

DuPont Performance Elastomers L.L.C.
300 Bellevue Parkway
Wilmington, DE 19809


DuPont
Performance Elastomers

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Office of Pollution Prevention and Toxics (OPPT)
Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

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ATTN: 8(d) Health and Safety Reporting Rule
(Notification/Reporting)

Dear 8(d) Coordinator:

RE: Docket ID Number EPA-HQ-OPPT-2005-0055
Final Rule: 71 FR 47130 (08.16.06) and as revised 71 FR 54434 (09.15.06)
Submission of Study Report on CAS number 592-45-0

In response to the above-referenced final rule, issued pursuant to section 8(d) of the Toxic Substances Control Act (TSCA), the following study report is submitted to EPA:

Chemical Name: 1,4-Hexadiene
CAS Number: 592-45-0
Study title: Mutagenicity Testing of 1,4-Hexadiene in the Salmonella Typhimurium Plate Incorporation Assay (DuPont HLR 418-88)

An IUCLID Data Set summary document is also included in this submission.

This submission is made on behalf of DuPont Performance Elastomers L.L.C., 300 Bellevue Parkway, Wilmington, DE 19809.

Sincerely,

Sheena Sinclair

Sheena Sinclair
Regulatory Affairs Consultant
Agent for DuPont Performance Elastomers



Enclosures:

- IUCLID Data Set Summary (5 pages)
- Study Report for DuPont HLR 418-88 (20 pages)

I U C L I D

Data Set

Existing Chemical : ID: 592-45-0
CAS No. : 592-45-0
Substance name : 1,4-Hexadiene

Producer related part
Company : E. I. du Pont de Nemours and Company
Creation date : 01.11.2006

Substance related part
Company : E. I. du Pont de Nemours and Company
Creation date : 01.11.2006

Status :
Memo :

Printing date : 02.11.2006
Revision date :
Date of last update : 02.11.2006

Number of pages : 5

Chapter (profile) : Chapter: 5
Reliability (profile) :
Flags (profile) :

5.0 TOXICOKINETICS, METABOLISM AND DISTRIBUTION**5.1.1 ACUTE ORAL TOXICITY****5.1.2 ACUTE INHALATION TOXICITY****5.1.3 ACUTE DERMAL TOXICITY****5.1.4 ACUTE TOXICITY, OTHER ROUTES****5.2.1 SKIN IRRITATION****5.2.2 EYE IRRITATION****5.3 SENSITIZATION****5.4 REPEATED DOSE TOXICITY****5.5 GENETIC TOXICITY 'IN VITRO'**

Type	: Ames test
System of testing	: Salmonella typhimurium TA97, TA98, TA100, TA1535
Test concentration	: 0, 10, 50, 100, 500, 1000 µg/plate
Cycotoxic concentr.	: 1000 µg/plate
Metabolic activation	: with and without
Result	: negative
Method	: other
Year	: 1988
GLP	: yes
Test substance	: as prescribed by 1.1 - 1.4

Method : The negative (solvent) control was DMSO. Positive indicators were 2-aminoanthracene, 2-nitrofluorene, sodium azide, and ICR-191 acridine. Distilled-deionized water was the solvent used for sodium azide. The solvent for all other positive indicators was DMSO.

The cytotoxicity of the test substance in the presence and absence of an activation system, as measured in strain TA98, was the basis for selecting concentrations for the mutagenicity experiments. The protocol used to determine cytotoxicity was identical to the mutagenicity protocol, except that approximately 10E3 rather than 10E8 bacteria were used per plate, excess histidine was present, and no positive indicators were tested. Concentrations of test sample that were nontoxic and, if possible, slightly toxic were selected for the mutagenicity assay.

The plate incorporation assay was performed in the presence and the absence of a rat liver homogenate activation system (S9 mix) at concentrations of 0, 10, 50, 100, 500, and 1000 µg/plate. Positive indicators and negative controls were included in all assays. Treatments without activation (nonactivated) were conducted by adding the solvent or a solution of the test substance and overnight culture containing approximately 10E8 bacteria to top agar (agar, NaCl, L-histidine, and biotin). These components were mixed and poured on the surface of a plate containing Davis minimal agar. Treatments with activation were conducted by adding S9 mix to the bacteria/test sample/top agar as described above and pouring the mixture onto a minimal agar plate. The S9 mix contained S9 diluted with phosphate buffered saline (PBS) and a cofactor solution containing MgCl₂, KCl, glucose-6-phosphate, NADP, and sodium phosphate (pH 7.4). The S9 was the 9000 x g supernatant of liver homogenate. The livers were obtained from 8-9 week old male CrI:CD®Br rats injected intraperitoneally with Aroclor® 1254 (500 mg/kg) 5 days before sacrifice. The revertant colonies were counted after the individually labeled plates were incubated at 37°C for approximately 48 hours.

