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Bio/dynamics Inc.

Division of Biology and Safety Evaluation

PROJECT NO. 81-2571

EMBRYOFETAL TOXICITY AND TERATOGENICITY STUDY

OF [redacted] BY INHALATION IN THE RAT

Final Report

Submitted to: E. I. DuPont DeNemours
Wilmington, Delaware 19898

Attention: Dr. H.M. Solomon

Date: December 31, 1981

Company Sanitized. Does not contain TSCA CBI

Bio/dynamics Inc.

PROJECT NO. 81-2571

EMBRYOFETAL TOXICITY AND TERATOGENICITY STUDY

OF [PERFLUOROBUTYLETHENE (PFBE)] BY INHALATION IN THE RAT

ABSTRACT

This pilot study was conducted for E.I. DuPont DeNemours and Company to evaluate the embryotoxic and/or teratogenic potential of [redacted] in the pregnant rat. Mated CD® female rats (seven females/group) were exposed by chamber inhalation to [redacted] at dose levels of 1,000 and 70,000 ppm. Animals were exposed for six hours/day during the Day 6-15 gestation interval. Included in the study was a sham-air, chamber-exposed control group (seven mated females). All females were sacrificed on Day 21 of gestation and fetuses recovered at this time were weighed, sexed and evaluated for external, soft tissue and/or skeletal malformations. Fetuses processed for skeletal evaluation were also examined for the presence of ossification variations.

No maternal mortality was encounte ed during the study and all females, control and treated groups were pregnant.

No treatment-related effect was evident in body weight or food consumption data for the low-dose group. At the high-dose level, mean body weight gain for the Day 6-16 gestation interval (exposure period) was significantly lower than control. Mean food consumption data for the high-dose group was lower than control for the exposure (Day 6-16) and post-exposure (Day 16-21) intervals; however, only during the exposure period was the difference from control data statistically significant.

No adverse maternal effects were seen in physical in-life examination data, liver weight data or gross postmortem examination data. Likewise, corpora lutea and uterine implantation data, fetal weight and sexing data and ossification variation data did not appear to be adversely affected by treatment.

No increase in malformation rate was seen in the treated groups during the fetal external or skeletal evaluations. During the soft tissue evaluations, a slight increase in the incidence of fetuses with malformations was seen only in the low-dose group. The most common observation noted during this evaluation involved distention and/or tortuous condition of the ureter. Similar type observations were seen in the concurrent control group with a lower incidence, and are noted frequently in our historical data for this strain of rat. In that a similar response was not noted at the higher exposure level, no treatment-related effect was indicated.

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81-2571 Bio/dynamics Inc.

Revised: R S Schnurr

Date: 1/13/82

I. INTRODUCTION:

This pilot study, conducted for E.I. DuPont DeNemours and Company, was designed to determine the teratogenic potential and/or degree of embryotoxicity of [redacted] in the pregnant rat. Test material was administered by chamber inhalation to mated rats (seven females/group) at dose levels of 1,000 and 70,000 ppm. Included in the study was a chambered, sham-air control group.

Species and strain of test animal, dose levels and route of test substance administration were established by the sponsor. Testing was conducted at Bio/dynamics, Inc., Mettlers Road, East Millstone, New Jersey 08873. Raw data to include fetal specimens were sent to sponsor for storage.

II. MATERIALS:

A. Test Substance: [redacted]

Supplier:	E. I. DuPont DeNemours Wilmington, Delaware 19898
Lot No.:	Haskell #14,100 [redacted]
Description:	Colorless liquid
Date Received:	6 July 1981
Amount Received:	Container #1: 24.900 kg Container #2: 26.475 kg Container #3: 26.650 kg Container #4: 26.500 kg
Storage:	Room Temperature

B. Test Animals:

Species:	Rat (albino)
Strain:	Crl:CD® (SD) BR
Supplier:	Charles River Breeding Laboratories Inc. Wilmington, Massachusetts

B. Test Animals (cont.):

Justification for Animal Selection:	Generally recognized laboratory animal. This strain of albino rat was selected because this rat was used in recent acute and subacute testing.
Number of Animals Purchased:	59 females
Placed on Test:	21 females
Date Received:	30 July 1980 (56 days of age)
Age at Initiation of Mating:	Females - 69 days of age
	Males from an in-house mating colony of the same strain of rat were used for breeding purposes only.

III. METHODS:

A. Husbandry:

Housing:	Females were housed individually (except during overnight mating intervals) in stainless steel, suspended cages with wire mesh floors.
Food:	Certified Purina Rodent Chow #5002 - available ad libitum during the non-exposure intervals.
Water:	Tap water (Elizabethtown Water Company) available via automatic watering system ad libitum during the non-exposure intervals.
	During the exposure intervals, animals did not have access to either food or water.
Environmental Conditions:	Temperature: 70 - 78°F
	Humidity: 42 - 70%
	Photo period: 12 hours light/dark cycle.

B. Mating: Females selected for mating were placed with male rats nightly in a 1:1 ratio. Vaginal smears were taken early in the morning and females were considered mated if sperm were observed microscopically in the vaginal rinse. The day evidence of mating was observed was designated Day 1 of gestation.

Dates of Mating: 11 August to 15 August 1981.

C. Assignment to Groups: Females which mated were assigned to groups daily in such a way as to most nearly equalize the Day 1 body weights.

D. Identification of Animals: Each mated female was identified with a metal eartag bearing it's animal number. The individual animal number plus the Bio/dynamics project number comprised a unique identification number for each female.

E. Experimental Outline:

Group No.	Test Substance[a]	Dose Level (ppm)	No. of Females Mated	No. of Females Sacrificed Day 21 of Gestation	Treatment Schedule[b] (Gestation Days)
I	Sham-air	0 (Chamber-Control)	7	7	6 - 15
II	[redacted]	1,000	7	7	6 - 15
III	[redacted]	70,000	7	7	6 - 15

[redacted]

[b]Chamber inhalation - 6 hours/day.

F. Test Substance Administration:

Route: Chamber inhalation

Treatment Schedule: Mated females were exposed daily, 6 hours/day from Day 6 to Day 15 of gestation, a 10-day treatment period.

Chamber Operation and Atmospheric Monitoring: See Appendix K.

IV. EVALUATIONS:

A. In-life:

Gross Physical Observations:	Twice daily (morning; afternoon) females were evaluated for obvious signs of pharmacologic or toxicologic effects and mortality.
Detailed Physical Examinations:	Each female was given a detailed physical examination on Days 1, 3, 9, 12, 15, 18 and 21 of gestation. During the treatment period, these examinations were performed in the afternoon following exposure.
Body Weights:	Females were weighed on Days 1, 6, 9, 13, 16 and 21 of gestation.
Food Consumption:	Food consumption was recorded during the exposure (Days 6-9, 9-13 and 13-16) and post-exposure (Days 16-21) periods.[1] Food spillage was weighed and recorded during the same intervals that feeder jars were weighed.

B. Postmortem:

1. Sacrifice Method and Maternal Postmortem Procedure: All females were sacrificed with an overdose of carbon dioxide on Day 21 of gestation. The abdominal and thoracic cavities were opened and each female was given a gross postmortem examination. The uterus was then removed intact (to include the ovaries) from the abdominal cavity and weighed. Likewise, the liver was dissected free and weighed for each female.

