)	2-Ethyl-1-hexanol (1,525 mg/kg)
Mean Body Weights (g) for Pregnant Females				
Gestation Day 7				
N	34	32	34	20
Mean	29.9	29.6	29.5	29.0
S[a]	2.35	1.79	2.42	2.07
Gestation Day 14				
N	34	32	34	20
Mean	37.1	33.1*	36.6	33.8*
S	2.89	2.85	2.86	3.58
Gestation Day 18				
N	34	32	34	20
Mean	47.9	33.5*	47.0	40.5*
S	5.01	4.40	5.46	6.38
Post-partum Day 3				
N	33	2	31	11
Mean	36.8	32.6[b]	36.4	33.6*
S	2.68	3.39	2.58	3.81

[a]S: Standard deviation.
[b]Too few animals to analyze statistically.
*$p < 0.05$.

Table 66 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block D

	Treatment			
	Vehicle Control (10 mL/kg)	Di(2-ethylhexyl) phthalate (9,860 mg/kg) (undiluted)	Di(isodecyl) phthalate (9,650 mg/kg) (undiluted)	2-Ethyl-1-hexanol (1,525 mg/kg)
Mean Body Weight Changes (g) for Pregnant Females				
During Treatment (Days 7-14 of gestation)				
N	34	32	34	20
Mean	7.2	3.5*	7.1	4.8*
S[a]	2.72	2.78	3.02	2.73
During Pregnancy (Days 7-18 of gestation)				
N	34	32	34	20
Mean	18.0	3.9*	17.5	11.4*
S	5.18	4.67	6.06	5.86

[a]S: Standard deviation.
*$p < 0.05$.

Table 67

Group Litter and Viability Data
for Experimental Block D

	Treatment			
	Vehicle Control (10 mL/kg)	Di(2-ethylhexyl) phthalate (9,860 mg/kg) (undiluted)	Di(isodecyl) phthalate (9,650 mg/kg) (undiluted)	2-Ethyl-1-hexanol (1,525 mg/kg)
Number of females that produced viable litters[a]	33	2	31	11
Post-partum Day 1				
Number of live pups/litter				
N	33	2	31	11
Mean	9.9	6.5[c]	9.9	6.4*
S[b]	2.36	6.36	2.46	3.23
Number of dead pups/litter				
N	33	2	31	11
Mean	0.1	1.0[c]	0.0	1.5*
S	0.29	1.41	0.00	1.63
Percent of live pups/litter				
N	33	2	31	11
Mean	99.2	75.0[c]	100.0	79.5*
S	2.56	35.36	0.00	21.18
Percent of dead pups/litter				
N	33	2	31	11
Mean	0.8	25.0[c]	0.0	20.5*
S	2.56	35.36	0.00	21.18
Litter weight (g)				
N	33	2	31	11
Mean	16.0	9.4[c]	15.7	8.9*
S	3.30	9.12	3.26	4.90
Mean pup weight/litter (g)				
N	33	2	31	11
Mean	1.6	1.5[c]	1.6	1.4*
S	0.13	0.04	0.14	0.17

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.
[c]Too few animals to analyze statistically.
*$p < 0.05$.

Table 67 (Continued)

Group Litter and Viability Data
for Experimental Block D

	Treatment			
	Vehicle Control (10 mL/kg)	Di(2-ethylhexyl) phthalate (9,860 mg/kg) (undiluted)	Di(isodecyl) phthalate (9,650 mg/kg) (undiluted)	2-Ethyl-1-hexanol (1,525 mg/kg)
Post-partum Day 3				
Number of live pups/litter				
N	33	2	31	11
Mean	9.8	6.0[b]	9.8	4.9*
S[a]	2.46	5.66	2.40	3.70
Number of dead pups/litter				
N	33	2	31	11
Mean	0.1	0.5[b]	0.1	1.5
S	0.24	0.71	0.40	2.02
Percent of live pups/litter				
N	33	2	31	11
Mean	98.2	95.5[b]	99.2	73.4
S	8.80	6.36	3.33	32.22
Percent of dead pups/litter				
N	33	2	31	11
Mean	1.8	4.5[b]	0.8	26.6
S	8.80	6.36	3.33	32.22
Mean pup viability/litter (Days 1 to 3)				
N	33	2	31	11
Mean	98.2	95.5[b]	99.2	73.4*
S	8.80	6.43	3.30	32.20
Litter weight (g)				
N	33	2	31	11
Mean	21.6	11.5[b]	21.2	9.1*
S	4.75	10.25	4.22	7.77
Mean pup weight/litter (g)				
N	33	2	31	11
Mean	2.2	2.0[b]	2.2	1.7*
S	0.19	0.19	0.25	0.31
Mean percent pup weight change (Days 1 to 3)				
N	33	2	31	11
Mean	35.3	36.7[b]	36.9	23.7*
S	6.28	9.38	6.39	13.15

[a]S: Standard deviation.
[b]Too few animals to analyze statistically.
*$p < 0.05$.