A spot test in a closed system was performed to aid in identifying samples with volatile mutagenic components. Experiments without activation were performed by adding 10E8 bacteria to standard mutagenesis top agar, mixing immediately, and pouring on a Davis minimal agar plate. Experiments with activation were performed in the same manner except that S9 mix was also added before mixing. All components used for this assay were identical to the ones used in the plate incorporation assay. A sterile disk saturated with test compound or solvent control was placed in the center of 1 of 2 duplicate plates. The plate with the disk was inverted and the duplicate plate was stacked on top of it. Both plates were sealed in a plastic bag and incubated for 48 hours at 37°C. Colonies were counted on the duplicate plates.

Doses (concentrations), with and without activation, were ranked and results from a strain were analyzed individually by multiple linear regression. Comparisons were made between each dose/concentration and the solvent control (0 rank), using the mean square error estimate. All comparisons were at the 95% level of confidence (alpha = 0.05).

A test sample was classified as positive when (1) the number of induced revertants at one or more of the test sample concentrations studied was at least 2 times greater than the number of revertants in the solvent control AND (2) there was a dose-response relationship. A test sample was classified as negative when (1) the number of induced revertants at each test sample concentration was similar to the number of revertants in the solvent control OR (2) there was no dose-response relationship. A test sample was classified as equivocal when neither of the criteria for a positive or negative was satisfied.

Result

: The results of the spot test were negative, and a standard toxicity and plate incorporation assay were performed.

In the cytotoxicity experiment, the test substance exhibited toxicity to strain TA98 with and without activation at 1000 µg/plate. Based on these results, 1000 µg/plate with and without activation was chosen as the highest dose for the mutagenicity assays.

No mutagenic activity was detected in any strain, either with or without activation, at levels up to 1000 µg/plate. Under the conditions of this assay, the test substance was negative.

Test substance
01.11.2006

: 1,4-Hexadiene, purity 99.5%

(1)

5. Toxicity

Id 592-45-0

Date 02.11.2006

5.6 GENETIC TOXICITY 'IN VIVO'

5.7 CARCINOGENICITY

5.8.1 TOXICITY TO FERTILITY

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

5.8.3 TOXICITY TO REPRODUCTION, OTHER STUDIES

5.9 SPECIFIC INVESTIGATIONS

5.10 EXPOSURE EXPERIENCE

5.11 ADDITIONAL REMARKS

9. References

Id 592-45-0

Date 02.11.2006

- (1) DuPont Co. (1988). Unpublished Data, Haskell Laboratory Report 418-88, Mutagenicity testing of 1,4-hexadiene in the Salmonella typhimurium plate incorporation assay (July 13).

FOR DU PONT USE ONLY

Study Title

MUTAGENICITY TESTING OF 1,4-HEXADIENE IN THE
SALMONELLA TYPHIMURIUM PLATE INCORPORATION ASSAY

Author

Vincent L. Reynolds

Study Completed on

July 13, 1988

Performing Laboratory

E. I. du Pont de Nemours and Company, Inc.
Haskell Laboratory for Toxicology and Industrial Medicine
Elkton Road, P. O. Box 50
Newark, Delaware 19714

Medical Research No.

8418-001

Laboratory Project ID

Haskell Laboratory Report No. 418-88

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted according to FDA Good Laboratory Practice Regulations (21 CFR 58, 1987). Any areas of noncompliance are documented in the study records. No deviations existed that significantly affected the validity of the study.

Submitter E. I. du Pont de Nemours and Company, Inc.

Sponsor Polymer Products Department

Study Director

Vincent L. Gaj. 11 July 1988
(Signature) (Date)

COMPOUND INFORMATION

Material Tested: 1,4-Hexadiene

Medical Research No.: 8418-001

Haskell No.: 17,328

Other Codes: Monomer C

CAS Registry No.: 592-45-0

Composition: The sample contains approximately 100 ppm p-tert-butylcatechol which is added as an 85% aqueous solution.