Maternal identifications were coded at time of sacrifice. Maternal sacrifices and subsequent fetal evaluations were performed blind in respect to dose group.

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[1]It was intended that food consumption be recorded during the pre-exposure interval (Days 1-3, 3-6 of gestation); however, due to technical error, these data were not recorded for either the control or treated groups.

B. Postmortem (cont.):

2. Examination of the Reproductive System:

Ovaries - each ovary was dissected free of the uterus, cleaned of connective tissues and evaluated for the presence and number of corpora lutea.

Uterus - the number and relative location of the following were recorded for each uterine horn:

Live fetuses

Dead fetuses (dead fetus with no visible degeneration)

Late resorptions (recognizable dead fetus undergoing degeneration regardless of size)

Early resorptions (evidence of implantation but no recognizable fetus)

Implantation sites (represents the sum total of fetuses [live and dead] and resorption sites [early and late]).

Once fetuses have been removed from uterus and all implantation sites identified, the placental/extra-embryonic tissues were removed and the uterus re-weighed (empty weight).

Dates of Maternal Day 21 Sacrifices: 1 September to 4 September 1981

3. Examination of Fetuses:

Upon removal from the uterus, each fetus was given a gross external examination for malformations, weighed, sexed and tagged for future identification.

Fetal Soft Tissue Examination:

Approximately one-half of the fetuses in each litter were evaluated for soft tissue malformations by the Staples microdissection technique.[2]

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[2]Staples, R.E. (1974). Detection of Visceral Alteration in Mammalian Fetuses. Teratology, 9:A37 (abstract).

B. Postmortem (cont.):

3. Examination of Fetuses (cont.):

Fetuses so processed were decapitated and heads fixed in Bouins solution to be processed by a razor blade slicing technique (Wilson[3]). At completion of the soft tissue evaluation, the decapitated, eviscerated fetal specimens were processed for staining of the skeletal structures using an Alizarian Red S staining procedure.[4] Stained fetal specimens were subsequently evaluated under a dissecting microscope for malformations and ossification variations.

Fetal Skeletal Examinations:

The remaining fetuses in each litter were eviscerated (external sexing data confirmed by internal inspection of the gonads) and processed for staining of the skeletal structures using an Alizarian Red S staining procedure.[4] Stained fetal specimens were evaluated under a dissecting microscope for skeletal malformations and ossification variations.

C. Statistical Evaluations:

Data were compared between the control and each treated group. Statistical evaluations used for this study are discussed in Appendix A.

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[3]Teratology, J.G. Wilson and J. Warkany, eds. (The University of Chicago Press, 1965) pp. 267-277.
[4]D.D. Crary, Stain Technology, 37 (1962) pp. 124-125.

V. RESULTS AND DISCUSSION:

A. Maternal Data:

1. Mortality Rate:

No mortality occurred in the control or treated groups.

2. Pregnancy Rates:

Pregnancy rates were 100% in each of the control and treated groups. All females (control and treated groups) were pregnant.

3. Body Weight Data:

Mean maternal body weights and weight gain data for the control and treated groups are presented in Table 1. These data are summarized from data for individual females presented in Appendix B.

Table 1 - Mean Maternal Body Weight Data - Gestation

Group (ppm)	Mean Body Weight (grams) Gestation Days							Mean Weight Gain (grams) Gestation Days				
	1	6	9	13	16	21		1-6	6-16	16-21		6-21
						Actual	Corr.[a]			Actual	Corr.[a]	Corr.[a]
I (0)	201	224	235	256	275	337	274	23	51	62	-1	50
II (1,000)	204	230	239	257	275	338	277	26	45	63	3	47
III (70,000)	206	234*	237	253	270	338	277	29	36**	68	6	42

Difference from the control group statistically significant; *$p \leq 0.05$; **$p \leq 0.01$.
[a]Corr. = Corrected Day 21 body weight = final Day 21 body weight - gravid uterine weight + empty uterine weight.

Mean body weight data were generally comparable between the control and each treated group throughout gestation; however, on Day 6 of gestation, mean body weight for the high-dose group was significantly higher than control.

3. Body Weight Data (cont.):

Mean body weight gain during the several gestation intervals were considered comparable between the control and low-dose group. In the high-dose group, mean weight gains were comparable to control during the pre-exposure (Days 1-6) and post-exposure intervals (Days 16-21 using actual and corrected Day 21 weight data). During the exposure interval (Days 6-16), mean weight gain for the high-dose group was significantly lower than control. Mean weight gain for the high-dose group during the Day 6-21 interval (using corrected Day 21 weight data) was also lower than control; however, this difference from control was not statistically significant.

4. Food Consumption Data:

Mean food consumption data for the exposure and post-exposure gestation intervals are presented in Table 2. These data are summarized from individual female data in Appendix E.

Table 2 - Mean Food Consumption Data

Group (ppm)	Mean Food Consumption (gram/animal/day) Gestation Days	
	6 - 16	16 - 21
I (0)	21.6	25.6
II (1,000)	20.4	24.8
III (70,000)	18.7**	23.0

Difference from the control group statistically significant:
**$p \leq 0.01$.

4. Food Consumption Data (cont.):

Mean food consumption data during the exposure period (Days 6-16) and post-exposure period (Days 16-21) were considered comparable between the control and low-dose group. In the high-dose group, mean food consumption during the exposure interval (Day 6-16) was significantly lower than control values. During the Day 16-21 interval, mean food consumption for the high-dose group was lower than control; however, this difference was not statistically significant.

5. Physical In-life Observation Data:

A similarity was seen between the control and treated groups in the types and incidences of observations noted during the detailed physical in-life evaluations. No treatment-related effect was evident.

6. Reproduction Data - Corpora Lutea and Uterine Implantation Data:

Corpora lutea and uterine implantation data for the control and treated groups are presented in Table 3. These data are summarized from individual female data in Appendix C.

The mean numbers of corpora lutea, implantations and fetuses (live and dead) were comparable between the control and each treated group. Likewise, the mean percentage of resorptions to implants and the mean percentage of resorptions plus dead fetuses to implants were comparable between these same groups.

The one dead fetus seen during the study was recovered from the litter of low-dose female No. 2579.

No treatment-related effect was evident in corpora lutea or uterine implantation data.

Table 3 - Corpora Lutea and Uterine Implantation Data

Parameter:	Group (ppm) I (0)	II (1,000)	III (70,000)
No. Pregnant Females:	7	7	7
Mean No./Pregnant Female -			
Corpora lutea:	14.9	13.0	15.0
Implantations:	13.3	12.7	12.6
Resorptions -			
Total No.:	2	5	3
Mean/pregnant female:	0.3	0.7	0.4
Mean % of Implants:	2.0	5.7	3.2
No. of dams with 100% resorptions:	0	0	0
Live Fetuses -			
Total No.:	91	83	85
Mean/pregnant female:	13.0	11.9	12.1
Dead Fetuses -			
Total No.:	0	1	0
Mean/pregnant female:	0.0	0.1	0.0
Mean % fetal deaths per implant:	0.0	1.0	0.0
Mean % resorption and fetal deaths/implant:	2.0	6.7	3.2

No statistically significant differences from the control group.