EXPERIMENTAL BLOCK E

MED Study No. 6125-109
Di(n-hexyl)phthalate (DNHP). (Tables 68-71)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg/body weight/day)
Group 2 - 3,000 mg DNHP/kg/day
Group 3 - 4,465 mg DNHP/kg/day
Group 4 - 6,650 mg DNHP/kg/day
Group 5 - 9,900 mg DNHP/kg/day (undiluted)

One death in the 9,900-mg/kg group was due to a dosing error; compound-related deaths occurred at all dose levels tested (Table 68) and included: one mouse in the 3,000-mg/kg group on Day 3 of treatment; three mice in the 4,465-mg/kg group on Days 2, 5, and 8 of treatment, respectively; two mice in the 6,650-mg/kg group on Day 2 of treatment; and one mouse in the 9,900-mg/kg group on Day 6 of treatment. Clinical signs of wet urogenital area and oily coat became more evident with increasing doses (Tables 69 and 70). With the exception of one animal in the 4,465-mg/kg group, languidity and prostration were reported only for animals that subsequently died. The only statistically significant differences in body weight data were smaller weight changes from Days 1-12 of treatment in the 6,650- and 9,900-mg/kg groups (Table 71).

Based on these data, a dose level of 9,900 mg DNHP/kg/day (i.e., undiluted DNHP) was used as the MED in the reproduction study.

Table 68

Survival of Mice in the MED Study
for Experimental Block E

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days								Mice Alive on Day 8		Mice Alive on Day 16	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
Di(n-hexyl)phthalate													
3,000	10	0	0	1	0	0	0	0	0	9	90	9	90
4,465	10	0	1	0	0	1	0	0	1	7	70	7	70
6,650	10	0	2	0	0	0	0	0	0	8	80	8	80
9,900†	10	0	0	0	0	0	1	1[a]	0	8[a]	80	8[a]	80
Di(n-octyl)phthalate													
3,000	10	0	0	0	0	0	0	0	0	10	100	10	100
4,450	10	0	0	0	0	0	0	0	0	10	100	10	100
6,595	10	0	0	0	0	0	0	0	0	10	100	10	100
9,780†	10	0	0	0	0	0	0	0	0	10	100	10	100
Mono(2-ethylhexyl)phthalate													
300	10	0	0	0	0	1	0	0	0	9	90	9	90
545	10	0	0	0	0	1	0	0	0	9	90	9	90
980	10	0	4	4	1	0	0	0	0	1	10	1	10
1,775	10	4[a]	6	-	-	-	-	-	-	0[a]	0	0[a]	0
3,210	10	2	8	-	-	-	-	-	-	0	0	0	0
5,805	10	7	3	-	-	-	-	-	-	0	0	0	0
10,500†	10	8	2	-	-	-	-	-	-	0	0	0	0

†Undiluted.
[a]One death due to dosing error.

Study No. 6125-109

Table 69

MED Study: Block E
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

Clinical Sign of Toxicity	Number of Animals Showing Sign
Languid	
Prostrate	
Ataxia	
Hunched	
Tremors	
Head tilt	
Thin	
Wheezing	
Dyspnea	
Urine stains	
Alopecia	
Rough hair coat	
Sores	
Piloerection	

Study No. 6125-109

Table 70

MED Study: Block E
Incidence of Clinical Signs: Di(n-hexyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day)			
	3,000	4,465	6,650	9,900
Languid	1	3	1	2[a]
Prostrate	1	1	1	1[a]
Ataxia				
Hunched				1
Tremors				
Head tilt				
Thin				1
Wheezing				
Dyspnea				
Urine stains				
Alopecia				
Rough hair coat				
Sores				
Piloerection				
Wet stains		4	9	10[a]
Nodules (lungs and thymus)	1			
Dry stains			6	10[a]
Oily coat			3	10[a]

[a]Includes one animal that died because of a dosing error.

T-12509A TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK E, STUDY NO. 6125-109

Table 71
Summary of Mean Body Weight Data (g)
Di(n-hexyl)phthalate

MG/KG		0	3.000	4.465	6.650	9.900
WEIGHT DAY 1						
	NO	50	10	10	10	10
	MEAN	26.8	26.9	26.0	27.0	26.3
	S [a]	1.03	1.15	1.40	0.98	0.90
	SE [b]	0.15	0.36	0.44	0.31	0.28
WEIGHT DAY 8						
	NO	50	9	7	8	8 [c]
	MEAN	27.0	27.8	26.9	27.3	27.1
	S	1.14	1.14	1.58	1.93	0.93
	SE	0.16	0.38	0.60	0.68	0.33
WEIGHT DAY 12						
	NO	50	9	7	8	8 [c]
	MEAN	28.0	28.2	27.2	27.4	26.9
	S	1.22	1.48	1.31	1.59	1.21
	SE	0.17	0.49	0.49	0.56	0.43
WEIGHT DAY 16						
	NO	50	9	7	8	8 [c]
	MEAN	28.8	28.7	27.8	27.9	27.8
	S	1.46	1.46	1.08	1.89	1.29
	SE	0.21	0.49	0.41	0.67	0.46
WT. CHANGE DAYS 1-8						
	NO	50	9	7	8	8 [c]
	MEAN	0.3	0.7	0.6	0.2	0.7
	S	0.96	0.67	0.64	1.09	0.50
	SE	0.14	0.19	0.24	0.38	0.18
WT. CHANGE DAYS 1-12						
	NO	50	9	7	8	8 [c]
	MEAN	1.3	1.0	0.8	0.3 *	0.4 *
	S	0.92	1.01	0.69	0.79	0.71
	SE	0.13	0.34	0.26	0.28	0.25
WT. CHANGE DAYS 1-16						
	NO	50	9	7	8	8 [c]
	MEAN	2.1	1.5	1.4	0.9	1.4
	S	1.20	1.24	0.98	1.23	0.85
	SE	0.17	0.41	0.37	0.44	0.30

a S: Standard deviation.
b SE: Standard error.
c One death due to dosing error.
* $p < 0.05$.