Purity: 99.5%

Impurities:
0.10% 3-Methyl-1,4-pentadiene
0.20% 1,5-Hexadiene
0.15% 3-Chloro-1-butene
0.05% 2,4-Hexadiene

Sponsor: Polymer Products Department

Material Submitted by: Bruce T. Nakata
Polymer Products Department
P. O. Box 3269
Beaumont, TX 77704

Study Initiated/Completed: Initiated May 25, 1988 and Completed June 27, 1988

Notebook(s): E-56688, pp. 1 to 16; E-48728; E-48853

There are 20 pages in this report.

Distribution: B. T. Nakata (1)
L. C. Pinder (1)

MUTAGENICITY TESTING OF 1,4-HEXADIENE IN THE
SALMONELLA TYPHIMURIUM PLATE INCORPORATION ASSAY

SUMMARY

1,4-Hexadiene was tested for mutagenic activity in Salmonella typhimurium strains TA1535, TA97, TA98 and TA100 in the presence and absence of an activation system. Under the conditions of this assay, 1,4-hexadiene is negative.

Work by: Catherine C. Matarese 7/13/88
Catherine C. Matarese Date
Technician

Approved by: Vincent L. Reynolds 27 June 1988
Vincent L. Reynolds Date
Study Director
Research Genetic Toxicologist

Approved by: Awni M. Sarrif 7/13/88
Awni M. Sarrif Date
Section Supervisor
Molecular and Genetic Toxicology

VLR/Ames 21-8

QUALITY ASSURANCE DOCUMENTATION

STUDY: MR 8418-001
H# 17,328

MUTAGENICITY TESTING OF 1,4-HEXADIENE IN THE
SALMONELLA TYPHIMURIUM PLATE INCORPORATION ASSAY

AUDITS:

<u>Items Audited</u>	<u>Audit Dates</u>
Protocol, Records, and Final Report	7/5-7/88

SHORT-TERM AUDIT REPORT NUMBER: C-391
DATE FINDINGS REPORTED TO MANAGEMENT AND STUDY DIRECTOR: 7/7/88

Reported by: William J. Lynam
William J. Lynam
Quality Assurance Auditor

7/11/88
Date

INTRODUCTION

The purpose of this study was to evaluate the mutagenic potential of 1,4-hexadiene in Salmonella typhimurium strains TA1535, TA97, TA98 and TA100. These strains cannot synthesize histidine, an essential amino acid, because of mutations in the genes coding for histidine biosynthetic enzymes. Chemically-induced mutations in the defective genes can result in individual bacteria regaining the ability to synthesize this amino acid. Back mutants or revertants can be scored by their ability to grow on agar plates lacking histidine. By comparing the number of chemically-induced revertants to the number of spontaneous revertants, the mutagenic potential of the test sample can be assessed.

MATERIALS/METHODS

A. PROTOCOL

This study was conducted according to protocol Ames-88, Edition 5 filed with the Quality Assurance Section of Haskell Laboratory on December 3, 1985; this protocol includes the Standard Operating Procedures: Routine Salmonella Typhimurium/Ames Mutagenicity Assay Nonvolatile Liquid, Solid, Gas and Volatile Liquid Samples and Routine Non-Culture Procedures Employed in Salmonella Typhimurium/Ames Test System for Mutagenicity Evaluation.

B. TEST MATERIALS

1. Test Compound

1,4-Hexadiene is a water-white mobile liquid. Based on information supplied with the test material, dimethylsulfoxide (DMSO) was chosen as the solvent. The compound was assumed to be stable under the conditions of this assay. No evidence of instability was observed during the study.

2. Negative Control and Positive Indicators

Negative (solvent) Control: DMSO, Baker Lot # A11334. Solvent was present where the concentration of test chemical is listed as "0".

Positive Indicators (known mutagens): 2-aminoanthracene (2AA), Sigma Lot # 33F-0816; 2-nitrofluorene (2NF), Aldrich Lot # JPO3222JJ, sodium azide (NAAZ), Sigma Lot # 26F-0434; ICR-191 Acridine (ICR-191) Raylo Chemicals Lot # 178. Distilled-deionized water was the solvent used for sodium azide. The solvent for all other positive indicators was DMSO, Baker Lot Number A11334.

The negative control and positive indicators were assumed to be stable under the conditions of this study; no evidence of instability was observed.