7. Liver Weight Data:

Mean liver weight data (absolute and relative to Day 21 corrected body weights) are summarized in Table 4. Liver weight data for individual females are presented in Appendix D.

Table 4 - Liver Weight Data

Group (ppm)	Mean Liver Weight (grams)	Mean Liver/Body Weight Ratio[a] (x 100)
I (0)	14.6	5.34
II (1,000)	14.0	5.06
III (70,000)	14.9	5.38

No statistically significant differences from the control group.
[a]Using the corrected Day 21 body weights.

Mean liver weights, both absolute and relative to Day 21 corrected body weights, were comparable between the control and treated groups.

8. Gross Postmortem Observations:

Observations noted during the gross postmortem evaluations of females sacrificed on Day 21 of gestation, are presented in Appendix F.

No treatment-related effect was evident from these evaluations.

B. Fetal Data:

1. Fetal Body Weight and Sex Distribution Data:

Mean fetal body weight and fetal sex distribution data are presented in Table 5. These data are presented for individual litters in Appendix C.

Table 5 - Mean Fetal Body Weights and Fetal Sex Distribution Data

Group (ppm)	Mean Fetal Weight (grams)	Sex Distribution[a,b] No.	Sex Distribution[a,b] Ratio	Percent Male Fetuses
I (0)	3.49	40/51	0.8	44.0
II[c] (1,000)	3.65	46/37	1.2	55.4
III (70,000)	3.57	48/37	1.3	56.5

No statistically significant differences from the control group.
[a]Total Number of Male Fetuses/Total Number of Female Fetuses.
[b]Data not analyzed statistically.
[c]Excludes data for the one dead fetus recovered in this group.

Mean fetal weight data were considered comparable between the control and treated groups. No stunted fetuses[5] were recovered in either the control or treated groups. Likewise, no fetuses were calculated as being stunted[6] in respects to littermates in these same groups.

Fetal sex distribution data revealed a preponderance of males in each of the treated groups with an increase in number of females in the control group. The differences in sex distribution are attributed to normal biological variability noted with this parameter and the relatively small group sizes involved.

No treatment-related effect was evident in fetal body weight or fetal sexing data.

[5]Stunted fetuses - fetuses that weigh 1 gram or less.
[6]Calculated stunted fetus (see Appendix A: page A-5).

2. Fetal External Examination Data:

Observations noted during the fetal external examinations are summarized in Appendix G.

No external malformations were seen in the 84 low-dose fetuses (seven litters), or 85 high-dose fetuses (seven litters).

In the control group, one fetus had a darkened, raised area on its back (thoracic region). No other external malformations were seen in the 90 remaining fetuses from this group (seven litters).

3. Fetal Soft Tissue Examination Data:

Observations noted during the fetal soft tissue evaluations are summarized in Appendix H. These malformation data are summarized in Table 6.

Table 6 - Fetal Soft Tissue Evaluation - Summary of Malformation Data

Group (ppm)	Observation	Fetuses Affected/ Total Examined		Litter Affected/ Total Examined	
		No.	%	No.	%
I (0)	Ureters - tortuous:				
	unilateral:	1/48	2.1	1/7	14.3
	bilateral:	1/48	2.1	1/7	14.3
	Total affected:	2/48	4.2	2/7	28.6
II (1,000)	Ureters - tortuous:				
	unilateral:	2/43	4.7	2/7	28.6
	bilateral:	2/43	4.7	1/7	14.3
	Ureters - tortuous and distended:				
	unilateral:	2/43	4.7	2/7	28.6
	Liver - small dark discolored area on median lobe:	1/43	2.3	1/7	14.3
	Total affected:	7/43	16.3	5/7	71.4
III (70,000)	Ureters - tortuous and distended:				
	unilateral:	1/44	2.3	1/7	14.3
	Ureters - tortuous:				
	bilateral:	1/44	2.3	1/7	14.3
	Total affected:	2/44	4.5	1/7	14.3

No statistically significant differences from the control group.

The most common observations noted during the soft tissue evaluations involved distention and/or the tortuous condition of the ureter(s). These types of observations were seen in two of 48 control fetuses (4.2%), six of 43 low-dose fetuses (14.0%) and two of 44 high-dose fetuses (4.5%). The significance of these types of ureter observations is unclear. In these same fetuses with ureter observations, the bladders were noted as filled (indicating a patency between ureter and bladder) and no kidney malformations (e.g., severely dis-

tended renal pelvis, absence of renal papillae) were seen. These types of ureter observations (i.e., distended, tortuous condition) are seen frequently in the term rat fetuses; historically, the incidence of such ureter observations is 8.7% (representing 1067 fetuses from 100 litters). In that no dose relationship was apparent in the incidence of these ureter observations, no treatment-related effect was indicated.

With the exception of ureter observations, the only other observation noted during the soft tissue evaluations was a small, dark, discolored area on the medium lobe of the liver. This observation was seen in one low-dose fetus.

No treatment-related effect on the fetus was evident from data collected during the soft tissue evaluations.

4. Ossification Variation Data:

The types of ossification variations seen during the skeletal examination and the incidence of fetuses (litters) affected are presented in Appendix J. Ossification variations were seen in 87/91 control fetuses, 75/84 low-dose fetuses and 77/85 high-dose fetuses, an incidence of 95.6%, 89.3% and 90.6%, respectively. These incidences were comparable between the control and treated groups.

The mean percentage of fetuses per litter with ossification variations for the control, low- and high-dose group was 95.3%, 89.1% and 91.0%, respectively. These data were comparable between the control and each treated group.

The types and incidence of ossification variations seen were similar between the control and treated groups. No treatment-related effect was evident in these data.

5. Fetal Skeletal Examination Data:

Observations noted during the fetal skeletal evaluations are summarized in Appendix I. Malformation data are summarized in Table 7.

Table 7 - Fetal Skeletal Examination - Summary of Malformation Data

Group (ppm)	Malformation	Fetuses Affected/ Total Examined		Litter Affected/ Total Litters Evaluated	
		No.	%	No.[a]	%
I (0)	Angulated Ribs and Shortened Ribs:	1/91	1.1	1/7	14.3
	Total:	1/91	1.1	1/7	14.3
II (1,000)	Fused Sternebrae	1/84	1.2	1/7	14.3
	Total:	1/84	1.2	1/7	14.3
III (70,000)	Fused Sternebrae and midline cleft through the sternum:	1/85	1.2	1/7	14.3
	Total:	1/85	1.2	1/7	14.3

No statistically significant differences from the control group.
[a]No. of litters containing a malformed fetus divided by the number of litters evaluated.

The incidence of skeletal malformations, both on a per fetus and per litter basis, was comparable between the control and treated groups.

In the control group, one fetus had an angulated/shortened rib defect. No other skeletal malformations were seen in this group.

Fused sternebrae were seen in two treated group fetuses. In the low-dose group, one fetus from the litter of female No. 2580 had a fusion between the first and second sternebrae. The fused sternebrae seen in one high-dose fetus, from the litter of female No. 3578, involved the second through the fifth sternebra. A mid-line cleft was also evident through this fused segment of the sternum.

The sternebral fusion defects seen in a single fetus from each of the treated groups were dissimilar in respects to the sternebrae involved and were not considered to represent a treatment-related effect.