Di(n-octyl)phthalate (DNOP). (Tables 68, 69, 72, and 73)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 3,000 mg DNOP/kg/day
Group 3 - 4,450 mg DNOP/kg/day
Group 4 - 6,595 mg DNOP/kg/day
Group 5 - 9,780 mg DNOP/kg/day (undiluted)

No deaths occurred in mice treated with DNOP at any of the doses tested (Table 68). Clinical signs of wet urogenital area and oily coat increased in frequency with increasing doses (Tables 69 and 72). There were no statistically significant differences from control data in body weights or body weight changes at any of the doses tested (Table 73).

Based on these data, a dose level of 9,780 mg DNOP/kg/day (i.e., undiluted DNOP) was used as the MED in the reproduction study.

Study No. 6125-109

Table 72

MED Study: Block E
Incidence of Clinical Signs: Di(n-octyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day)			
	3,000	4,450	6,595	9,780
Languid				
Prostrate				
Ataxia				
Hunched				
Tremors				
Head tilt				
Thin				
Wheezing				
Dyspnea				
Urine stains				
Alopecia				
Rough hair coat				
Sores				
Piloerection				
Wet stains		6	7	10
Dry stains				10
Oily coat				10

T-12509B TREATMENT SUMMARY
MED STUDY IN MICE - EXPERIMENTAL BLOCK E, STUDY NO. 6125-109

Table 73
Summary of Mean Body Weight Data (g)

Di(n-octyl)phthalate

MG/KG		0	3,000	4,450	6,595	9,780
WEIGHT DAY 1						
	NO	50	10	10	10	10
	MEAN	26.8	26.8	27.1	26.2	26.5
	S [a]	1.03	0.92	0.69	0.86	1.16
	SE [b]	0.15	0.29	0.22	0.27	0.37
WEIGHT DAY 8						
	NO	50	10	10	10	10
	MEAN	27.0	27.0	27.3	26.7	26.2
	S	1.14	1.31	1.15	1.21	1.37
	SE	0.16	0.41	0.36	0.38	0.43
WEIGHT DAY 12						
	NO	50	10	10	10	10
	MEAN	28.0	27.5	28.1	27.4	27.6
	S	1.22	1.37	1.36	1.50	1.26
	SE	0.17	0.43	0.43	0.47	0.40
WEIGHT DAY 16						
	NO	50	10	10	10	10
	MEAN	28.8	28.9	28.6	27.8	28.6
	S	1.46	1.32	1.85	1.69	1.35
	SE	0.21	0.42	0.59	0.50	0.43
WT. CHANGE DAYS 1-8						
	NO	50	10	10	10	10
	MEAN	0.3	0.2	0.2	0.4	-0.3
	S	0.96	0.90	0.80	1.41	1.00
	SE	0.14	0.28	0.25	0.45	0.32
WT. CHANGE DAYS 1-12						
	NO	50	10	10	10	10
	MEAN	1.3	0.8	1.0	1.2	1.1
	S	0.92	1.01	0.99	1.25	0.85
	SE	0.13	0.32	0.31	0.39	0.27
WT. CHANGE DAYS 1-16						
	NO	50	10	10	10	10
	MEAN	2.1	2.1	1.5	1.6	2.1
	S	1.20	1.06	1.78	1.25	0.92
	SE	0.17	0.34	0.56	0.40	0.29

a S: Standard deviation.
b SE: Standard error.

Mono(2-ethylhexyl)phthalate (MEHP). (Tables 68, 69, 74, and 75)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 300 mg MEHP/kg/day
Group 3 - 545 mg MEHP/kg/day
Group 4 - 980 mg MEHP/kg/day
Group 5 - 1,775 mg MEHP/kg/day
Group 6 - 3,210 mg MEHP/kg/day
Group 7 - 5,805 mg MEHP/kg/day
Group 8 - 10,500 mg MEHP/kg/day (undiluted)

One animal in the 1,775-mg/kg group died because of a dosing error; compound-related deaths included one animal each in the 300- and 545-mg/kg groups on Day 5 of treatment, and nine animals in the 980-mg/kg group (four on Day 2, four on Day 3, and one on Day 4). All of the animals (10/group) in the 1,775-, 3,210-, 5,805-, and 10,500-mg/kg groups were dead after Day 2 of treatment (Table 68). The most common clinical signs reported were languidity, prostration, and ataxia (Tables 69 and 74). There were no biologically significant differences from control data in body weights or body weight changes at any of the doses tested; the only statistically significant difference was a higher body weight for the 545-mg/kg group on Day 8 of treatment (Table 75). There were too few animals in the 980-mg/kg group for the data to be analyzed statistically.

Based on these data, a dose level of 545 mg MEHP/kg/day was used as the MED in the reproduction study.

Study No. 6125-109

Table 74

MED Study: Block E
Incidence of Clinical Signs: Mono(2-ethylhexyl)phthalate

Clinical Sign of Toxicity	Dose Level (mg/kg/day)						
	300	545	980	1,775	3,210	5,805	10,500
Languid	1		6	7	7	5	5
Prostrate			4	6	3	5	9
Ataxia	1		3	1	4	4	6
Cold to touch			1		2		1
Hunched		1					
Tremors							
Head tilt							
Thin							
Wheezing							
Dyspnea			1	2			
Urine stains							
Alopecia							
Rough hair coat							
Sores							
Piloerection							
Nodules (lungs and thymus)		1					

Table 75
Summary of Mean Body Weight Data (g)
Mono(2-ethylhexyl)phthalate

MG/KG		0	300	545	980	1,775	3,210	5,805	10,500
WEIGHT DAY 1									
	NO	50	10	10	10	10	10	10	10
	MEAN	26.8	26.2	26.8	26.5	26.8	26.1	26.2	26.8
	S [a]	1.03	1.19	0.92	0.98	0.63	0.78	0.86	1.01
	SE [b]	0.15	0.38	0.29	0.31	0.20	0.25	0.27	0.32
WEIGHT DAY 8									
	NO	50	9	9	1 [c]	0 [d]	0	0	0
	MEAN	27.0	26.7	28.0 *	29.7 [c]	--	--	--	--
	S	1.14	1.69	1.13	0.0	0.0	0.0	0.0	0.0
	SE	0.16	0.56	0.38	0.0	0.0	0.0	0.0	0.0
WEIGHT DAY 12									
	NO	50	9	9	1	0 [d]	0	0	0
	MEAN	28.0	27.5	28.3	30.9 [c]	--	--	--	--
	S	1.22	1.76	1.65	0.0	0.0	0.0	0.0	0.0
	SE	0.17	0.59	0.55	0.0	0.0	0.0	0.0	0.0
WEIGHT DAY 16									
	NO	50	9	9	1	0 [d]	0	0	0
	MEAN	28.8	27.6	28.6	28.8 [c]	--	--	--	--
	S	1.46	1.74	1.90	0.0	0.0	0.0	0.0	0.0
	SE	0.21	0.58	0.63	0.0	0.0	0.0	0.0	0.0
WT. CHANGE DAYS 1-8									
	NO	50	9	9	1	0 [d]	0	0	0
	MEAN	0.3	0.3	1.1	2.2 [c]	--	--	--	--
	S	0.96	1.14	1.04	0.0	0.0	0.0	0.0	0.0
	SE	0.14	0.38	0.35	0.0	0.0	0.0	0.0	0.0
WT. CHANGE DAYS 1-12									
	NO	50	9	9	1	0 [d]	0	0	0
	MEAN	1.3	1.1	1.4	3.4 [c]	--	--	--	--
	S	0.92	1.36	1.23	0.0	0.0	0.0	0.0	0.0
	SE	0.13	0.45	0.41	0.0	0.0	0.0	0.0	0.0
WT. CHANGE DAYS 1-16									
	NO	50	9	9	1	0 [d]	0	0	0
	MEAN	2.1	1.2	1.7	1.3 [c]	--	--	--	--
	S	1.20	1.47	1.36	0.0	0.0	0.0	0.0	0.0
	SE	0.17	0.49	0.45	0.0	0.0	0.0	0.0	0.0

a S: Standard deviation.
b SE: Standard error.
c Too few animals to analyze statistically.
d One death due to dosing error.
* $p < 0.05$.

Reproduction Study No. 6125-110
Di(n-hexyl)phthalate (DNHP). (Tables 76-78, 81 and 82)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 9,900 mg DNHP/kg/day (undiluted)

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 76 and 77).

One death due to dosing error occurred on Day 14 of gestation (i.e., Day 8 of treatment); one chemically-related death and one death due to dosing error occurred on Day 15 of gestation (Table 76). Instances of languidity, coldness to the touch, wet and dry stains around the urogenital area, oily coat, and reddish-brown discharge from the vagina were recorded (Table 78).

Group pregnancy and female body weight data are presented in Table 81. The reproductive index, body weights on Days 14 and 18 of gestation, and body weight changes during treatment and pregnancy were significantly lower than those of the control group.

No viable litters were produced by females treated with 9,900 mg DNHP/kg/day (Table 82).

Di(n-octyl)phthalate (DNOP). (Tables 76, 77, 79, 81, and 82)

Dose levels used: Group 1 - vehicle control (10-mL corn oil/kg body weight/day)
Group 2 - 9,780 mg DNOP/kg/day (undiluted)

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 76 and 77).

No deaths occurred in animals treated with DNOP during the study period (Table 76); two instances of oily coat and three instances of wet stains around the urogenital area were recorded (Table 79).