During the external, soft tissue and skeletal evaluations, malformations were seen in four of 91 control fetuses (4.4%), eight of 84 low-dose fetuses (9.5%) and three of 85 high-dose fetuses (3.5%). The mean percentages of malformed fetuses per litter for the control, low- and high-dose groups were 4.3%, 9.8% and 3.3%, respectively. Though these values were increased in the low-dose group, differences from control were not statistically significant. Likewise, no dose-relationship was evident in these values.

Thus, [redacted] administered by chamber inhalation at dose levels of 1,000 and 70,000 ppm during the Day 6-15 gestation interval, did not appear to be embryotoxic or teratogenic in the seven pregnant rats evaluated per dose level.

Raymond E. Schroeder, M.S.
Study Director

12/3/81
Date

Geoffrey K. Hogan, Ph.D.
Vice President of Toxicology

12/31/81
Date

Appendix A

Embryofetal Toxicity and Teratogenicity Study of [redacted] by Inhalation in the Rat

Statistical Analysis

Statistical Analysis of Data:

Results of analysis by the indicated statistical procedure were reported for the following:

Parameter	Method
1. Body Weight - Days 1, 6, 9, 13, 16 and 21 of gestation (actual and corrected by subtracting gravid uterus):	A
2. Body weight change - Days 1-6, 6-16, 16-21 and 6-21 of gestation (actual and corrected by subtracting gravid uterus):	A
3. Reproduction Data	
a. Mean number of corpora lutea	A
b. Mean number of implantation sites:	A
c. Mean number of resorptions:	A
d. Mean number male and female fetuses:	A
e. Mean number of live fetuses:	A
f. Mean fetal weight:	A
4. Mean Percentage of Resorptions to Implants	A
5. Mean Liver weights (absolute and relative to Day 30 corrected body weights):	A
6. Food Consumption Data - Days 3-6, 6-16 and 16-21:	A
7. Number and percentage of fetuses with external, soft tissue or skeletal malformations:	B
8. Number and percentage of litters containing at least one fetus with a malformation:	B
9. Incidence of fetuses with ossification variations:	B
10. Mean percentage of malformed fetuses per litter.	A
11. Mean percentage of fetuses with ossification variations per litter.	A

Statistical Analysis (cont.)

Statistical Analysis of Data (cont.)

Method A[a]:

Statistical evaluation of equality of means was made by the appropriate one-way analysis of variance technique, followed by a multiple comparison procedure if needed. First, Bartlett's test was performed to determine if groups had equal variances. If the variances were equal, parametric procedures were used; if not, nonparametric procedures were used. The parametric procedures were the standard one-way ANOVA using the F-distribution to assess significance. If significant differences among the means were indicated, Dunnett's test was used to determine which means were significantly different from the control. If a nonparametric procedure for testing equality of means was needed, the Kruskal-Wallis test was used, and if differences were indicated, a summed rank test (Dunn) was used to determine which treatments differed from control.

When statistically significant differences were identified by the Dunnett's test, a standard two-way analysis of variance was used to detect interaction between breeding lots and test groups.

A statistical test for trend in the dose level was also performed. In the parametric case (i.e. equal variance), standard regression techniques with a test for trend and lack of fit were used. In the nonparametric case, Jonckheere's test for monotonic trend was used.

The test for equal variance (Bartlett's) was conducted at the 1%, two-sided risk level. All other statistical tests were conducted at the 5% and 1% two-sided risk levels.

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[a]Snedecor, G.W., and Cochran, W.G., Statistical Methods, 6th ed., Iowa State Univ. Press (1967), Hollander and Wolfe, Nonparametric Statistical Methods, John Wiley and Sons, New York (1973), Dunnett, C.W., J. Am. Sta. Assn., Vol. 50 (1955) and Biometrics, Vol. 20 (1964).

Bartletts's Test		
ANOVA	pp. 296-298	
Dunnett's Test	pp. 277-279	S&C
Kruskal-Wallis	pp. 1096-1121, 482	S&C
Summed Rank Test (Dunn)	pp. 114-116	D
Regression Analysis	pp. 131	H&W
Trend		H&W
Lack of Fit	pp. 149-152	
Jonckheere's Statistic	pp. 160-164	S&C
Arc Sine	pp. 120-123	S&C
	pp. 327-329	H&W
		S&C

Statistical Analysis (cont.)

Statistical Analysis of Data (cont.)

Method B[b]:

Statistical analysis of incidence data was performed using contingency tables. First, a standard chi-square analysis was performed to determine if the proportion of incidences differed between the groups tested. In keeping with standard statistical practice, if any one cell had an expected value of less than 5, this step was not reported. Next, each treatment group was compared to the control group using a 2x2 Fisher Exact test and the significance level was corrected via the Bonferroni inequality to assure an overall test of the stated significance level. Thirdly, Armitage's test for linear trend in the dosage groups was performed.

[b]Chi-square: Snedecor, G.W., and Cochran, W.G., Statistical Methods, 6th ed., Iowa State Univ. Press. Ames, Iowa, 1971, pp. 250-253.
Fisher Exact Test: Bradley, J.V., Distribution Free Statistical Tests, Prentice Hall Englewood Cliffs, N.J., 1968, pp. 195-203.
Bonferroni Inequality: Miller, R.G., Jr., Simultaneous Statistical Inference McGraw-Hill Book Co., N.Y. 1966, p. 15.
Armitage's Test: Armitage, P., "Tests for Linear Trends in Proportions and Frequencies", Biometrics, Sept. 1955, pp. 375-386.

Statistical Analysis (cont.)

Statistical Analysis of Data (cont.)

Statistical Notations

No Sig	Symbol p<0.05	p<0.01	Statistical Statement
			Absolute Data
A-			No statistical differences among the means (parametric ANOVA).
	A+	A++	The means differ significantly (parametric ANOVA).
	*	**	Significantly different from control (Dunnett's)
K-			No statistical differences among the means (Kruskal-Wallis, non-parametric)
	K+	K++	The means differ significantly (Kruskal-Wallis, non-parametric).
	†	††	Significantly different from control (Dunn's Rank Sum).
	L	L+	The response is linearly related to the dose levels.
	F	F+	Lack of fit.
	J	J+	Jonckheere's test.
	#	##	Incidence data indicates difference from control by the Fishers Exact test.

Statistical Analysis (cont.)

Procedures for calculating stunted fetuses, mean litter weight and maximum stunted weight calculations.

Stunted fetuses (S) - Fetuses that weigh 1 gram or less are considered stunted.

Calculated stunted fetuses (Sc) - Mean litter weight and maximum stunted weight must be calculated to identify which fetuses are stunted by calculation (based upon comparison to the weight of its litter mates only).

Mean litter weight (MLW) is determined by adding the weights of all live fetuses excluding "stunteds" and "calculated stunteds" and dividing this sum by the number of live fetuses except "stunteds" and "calculated stunteds."

Maximum stunted weight (MSW) is determined by calculating the mean weight of all live fetuses excluding "stunteds" and the smallest of the remaining fetuses, and multiplying this sum by 0.666. This product is then compared to the weight of the fetus that was excluded. If the fetus weighs less than or equal to the above figure it is labeled as "calculated stunted" (Sc). If the fetus weighs more than the figure, then this value is the maximum stunted weight (MSW).