Group pregnancy and female body weight data are presented in Table 81; no significant differences from the control group were seen in the parameters examined.

Group litter and viability data are presented in Table 82. The mean number of live pups per litter on Days 1 and 3 post-partum, mean litter weight on Day 3 post-partum, and mean percent pup weight change from Days 1 to 3 post-partum were significantly lower than those of controls.

Mono(2-ethylhexyl)phthalate (MEHP). (Tables 76, 77, and 80-82)

Dose levels used: Group 1 - vehicle control (10 mL corn oil/kg body weight/day)
Group 2 - 545 mg MEHP/kg/day

No deaths or clinical signs of toxicity were recorded for the vehicle control group (Tables 76 and 77).

Six chemically-related deaths occurred [one on Day 10 of gestation, one on Day 13 of gestation, two on Day 14 of gestation (i.e., Days 4, 7, and 8 of treatment, respectively), one on Day 15 of gestation, and one on Day 17 of gestation (Days 1 and 3 post-treatment, respectively)]; one death due to a dosing error occurred on Day 14 of gestation (Table 76). Clinical signs of toxicity included one animal that had a small mass on its tail during the study period, single instances of wet stains around the urogenital region and a reddish-brown discharge from the vagina, and two animals that were languid during the dosing period (Table 80).

Group pregnancy and female body weight data are presented in Table 81. The reproductive index, body weights on Days 14 and 18 of gestation, and body weight changes during treatment and pregnancy were significantly lower than those of controls.

Group litter and viability data are presented in Table 82. There were too few animals that produced litters for the data to be analyzed statistically, but the mean percent of survivors on Days 1 and 3 post-partum and mean litter weights on Day 3 post-partum, as well as mean pup viability per litter from Days 1-3 post-partum, were considerably lower than those of the control group.

Table 76

Survival of Mice in the Reproduction Study
for Experimental Block E

Treatment (mg/kg body weight/day)	No. of Animals	Mice Dead on Treatment Days[a]								Mice Alive on Day 8		Mice Alive at Study End	
		1	2	3	4	5	6	7	8	Number	Percent	Number	Percent
0	50	0	0	0	0	0	0	0	0	50	100	50	100
Di(n-hexyl)phthalate													
9,900†	50	0	0	0	0	0	0	0	1[b]	49[b]	98	47[c]	94
Di(n-octyl)phthalate													
9,780†	50	0	0	0	0	0	0	0	0	50	100	50	100
Mono(2-ethylhexyl)phthalate													
545	50	0	0	0	1	0	0	1	3[b]	45[b]	90	43[b]	86

†Undiluted.
[a]Treatment Days 1-8 = Gestation Days 7-14.
[b]One death due to dosing error.
[c]Two deaths due to dosing error.

Study No. 6125-110

Table 77

Reproduction Study: Block E
Incidence of Clinical Signs: Vehicle Control
10 mL corn oil/kg body weight/day

	Number of Animals Showing Sign		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			

Study No. 6125-110

Table 78

Reproduction Study: Block E
Incidence of Clinical Signs: Di(n-hexyl)phthalate

	Dose Level: 9,900 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	2[a]		
Prostrate			
Ataxia			
Cold to touch	2[a]		
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Wet stains	21[b]	1	
Dry stains	3	1	
Oily coat		5	
Reddish-brown discharge (vagina)	2		

[a]Includes one animal that died because of a dosing error.
[b]Includes two animals that died because of dosing errors.

Study No. 6125-110

Table 79

Reproduction Study: Block E
Incidence of Clinical Signs: Di(n-octyl)phthalate

	Dose Level: 9,780 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid			
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Wet stains	3		
Oily coat		2	

Study No. 6125-110

Table 80

Reproduction Study: Block E
Incidence of Clinical Signs: Mono(2-ethylhexyl)phthalate

	Dose Level: 545 mg/kg/day		
Clinical Sign of Toxicity	Days 7-14 of Gestation	Days 15-18 of Gestation	Day 19 of Gestation to Day 3 Post-partum
Languid	2		
Prostrate			
Ataxia			
Hunched			
Tremors			
Head tilt			
Thin			
Wheezing			
Dyspnea			
Urine stains			
Alopecia			
Rough hair coat			
Sores			
Piloerection			
Wet stains	1		
Reddish-brown discharge (vagina)		1	
Mass, small (tail)	1	1	1

Table 81

Group Pregnancy and Female Body Weight Data
for Experimental Block E

	Treatment			
	Vehicle Control (10 mL/kg)	Di(n-hexyl) phthalate (9,900 mg/kg) (undiluted)	Di(n-octyl) phthalate (9,780 mg/kg) (undiluted)	Mono(2-ethylhexyl) phthalate (545 mg/kg)
Number of females on test	50	50	50	50
Number of females found dead (excluded from results)	0	3[a]	0	7[b]
Number of females that survived	50	47	50	43
Number of females that produced viable litters[c]	34	0	39	2
Number of females with resorbed or nonviable litters	4	34	1	31
Number of proven pregnant females	38	34	40	33
Reproductive index				
[(Number of females that produced viable litters/number of proven pregnant females) x 100]	89	0*	98	6*

[a]Two deaths due to dosing error.
[b]One death due to dosing error.
[c]A viable litter was defined as a litter that had at least one live pup on Day 1.
*$p < 0.05$.

Table 81 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block E

	Treatment			
	Vehicle Control (10 mL/kg)	Di(n-hexyl) phthalate (9,900 mg/kg) (undiluted)	Di(n-octyl) phthalate (9,780 mg/kg) (undiluted)	Mono(2-ethylhexyl) phthalate (545 mg/kg)
Mean Body Weights (g) for Pregnant Females				
Gestation Day 7				
N	38	34	40	33
Mean	28.8	28.5	28.3	29.1
S[a]	1.92	2.41	2.10	2.21
Gestation Day 14				
N	38	34	40	33
Mean	38.2	32.0*	38.1	33.1*
S	3.63	3.49	3.38	2.83
Gestation Day 18				
N	38	34	40	33
Mean	48.8	30.1*	49.1	32.5*
S	6.88	2.54	6.00	5.24
Post-partum Day 3				
N	34	0[b]	39	2
Mean	39.3	-	37.8	38.5[c]
S	2.90	-	2.85	3.25

[a]S: Standard deviation.
[b]No litters produced.
[c]Too few animals to analyze statistically.
*$p < 0.05$.

Table 81 (Continued)

Group Pregnancy and Female Body Weight Data
for Experimental Block E

	Treatment			
	Vehicle Control (10 mL/kg)	Di(n-hexyl) phthalate (9,900 mg/kg) (undiluted)	Di(n-octyl) phthalate (9,780 mg/kg) (undiluted)	Mono(2-ethylhexyl) phthalate (545 mg/kg)
Mean Body Weight Changes (g) for Pregnant Females				
During Treatment (Days 7-14 of gestation)				
N	38	34	40	33
Mean	9.4	3.5*	9.7	4.0*
S[a]	3.17	3.06	2.41	2.88
During Pregnancy (Days 7-18 of gestation)				
N	38	34	40	33
Mean	20.0	1.7*	20.8	3.3*
S	6.64	2.25	5.11	5.14

[a]S: Standard deviation.
*$p < 0.05$.

Table 82

Group Litter and Viability Data
for Experimental Block E

	Treatment			
	Vehicle Control (10 mL/kg)	Di(n-hexyl) phthalate (9,900 mg/kg) (undiluted)	Di(n-octyl) phthalate (9,780 mg/kg) (undiluted)	Mono(2-ethylhexyl) phthalate (545 mg/kg)
Number of females that produced viable litters[a]	34	0	39	2
Post-partum Day 1				
Number of live pups/litter				
N	34	0	39	2
Mean	11.5	-	10.2*	9.0[c]
S[b]	1.73	-	2.80	1.41
Number of dead pups/litter				
N	34	0	39	2
Mean	0.0	-	0.1	0.5[c]
S	0.00	-	0.22	0.71
Percent of live pups/litter				
N	34	0	39	2
Mean	100	-	98.9	95.5[c]
S	0.00	-	5.44	6.36
Percent of dead pups/litter				
N	34	0	39	2
Mean	0.0	-	1.1	4.5[c]
S	0.00	-	5.44	6.36
Litter weight (g)				
N	34	0	39	2
Mean	18.6	-	17.1	14.9[c]
S	2.24	-	4.22	1.84
Mean pup weight/litter (g)				
N	34	0	39	2
Mean	1.6	-	1.7	1.7[c]
S	0.12	-	0.14	0.06

[a]A viable litter was defined as a litter that had at least one live pup on Day 1.
[b]S: Standard deviation.
[c]Too few animals to analyze statistically.
*$p < 0.05$.

Table 82 (Continued)

Group Litter and Viability Data
for Experimental Block E

	Treatment			
	Vehicle Control (10 mL/kg)	Di(n-hexyl) phthalate (9,900 mg/kg) (undiluted)	Di(n-octyl) phthalate (9,780 mg/kg) (undiluted)	Mono(2-ethylhexyl) phthalate (545 mg/kg)
Post-partum Day 3				
Number of live pups/litter				
N	34	0	39[b]	2
Mean	11.5	-	10.2*	7.5[c]
S[a]	1.71	-	2.94	0.71
Number of dead pups/litter				
N	34	0	39[b]	2
Mean	0.0	-	0.1	1.5[c]
S	0.17	-	0.35	0.71
Percent of live pups/litter				
N	34	0	39[b]	2
Mean	99.8	-	97.2	84.0[c]
S	1.37	-	16.03	5.66
Percent of dead pups/litter				
N	34	0	39[b]	2
Mean	0.2	-	2.8	16.0[c]
S	1.37	-	16.03	5.66
Mean pup viability/litter (Days 1 to 3)				
N	34	0	39[b]	2
Mean	99.8	-	97.2	83.8[c]
S	1.32	-	16.03	5.30
Litter weight (g)				
N	34	0	38	2
Mean	25.8	-	23.5*	18.6[c]
S	3.24	-	4.80	0.14
Mean pup weight/litter (g)				
N	34	0	38	2
Mean	2.3	-	2.3	2.5[c]
S	0.20	-	0.23	0.22
Mean percent pup weight change (Days 1 to 3)				
N	34	0	38	2
Mean	39.0	-	35.7*	49.9[c]
S	6.07	-	6.73	7.90

[a]S: Standard deviation.
[b]Includes one female whose pups were dead on Day 3.
[c]Too few animals to analyze statistically.
*$p < 0.05$.