If the lightest fetus was a Sc then the next lightest fetus must also be analyzed. In this step the Sc and the next lightest fetus are excluded from the calculations. Again, the mean value is multiplied by 0.666 and this product is compared to the weight of the fetus under examination. This procedure is repeated until a MSW larger than the next fetus to be excluded is found. Lastly, the MLW must be recalculated after excluding all Sc fetuses in the litter.

Staples, R. E. (January 1978). Teratology Laboratory Techniques, collated from information and training provided to Research Triangle Institute by R. E. Staples.

Individual Female Body Weight and Body Weight Change Data - Gestation Period

Group (ppm)	Female No.	Body Weight (grams) Gestation Day 1	6	9	13	16	21 Actual	21 Corrected[a]	Uterine Weight (grams) Gravid	Empty	Mean Body Weight Change (grams) Gestation Days 1-6	6-16	16-21 Actual	16-21 Corrected[a]	6-21 (Corrected)[a]
I (0) (Chamber Control)	1576	A- 193	A+L 221	A- 226	A- 247	269	A- 325	A- 271	K- 59	A- 5	A- 28	A++L+ 48	A- 56	A- 2	A- 50
	1577	203	234	244	260	287	352	286	72	6	31	53	65	-1	52
	1578	204	229	235	260	277	350	283	73	6	25	48	73	6	54
	1579	199	212	232	254	272	328	266	68	6	13	60	56	-6	54
	1580	200	227	233	256	277	342	279	69	6	27	50	65	2	54
	1581	201	223	233	256	272	330	267	67	4	22	49	58	-5	52
	1582	207	222	241	262	272	330	264	71	5	15	50	58	-8	44
	Mean :	201	224	235	256	275	337	274	68	5	23	51	62	-1	42
	S.E.M.:	2	3	2	2	2	4	3	2	0	3	2	2	2	50
	N :	7	7	7	7	7	7	7	7	7	7	7	7	7	2
II (1,000)	2576	194	224	233	250	274	324	264	65	5	30	50	50	-10	7
	2577	201	224	230	252	261	323	273	56	6	23	37	62	12	40
	2578	204	237	237	255	272	340	278	67	5	33	35	68	6	49
	2579	211	239	246	268	294	366	297	74	5	28	55	72	3	41
	2580	201	224	241	258	275	338	278	65	5	23	51	63	3	58
	2581	207	231	242	254	273	335	280	61	6	24	42	63	7	54
	2582	209	233	241	261	275	339	272	72	5	24	42	64	-3	49
	Mean :	204	230	239	257	275	338	277	66	5	26	45	63	3	39
	S.E.M.:	2	2	2	2	4	5	4	2	0	1	3	3	3	47
	N :	7	7	7	7	7	7	7	7	7	7	7	7	7	3
III (70,000)	3576	198	233	224	237	252	327	262	70	5	35	19	75	10	7
	3577	199	227	234	241	263	338	273	70	5	28	36	75	10	29
	3578	204	234	242	251	269	342	272	76	6	30	35	73	3	46
	3579	210	246	246	265	286	372	293	85	6	36	40	86	7	38
	3580	216	237	244	262	281	327	298	34	5	21	44	46	17	47
	3581	201	229	241	260	279	331	268	69	6	28	50	52	-11	61
	3582	211	235	231	252	262	331	270	65	4	24	27	69	8	39
	Mean :	206	234*	237	253	270	338	277	67	5	29	36**	68	6	35
	S.E.M.:	3	2	3	4	5	6	5	6	0	2	4	5	3	42
	N :	7	7	7	7	7	7	7	7	7	7	7	7	7	4

For explanation of statistical symbols (i.e., A+, A++, A-, K-, L, L+) see Appendix A.
Differences from control statistically significant: *$p \leq 0.05$; **$p \leq 0.01$.
[a]The "corrected" Day 21 body weight = final Day 21 body weight - gravid uterine weight + empty uterine weight.

Embryofetal Toxicity and Teratogenicity Study of [redacted] by Inhalation in the Rat

Individual Female Reproduction Data

Group (ppm)	Female No.	No. of Corpora Lutea	No. of Implants	Resorptions No.	Resorptions % of Implants	Fetuses No. Live Male	Fetuses No. Live Female	Fetuses No. Live Total	Fetuses No. Dead	Mean Fetal Weight (grams)
I (0) (Chamber Control)	1576	A- 15	A- 11	A- 0	A- 0.0	A- 4	A- 7	A- 11	0	A- 3.59
	1577	21	15	1E	6.7	5	9	14	0	3.32
	1578	15	15	0	0.0	9	6	15	0	3.11
	1579	13	13	0	0.0	9	4	13	0	3.31
	1580	14	14	1E	7.1	2	11	13	0	3.61
	1581	13	13	0	0.0	5	8	13	0	3.51
	1582	13	12	0	0.0	6	6	12	0	3.98
	Total :	104	93	2	13.8	40	51	91	0	24.43
	Mean :	14.9	13.3	0.3	2.0	5.7	7.3	13.0	0.0	3.49
	S.E.M.:	1.1	0.6	0.2	1.3	1.0	0.9	0.5	0.0	0.11
	N :	7	7	7	7	7	7	7	7	7
II (1,000)	2576	12	12	1E	8.3	9	2	11	0	4.01
	2577	12	11	1E	9.1	4	6	10	0	3.53
	2578	13	13	1E	7.7	7	5	12	0	3.71
	2579	14	14	0	0.0	7	6	13	1(7.1)[b]	3.44
	2380	15	13	1E	7.7	7	5	12	0	3.44
	2581	14	14	1E	7.1	3	10	13	0	3.46
	2582	11	12	0	0.0	9	3	12	0	3.98
	Total :	91	89	5	39.9	46	37	83	1	25.57
	Mean :	13.0	12.7	0.7	5.7	6.6	5.3	11.9	0.1	3.65
	S.E.M.:	0.5	0.4	0.2	1.5	0.9	1.0	0.4	0.1	0.10
	N :	7	7	7	7	7	7	7	7	7
III (70,000)	3576	14	13	1E	7.7	7	5	12	0	3.87
	3577	16	15	1E	6.7	11	3	14	0	3.36
	3578	14	13	0	0.0	5	8	13	0	3.93
	3579	17	16	0	0.0	7	9	16	0	3.42
	3580	15	6	0	0.0	1	5	6	0	3.25
	3581	16	13	1E	7.7	8	4	12	0	3.77
	3582	13	12	0	0.0	9	3	12		3.49
	Total :	105	88	3	22.1	48	37	85	0	24.99
	Mean :	15.0	12.6	0.4	3.2	6.9	5.3	12.1	0.0	3.57
	S.E.M.:	0.5	1.2	0.2	1.5	1.2	0.9	1.2	0.0	0.09
	N :	7	7	7	7	7	7	7	7	7

For explanation of statistical symbol (A-) see Appendix A.
No statistically significant differences from the control group.
[a]E = Early resorption.
[b]Number in parenthesis represents the percentage of dead fetuses to implants.