CONCLUSIONS

A summary of the positive effects observed in the reproduction studies for this contract is presented in Table 83 which immediately follows this section. Based upon these effects, the following conclusions were drawn for chemicals tested in this contract.

- Di(n-butyl)phthalate, di(isobutyl)phthalate, N-isopropyl-N'-phenyl-p-phenylenediamine, di(2-ethylhexyl)phthalate, 2-ethyl-1-hexanol, di(n-hexyl)phthalate, and mono(2-ethylhexyl)phthalate warrant high priority consideration for more detailed reproductive toxicity testing.

- Diethyl phthalate, dimethyl methylphosphonate, tris(2-chloroethyl)-phosphate, d-phenylalanine, probenecid, trans-cinnamaldehyde, di(isodecyl)phthalate, and di(n-octyl)-phthalate warrant lower priority consideration for more detailed reproductive toxicity testing.

APPROVAL

Daniel F. Kehoe — December 6, 1983

Daniel F. Kehoe
Study Supervisor, Reproductive Toxicology

Karen M. MacKenzie — 12-6-83

Karen M. MacKenzie, PhD
Principal Investigator
Assistant Director, Toxicology

Date

by and for Hazleton Laboratories America, Inc.

(1331N/tt)

Table 83

Summary of Positive Effects on Parameters Examined in Reproduction Studies[a]

Parameter	Experimental Block A			Experimental Block B		
	Diethyl phthalate	Di(n-butyl) phthalate	Di(isobutyl) phthalate	Dimethyl methyl-phosphonate	tris(2-Chloro-ethyl)phosphate	N-isopropyl-N'-phenyl-p-phenylenediamine
Maternal mortality		(+)	+			+
Clinical signs			+			+
Reproductive index		+	+			+
Maternal body weight		+	+			+
Litter/pup weight		b	b			b
Pups dead at birth (Post-partum Day 1)	(+)	b	b			b
Pup viability (Post-partum Days 1-3)	(+)	b	b			b

[a]Positives in parentheses indicate slight effect.
[b]No litters produced.

Table 83 (Continued)

Summary of Positive Effects on Parameters
Examined in Reproduction Studies[a]

Parameter	Experimental Block C			Experimental Block D			Experimental Block E		
	D-phenyl-alanine	Probenecid	trans-Cinna-maldehyde	Di(2-ethylhexyl) phthalate	Di(isodecyl) phthalate	2-Ethyl-1-hexanol	Di(n-hexyl) phthalate	Di(n-octyl) phthalate	Mono(2-ethyl-hexyl)phthalate
Maternal mortality						+			(+)
Clinical signs				(+)	(+)	+	(+)		
Reproductive index	(+)	(+)		+		+	+		+
Maternal body weight				+		+	+		+
Litter/pup weight				+		+	b	(+)	+
Pups dead at birth (Post-partum Day 1)				+		+	b		(+)
Pup viability (Post-partum Days 1-3)				(+)		+	b		+

[a]Positives in parentheses indicate slight effect.
[b]No litters produced.