Appendix D

EMBRYOFETAL TOXICITY AND TERATOGENICITY STUDY
OF [redacted] BY INHALATION IN THE RAT

INDIVIDUAL FEMALE LIVER WEIGHT DATA

AN NO	TERMINAL BODY WT.[a] (GM)	LIVER WGHT WT. (GM)	ORG/TBW[b] (X 100)
GROUP 1 - (0 PPM)			
	A-	A-	A-
1576	271.0	14.4	5.31
1577	266.0	16.2	5.66
1578	283.0	15.3	5.41
1579	256.0	13.9	5.23
1580	279.0	14.9	5.30
1581	267.0	13.1	4.91
1582	264.0	14.5	5.53
MEAN	272.7	14.6	5.34
SD	6.9	1.0	0.24
SE	2.4	0.4	0.09
N	7	7	7
MIN	264.0	13.1	4.91
MAX	266.0	16.2	5.66

For explanation of statistical symbol (A-) see Appendix A.
[a]Corrected Day 21 body weights (see Appendix B).
[b]Liver weight divided by corrected Day 21 body weight X 100.

Appendix D (cont.)

EMBRYOFETAL TOXICITY AND TERATOGENICITY STUDY OF [redacted] BY INHALATION IN THE RAT

INDIVIDUAL FEMALE LIVER WEIGHT DATA

AN NO	TERMINAL BODY WT. a (GM)	LIVER WGHT WT. (GM)	LIVER WGHT/TBW b (X 100)
GROUP 11 - (1000 PPM)			
2576	264.0	12.6	4.77
2577	273.0	14.8	5.55
2578	278.0	13.7	4.93
2579	297.0	15.5	5.22
2580	278.0	15.2	5.47
2581	280.0	13.3	4.82
2582	272.0	13.2	4.85
MEAN	277.4	14.0	5.06
SD	10.2	1.1	0.28
SE	3.8	0.4	0.11
N	7	7	7
MIN	264.0	12.6	4.77
MAX	297.0	15.5	5.47

[a] Corrected Day 21 body weights (see Appendix 3).
[b] Liver weight divided by corrected Day 21 body weight X 100.

Appendix D (cont.)

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EMBRYOFETAL TOXICITY AND TERATOGENICITY STUDY
OF [redacted] BY INHALATION IN THE RAT

INDIVIDUAL FEMALE LIVER WEIGHT DATA

AN NO	TERMINAL BODY WT.[a] (GM)	LIVER WGHT WT. (GM)	ORG/TBW[b] (X 100)
GROUP III - (70000 PPM)			
3576	262.0	12.7	4.85
3577	273.0	14.4	5.27
3578	272.0	15.0	5.51
3579	293.0	15.8	5.39
3580	293.0	16.6	5.64
3581	266.0	14.4	5.37
3582	270.0	15.2	5.63
MEAN	276.6	14.9	5.38
SD	13.5	1.3	0.27
SE	5.1	0.5	0.10
N	7	7	7
MIN	262.0	12.7	4.85
MAX	293.0	15.8	5.64

[a]Corrected Day 21 body weights (see Appendix B).
[b]Liver weight divided by corrected Day 21 body weight X 100.

Appendix E

81-2571

of [redacted] Embryofetal Toxicity and Teratogenicity Study by Inhalation in the Rat

Individual Female Food Consumption Data (g/animal/day)

Group (ppm)	Female No.	Day: 3 - 6	6 - 16	16 - 21
I (0) (Chamber Control)	1576	A	A++L+	A-
	1577	-	20.7	26.4
	1578	-	21.1	25.4
	1579	-	21.4	26.6
	1580	-	21.1	22.4
	1581	-	21.2	23.0
	1582	17.0	20.7	25.6
		18.0	24.7	29.8
	Total :	35.0	150.9	179.2
	Mean :	17.5	21.6	25.6
	S.E.M.:	0.5	0.5	0.9
	N :	2	7	7
II (1,000)	2576	-	20.7	24.8
	2577	-	18.3	22.8
	2578	-	19.6	24.6
	2579	-	22.2	27.4
	2580	-	23.9	20.8
	2581	-	17.5	25.6
	2582	18.7	20.7	27.6
	Total :	18.7	142.9	173.6
	Mean :	18.7	20.4	24.8
	S.E.M.:	0.0	0.8	0.9
	N :	1	7	7
III (70,000)	3576	-	18.2	26.2
	3577	-	18.1	20.0
	3578	-	19.4	19.2
	3579	-	20.0	26.6
	3580	-	18.3	27.6
	3581	-	19.2	17.4
	3582	18.3	17.6	24.0
	Total :	18.3	130.8	161.0
	Mean :	18.3	18.7**	23.0
	S.E.M.:	0.0	0.3	1.5
	N :	1	7	7

For explanation of statistical symbols (A-, A++, L+) see Appendix A.

Appendix F

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Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat
Pathology Report

Summary of Adult Gross Postmortem Observations

All of the animals on test survived to the termination of the study when they were killed for postmortem examination. Morphologic abnormalities observed grossly were sporadically in the treated and control animals; they did not appear to be related to the administration of the test article.

Henry F. Bolte, D.V.M., Ph.D.
Pathologist

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Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat

Adult Gross Postmortem Observations

Group (ppm)	Female No.	Type of Death[a]	Pregnancy Status[b]	Observation(s)[c]
I (0)	1576	T	+	N.O.A.
	1577	T	+	N.O.A.
	1578	T	+	Kidney (R): moderately hollow.
	1579	T	+	N.O.A.
	1580	T	+	N.O.A.
	1581	T	+	N.O.A.
	1582	T	+	N.O.A.
II (1,000)	2576	T	+	N.O.A.
	2577	T	+	N.O.A.
	2578	T	+	N.O.A.
	2579	T	+	N.O.A.
	2580	T	+	Lungs: small and dark red in color; diaphragmatic hernia.
	2581	T	+	Kidney (R): moderately hollow.
	2582	T	+	N.O.A.
III (70,000)	3576	T	+	N.O.A.
	3577	T	+	N.O.A.
	3578	T	+	N.O.A.
	3579	T	+	N.O.A.
	3580	T	+	N.O.A.
	3581	T	+	N.O.A.
	3582	T	+	N.O.A.

[a] T = Terminal (Day 21 of Gestation).
[b] + = Pregnant.
[c] R = Right.

Appendix G

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Embryofetal Toxicity and Teratogenicity Study of [redacted] by Inhalation in the Rat

Summary of Fetal External Examination Data

Group (ppm)	Female No.	Fetus No.	Malformation[a]
I[b] (0)	1578	5	Upper mid-dorsal - dark, blue-black, raised areas.
II (1,000)	A total of 84 fetuses were evaluated representing 7 litters.		
III (70,000)	A total of 85 fetuses were evaluated representing 7 litters.		

[a]Represents only fetuses with malformations.
[b]A total of 91 fetuses were evaluated representing 7 litters.

Appendix H
Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat

Fetal Soft Tissue Examination Data

Group (ppm)	Female No.	Fetus No.	Fetus Sex	Malformation(s)[a,b]
I[c] (0)	1579	7	M	Ureter (L) - (S) tortuous.
	1580	13	F	Ureters (B) - (S) distended and tortuous.
II[d] (1,000)	2576	3	M	Ureter (R) - (S) tortuous; Ureter (L) - (M) tortuous.
		7	M	Liver - small, dark discolored area on median lobe near cleft (approx. 0.3 cm in diam.).
		11	M	Ureters (B) - (S) tortuous.
	2577	5	F	Ureter (L) - (S) tortuous.
	2578	11	M	Ureter (R) - (S) tortuous.
	2579	3	F	Ureter (L) - (S) distended and tortuous.
	2581	9	F	Ureter (R) - (S) distended and tortuous.
III[e] (70,000)	3578	1	F	Ureters (B) - (S) tortuous.
		9	F	Ureter (R) - (S) distended and (M) tortuous.

[a]Represents only fetuses with observable malformations.
[b]R = Right; L = Left; B = Both; S = Slightly; M = Moderately.
[c]A total of 48 fetuses were evaluated representing 7 litters.
[d]A total of 43 fetuses were evaluated representing 7 litters.
[e]A total of 44 fetuses were evaluated representing 7 litters.

Appendix I

81-2571

of [redacted] Embryofetal Toxicity and Teratogenicity Study by Inhalation in the Rat

Fetal Skeletal Examination Data

Group (ppm)	Female No.	Fetus No.	Malformation(s)[a]
I[b] (0)	1579	1	Ribs (Right side) - 6th-8th, 11th and 12th angulated; 9th and 10th ribs shortened.
II[c] (1,000)	2580	10	1st and 2nd sternebrae fused.
III[d] (70,000)	3578	4	2nd-5th sternebrae fused; cleft evident down ventral mid-line of fused sternebrae.

[a]Represents only fetuses with observable malformations.
[b]A total of 91 fetuses were evaluated representing 7 litters.
[c]A total of 84 fetuses were evaluated representing 7 litters.
[d]A total of 85 fetuses were evaluated representing 7 litters.

Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat

Fetal Ossification Variation Data Summarized

Group: (ppm)	I (0)		II (1,000)		III (10,000)	
No. of Fetuses Examined -						
Intact:	43		41		41	
Without heads:	48		43		44	
Total:	91		84		85	
No. of Litters Evaluated:	7		7		7	
No. of Fetuses With at Least One Ossification Variation:	87		75		77	
No. of Litters Containing at Least One Fetus with an Ossification Variation:	7		7		7	
Mean % Fetuses with Variation/Litter:	95.3		89.1		91.0	
	No. of Fetuses (Litters) with Ossification Variations[a,b,c]					
	No.	%	No.	%	No.	%
Type of Ossification Variations:						
Cranial Ossifications[d]:						
Parietal Incompletely Ossified:	1 (1)	2.3	0	0.0	0	0.0
Malar Incompletely Ossified -						
Unilateral:	1 (1)	2.3	0	0.0	0	0.0
Bilateral:	2 (2)	4.7	0	0.0	0	0.0
Hyoid -						
Incompletely Ossified:	0	0.0	1 (1)	2.4	1 (1)	2.4
Unossified:	9 (3)	20.9	5 (2)	12.2	1 (1)	2.4
Sternebrae -						
Asymmetric (small) -						
Sternebrae No. 1:	0	0.0	3 (2)	3.6	2 (2)	2.4
2:	15 (5)	16.5	15 (7)	17.9	19 (6)	22.4
3:	7 (5)	7.7	8 (5)	9.5	8 (6)	9.4
4:	27 (6)	29.7	26 (7)	31.0	19 (7)	22.4
5:	0	0.0	0	0.0	0	0.0
6:	57 (7)	62.6	56 (7)	66.7	55 (7)	64.7
Unossified -						
Sternebrae No. 1:	0	0.0	0	0.0	0	0.0
2:	0	0.0	1 (1)	1.2	0	0.0
3:	0	0.0	0	0.0	0	0.0
4:	0	0.0	0	0.0	0	0.0
5:	26 (6)	28.6	9 (3)	10.7	19 (3)	22.4
6:	2 (1)	2.2	2 (1)	2.4	6 (2)	7.1
Ribs:						
Rudimentary Structures[e]						
Unilateral:	3 (2)	3.3	1 (1)	1.2	5 (3)	5.9
Bilateral:	2 (1)	2.2	0	0.0	2 (1)	2.4
12 Rib pairs:	2 (1)	2.2	0	0.0	0	0.0
14 Rib pairs:	0	0.0	0	0.0	1 (1)	1.2

[a]Data not analyzed statistically.
[b]This is a summary of representative ossification variations as noted during the skeletal evaluations. It is not the intent to present every type of ossification variation observed during the study.
[c]% = No. of fetuses with the variation/Total No. of fetuses evaluated.
[d]% = No. of fetuses with the variation/Total No. of intact fetuses evaluated.
[e]Small discrete ossification(s) adjacent to the last thoracic or first lumbar vertebral transverse process(es).

of [redacted] Embryofetal Toxicity and Teratogenicity Study by Inhalation in the Rat

Fetal Ossification Variation Data Summarized

Group: (ppm)	I (0)		II (1,000)		III (10,000)	
	No. of Fetuses (Litters) with Ossification Variations[a,b,c]					
	No.	%	No.	%	No.	%
Vertebral Column:[f]						
Cervical V. Trans. Proc. Incompletely Ossified -						
Unilateral:	1 (1)	1.1	0	0.0	0	
Bilateral:	1 (1)	1.1	0	0.0	1 (1)	1.2
Cervical Ossifications -						0.0
Unilateral:	1 (1)	1.1	0	0.0	0	0.0
Thoracic V. Centra -						
Incompletely Ossified:	43 (7)	47.3	41 (7)	48.8	33 (7)	38.8
Unossified:	0	0.0	2 (2)	2.4	0	0.0
Lumbar V. Trans. Proc.						
Incompletely Ossified:	1 (1)	1.1	1 (1)	1.2	0	0.0
Sacral V. Trans. Proc. -						
Fused to Centra -						
Unilateral:	4 (2)	4.4	3 (3)	3.6	0	0.0
Bilateral:	2 (2)	2.2	5 (2)	6.0	0	0.0
Incompletely Ossified -						
Unilateral:	7 (4)	7.7	1 (1)	1.2	1 (1)	1.2
Bilateral:	6 (3)	6.6	3 (1)	3.6	0	0.0
Unossified -						
Unilateral:	3 (3)	3.3	1 (1)	1.2	0	0.0
Bilateral:	1 (1)	1.1	4 (2)	4.8	0	0.0
Pelvic Ossifications						
Pubis Incompletely Ossified -						
Unilateral:	0	0.0	1 (1)	1.2	0	0.0
Bilateral:	1 (1)	1.1	0	0.0	0	0.0
Pubis Unossified (bilateral):	0	0.0	1 (1)	1.2	0	0.0
Distal Phalanges Unossified -						
Forelimbs:	4 (3)	4.4	12 (1)	14.3	5 (3)	5.9
Hindlimbs:	40 (6)	44.0	30 (5)	35.7	23 (6)	27.1
Metacarpals Unossified:	1 (1)	1.1	1 (1)	1.2	0	0.0

[a]Data not analyzed statistically.
[b]This is a summary of representative ossification variations as noted during the skeletal evaluations. It is not the intent to present every type of ossification variation observed during the study.
[c]% = No. of fetuses with the variation/Total No. of fetuses evaluated.
[f]One or more vertebral elements affected (Key: V.=Vertebral; Trans. Proc.= Transverse Process).