Appendix A

Study Summary Information for Experimental Blocks A through E

Experimental Block	Chemical	Type of Study[a]	Study No.	Dates for Initiation	Dates for Completion	Dates for Report	Dose (mg/10 mL/kg)
A	Diethyl phthalate	MED	6125-101	11/17/82	12/02/82	12/10/82	1000, 1830, 3345, 6120, 11200 (undiluted)
		REPRO	6125-102	01/17/83	02/03/83	10/11/83	4500
	Di(n-butyl) phthalate	MED	6125-101	11/17/82	12/02/82	12/10/82	1000, 1800, 3240, 5035, 10500 (undiluted)
		REPRO	6125-102	01/17/83	02/03/83	10/11/83	2500
	Di(isobutyl) phthalate	MED	6125-101	11/17/82	12/02/82	12/10/82	1000, 1795, 3225, 5790, 10400 (undiluted)
		REPRO	6125-102	01/17/83	02/03/83	10/11/83	4000
B	Dimethyl methylphosphonate	MED	6125-103	12/17/82	01/01/83	01/10/83	1000, 1750, 3150, 5600, 10000
		REPRO	6125-104	02/21/83	03/11/83	10/11/83	4175
	tris(2-Chloroethyl) phosphate	MED	6125-103	12/17/82	01/01/83	01/10/83	1000, 1315, 1730, 2280, 3000
		REPRO	6125-104	02/21/83	03/11/83	10/11/83	940
	N-isopropyl-N'-phenyl-p-phenylenediamine	MED	6125-103	12/17/82	01/01/83	01/10/83	100, 175, 315, 560, 1000
		REPRO	6125-104	02/21/83	03/11/83	10/11/83	800
C	D-phenylalanine	MED	6125-105	01/24/83	02/08/83	03/10/83	500, 890, 1580, 2810, 5000
		REPRO	6125-106	03/14/83	04/01/83	10/11/83	5000
	Probenecid	MED	6125-105	01/24/83	02/08/83	03/10/83	200, 355, 630, 1125, 2000
		REPRO	6125-106	03/14/83	04/01/83	10/11/83	1200
	trans-Cinnamaldehyde	MED	6125-105	01/24/83	02/08/83	03/10/83	250, 445, 790, 1405, 2500
		REPRO	6125-106	03/14/83	04/01/83	10/11/83	1200
D	Di(2-ethylhexyl) phthalate	MED	6125-107	03/08/83	03/23/83	04/10/83	6000, 7690, 9860 (undiluted)
		REPRO	6125-108	05/02/83	05/19/83	10/11/83	9860 (undiluted)
	Di(isodecyl) phthalate	MED	6125-107	03/08/83	03/23/83	04/10/83	1000, 1575, 2475, 3900, 6130, 9650 (undiluted)
		REPRO	6125-108	05/02/83	05/19/83	10/11/83	9650 (undiluted)
	2-Ethyl-1-hexanol	MED	6125-107	03/08/83	03/23/83	04/10/83	1000, 1525, 2330, 3560, 5435, 8300 (undiluted)
		REPRO	6125-108	05/02/83	05/19/83	10/11/83	1525
E	Di(n-hexyl)phthalate	MED	6125-109	04/26/83	05/11/83	06/10/83	3000, 4465, 6650, 9900 (undiluted)
		REPRO	6125-110	06/27/83	07/14/83	10/11/83	9900 (undiluted)
	Di(n-octyl)phthalate	MED	6125-109	04/26/83	05/11/83	06/10/83	3000, 4450, 6595, 9780 (undiluted)
		REPRO	6125-110	06/27/83	07/14/83	10/11/83	9780 (undiluted)
	Mono(2-ethylhexyl) phthalate	MED	6125-109	04/26/83	05/11/83	06/10/83	300, 545, 980, 1775, 3210, 5805, 10500 (undiluted)
		REPRO	6125-110	06/27/83	07/14/83	10/11/83	545

[a] MED: Minimum effective dose study
REPRO: Reproduction study

APPENDIX B

Clinical Observations

Animal and Housing

Normal
Abnormal

Animal Status (6-Digit Date Required)

Found dead
Terminal sacrifice
Accidental death
Animal escaped
Cannibalized
Mis-sexed
Replaced (explanation will be provided)
Other

Behavior

Languid
Anorexia
Hyperactive
Circling
Prostrate
Ataxia
Other

Excretion

Emesis
Salivation
Soft feces
Polyuria
Anuria
Compound-colored stool
Other

Housing, Food, and Water

Spillage - wasting feed
No feed
No water
Other

Appearance

Hunched
Thin
Obese
Teeth cut
Malocclusion
Head tilt
Paralysis
Tremors
Other

Eyes (Body Locator Codes Must be Used)

Squinted
Opaque
Lacrimation
Chromodacryorrhea
Exophthalmus
Mydriasis
Miosis

Respiration

Wheezing
Dyspnea
Polypnea
Rhinorrhea
Epistaxis
Other

APPENDIX B (Continued)

Clinical Observations

Skin and Pelage (Should be Used With Body Locator Codes)	Additional Modifiers (Body Locator Codes Must be Used)
Urine stains	Exudate
Alopecia	Enlarged
Rough hair coat	Small
Sores	Red
Cyanotic	Pale
Desquamation	Swollen
Piloerection	Abcessed
Scab formation involving hair	Necrotic
Discolorations of hair	Ulcerated
Skin with scab peeled off	Other
Other	

Body Locator Codes

ABD	Abdomen	HED	Head	PFR	Paw - Fore - Right
ANS	Anus	HPL	Hip - Left	PHL	Paw - Hind - Left
AXL	Axilla - Left	HPR	Hip - Right	PHR	Paw - Hind - Right
AXR	Axilla - Right	HPB	Hips - Both	PAL	Paws - All
AXB	Axilla - Both	INL	Inguinal - Left	SHL	Shoulder - Left
BCK	Back	INR	Inguinal - Right	SHR	Shoulder - Right
BDY	Entire Body	INB	Inguinal - Both	SHB	Shoulders - Both
CHS	Chest	LFL	Leg - Fore - Left	SDL	Side - Left
EAL	Ear - Left	LFR	Leg - Fore - Right	SDR	Side - Right
EAR	Ear - Right	LHL	Leg - Hind - Left	SDB	Sides - Both
EAB	Ears - Both	LHR	Leg - Hind - Right	TAL	Tail
ELL	Eyelid - Left	LAL	Legs - All	TEE	Teeth
ELR	Eyelid - Right	MTH	Mouth	TSL	Testis - Left
ELB	Eyelids - Both	NSE	Nose	TSR	Testis - Right
EYL	Eye - Left	NCK	Neck	TSB	Testes - Both
EYR	Eye - Right	PNS	Penis	VAG	Vagina
EYB	Eyes - Both	PFL	Paw - Fore - Left		

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