PROJECT NO. 81-2571

EMBRYOFETAL TOXICITY AND TERATOGENICITY STUDY OF [redacted] BY INHALATION IN THE RAT

I. INTRODUCTION

The purpose of this study was to determine the teratogenic potential and degree of embryotoxicity of [redacted] in the pregnant rat. Two groups of female rats were exposed to atmospheres containing [redacted] for 6 hr/day from Day 6 through Day 15 of gestation. A concurrent control group was exposed only to houseline air. Target concentrations selected for testing were 1000, and 70,000 parts per million (ppm).

II. METHODS

A. Test Material Delivery

The test material was placed in a 50 milliliter (ml) glass syringe mounted on a Sage syringe pump (Model #352) for the low exposure (Group II). For the high exposure (Group III), approximately 1600 mls of [redacted] was placed in a 2000 ml flask connected to an FMI Lab Pump (Model G-20). From these reservoirs, the test material was metered to a 4-neck round-bottom flask that was maintained at a temperature of 45°C using a water bath. Houseline dry air, at a flow rate of 7.0 liters per minute (lpm), was directed into the flask. The resultant vapor-laden airstream was delivered into the exposure chamber.

B. Chamber Operation

The animals were exposed in a glass chamber with a volume of 10.7 liters. The average airflow rate through the chamber was 7.0 lpm. This flow is calculated to provide one complete air change every 2.8 minutes and a 99% equilibrium time of 12.9 minutes.

C. Atmospheric Sampling

Chamber concentrations were determined in chamber including the control every half hour using a GOW-MAC Model #750 gas chromatograph. A six foot 1/8" Chromosorb 102 column, at a temperature of 160°C, was employed for the chromatographic separation and determination. Chamber concentrations were

II. METHODS (Cont.)

C. Atmospheric Sampling (cont.)

determined by comparing the integrated G.C. area to responses obtained with primary gas standards. The G.C. sampling sequence was Groups I, II, III, I, etc. However, because of the carry-over of [redacted] in the sampling lines from high dose to control, this sequence was reversed on Day 2 and on Day 6 the sampling time was increased from 8 to 9 minutes.

Chamber oxygen was monitored daily (4 times per day for the first two days and twice daily thereafter) using an MSQ Portable Oxygen Indicator (Type E).

Chamber air temperatures were recorded hourly during the exposures.

III. RESULTS AND DISCUSSION

The mean nominal concentrations were 1050 and 69,600 ppm [redacted] for Groups II and III, respectively. The cumulative analytical concentrations of [redacted] were 994 and 70,700 ppm (Table 1). The differences between nominal and analytical are within the limits of experimental error. For the first two days of the exposure, concentrations of approximately 25 ppm were observed for samples taken from the control chamber. This was attributed to the carry-over in the sampling lines from high dose to control. This problem was eliminated by reversing the sampling sequence and increasing the sampling time. During the remainder of the control exposures, peak areas observed were either "very small" or not present at all.

The oxygen concentration in the exposure chambers ranged from 19.0 to 20.8%. The mean chamber temperatures and ranges are presented below:

Group	Mean	Range
I (Control)	24.6	20.0-28.0
II (900 ppm)	25.0	21.5-28.0
III (70,000 ppm)	24.6	22.0-26.5

In some cases, higher thermometer readings were noted but these were due to intimate contact between the rats and the thermometer. These findings were shown to be much higher (1-8°C) than the atmospheric temperature of the exposure chamber. When this occurred, a second temperature reading was taken after the thermometer was free of physical contact. High chamber temperatures, not associated with improper placement of the thermometer, were lowered by placing ice-bags on the chamber.

James B. Terrill
James B. Terrill, Ph.D.
Study Director, Inhalation

12/3/81
Date

Table 1
Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat
Group I (Control)

Date	Test Day	Exposure Number	Analytical* Daily Mean Concentration (ppm)	Analytical* Cumulative Mean Concentration (ppm)
8/17/81	1	1	25.2	25.2
8/18/81	2	2	26.1	25.7
8/19/81	3	3	7.60	19.6
8/20/81	4	4	3.65	15.6
8/21/81	5	5	6.10	13.7
8/22/81	6	6	2.80	11.9
8/23/81	7	7	1.00	10.4
8/24/81	8	8	0.00	9.06
8/25/81	9	9	6.12	8.73
8/26/81	10	10	3.35	8.19
8/27/81	11	11	0.00	7.45
8/28/81	12	12	0.00	6.83
8/29/81	13	13	0.81	6.36

*These concentrations were attributed to carry-over of test material in the multiport sampling system.

Table 1 (cont.)
Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat
Group II (1000 ppm)

Date	Test Day	Exposure Number	Analytical Daily Mean Concentration (ppm)	Analytical Cumulative Mean Concentration (ppm)	Nominal Daily Mean Concentration (ppm)
8/17/81	1	1	922	922	995
8/18/81	2	2	942	932	1080
8/19/81	3	3	966	943	1050
8/20/81	4	4	993	956	1060
8/21/81	5	5	1050	975	1040
8/22/81	6	6	1010	981	996
8/23/81	7	7	1010	985	1040
8/24/81	8	8	1000	987	1040
8/25/81	9	9	1020	990	1100
8/26/81	10	10	1020	993	1020
8/27/81	11	11	995	993	1070
8/28/81	12	12	1000	994	1060
8/29/81	13	13	993	994	1100
				Mean:	1050

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Table 1 (cont.)
Embryofetal Toxicity and Teratogenicity Study
of [redacted] by Inhalation in the Rat
Group III (70,000 ppm)

Date	Test Day	Exposure Number	Analytical: Daily Mean Concentration (ppm)	Analytical: Cumulative Mean Concentration (ppm)	Nominal: Daily Mean Concentration (ppm)
8/17/81	1	1	74000	74000	72000
8/18/81	2	2	75300	74700	72200
8/19/81	3	3	69200	72800	69300
8/20/81	4	4	72100	72700	70700
8/21/81	5	5	69500	72000	72200
8/22/81	6	6	71200	71900	65500
8/23/81	7	7	69300	71500	68200
8/24/81	8	8	69500	71300	69100
8/25/81	9	9	69100	71000	70100
8/26/81	10	10	70400	71000	69100
8/27/81	11	11	69900	70900	69100
8/28/81	12	12	70200	70800	68700
8/29/81	13	13	69600	70700	68100
				Mean:	69600

of [redacted] by Inhalation in the Rat

PERSONNEL

DEPARTMENT/TITLE	NAME	DEGREE
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QUALITY ASSURANCE STATEMENT

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STUDY NO.: 81-2571 TEST SUBSTANCE/ARTICLE: [redacted]

STUDY SPONSOR: E.I. Dupont DeNemours

Listed below are dates that this study was inspected by the Quality Assurance Unit and the dates findings were reported to the Study Director and Management

DATE(S) OF INSPECTION	REPORTED TO STUDY DIRECTOR	REPORTED TO MANAGEMENT
9/1/81	9/2/81	9/21/81 & 10/9/81
9/4/81	9/4/81	9/8/81 & 9/22/81
11/23-11/25/81	12/3/81	12/30/81 & 1/5/82
12/9/81 & 12/10/81	12/17/81	12/18/81 & 12/23/81

Company Sanitized. Does not contain TSCA CBI

CRAIG LAMB
MANAGER, QUALITY ASSURANCE

1-6-82
DATE