C. DOSE SELECTION

The cytotoxicity of 1,4-hexadiene in the presence and absence of an activation system, as measured in strain TA98, was the basis for selecting concentrations for the mutagenicity experiments. The protocol used to determine the cytotoxicity was identical to the mutagenicity protocol described under PLATE INCORPORATION ASSAY except that approximately 10^3 rather than 10^8 bacteria were used per plate, excess histidine was present and no positive indicators were tested. Concentrations of test sample that were nontoxic and, if possible, slightly toxic were selected for the mutagenicity assay. The concentrations of test sample per mL of treatment medium per plate were calculated with the assumption that the addition of test sample did not change the volume of solvent.

D. PLATE INCORPORATION ASSAY

The assay was performed in the presence and the absence of a rat liver homogenate activation system (S-9 mix) similar to the method described by Ames and co-workers (Mutation Res. 113: 173-215, 1983). All tester strains were obtained from Dr. B. Ames, Berkeley, CA. Positive indicators and negative controls were included in all assays. Treatments without activation (nonactivated) were conducted by adding 0.1 mL of the solvent or a solution of 1,4-hexadiene and 0.1 mL of an overnight culture containing approximately 10^8 bacteria to 2 mL of top agar (0.6% agar, 0.6% NaCl, 0.05 mM L-histidine, 0.05 mM biotin). These components were mixed and poured on the surface of a plate containing 25 mL of Davis minimal agar. Treatments with activation were conducted by adding 0.5 mL of S-9 mix to the bacteria/test sample/top agar as described above and pouring the mixture onto a minimal agar plate. The S-9 mix contained per mL: 0.3 mL of S-9 diluted to 1.6 mg/mL with phosphate buffered saline (PBS), and 0.7 mL of a cofactor solution containing 8 micromoles $MgCl_2$, 33 micromoles KCl, 5 micromoles glucose-6-phosphate, 4 micromoles NADP and 100 micromoles sodium phosphate (pH 7.4). The S-9 (Sitek Research Laboratories, Rockville, MD Lot # 871215) was the 9,000 x g supernatant of liver homogenate (1 g wet liver: 3.0 mL PBS). The livers were obtained

from 8 to 9 week old male Cr1:CD®BR (Charles River, Kingston, NY) rats injected intraperitoneally with Aroclor® 1254 (500 mg/kg) 5 days before sacrifice. The revertant colonies were counted after the individually labeled plates were incubated at 37°C for approximately 48 hours.

E. SPOT TEST IN A CLOSED SYSTEM

A spot test in a closed system is performed on all nonaqueous liquids to aid in identifying samples with volatile mutagenic components. These samples are tested by different protocols.

Experiments without activation were performed by adding 10^8 bacteria (in 0.1 mL) to 2.0 mL of standard mutagenesis top agar, mixing immediately, and pouring on a Davis minimal agar plate. Experiments with activation were performed in the same manner except that 0.5 mL of S-9 mix was also added before mixing. All components used for this assay were identical to the ones used in the plate incorporation assay.

A sterile disk saturated with test compound or solvent control article was placed in the center of one of two duplicate plates. The plate with the disk was inverted and the duplicate plate was stacked on top of it. Both plates were sealed in a plastic bag and incubated for 48 hours at 37°C. Colonies were counted on the duplicate plates.

F. STATISTICAL ANALYSES

Doses (concentrations), with and without activation, were ranked and results from a strain were analyzed individually by multiple linear regression. Comparisons were made between each dose/concentration and the solvent control (0 rank), using the mean square error estimate. All comparisons were at the 95% level of confidence ($\alpha = 0.05$).

G. CLASSIFICATION GUIDELINES

The guidelines below are used to aid in the classification of a test sample along with sound scientific judgement and experience.

A test sample is classified as a POSITIVE when:

- A. The number of induced revertants at one or more of the test sample concentrations studied is at least two times greater than the number of revertants in the solvent control.

AND

B. There is a dose-response relationship.

A test sample is classified as a NEGATIVE when:

A. The number of induced revertants at each test sample concentration is similar to the number of revertants in the solvent control.

OR

B. There is no dose-response relationship.

A test sample is classified as EQUIVOCAL when:

A. Neither of the criteria for a positive or negative is satisfied.

H. RETENTION OF RECORDS

All raw data and the final report are stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine or in the Du Pont Records Management Center, E. I. du Pont de Nemours and Co., Inc., Wilmington, DE.

RESULTS/DISCUSSION

A spot test in a closed system was performed prior to toxicity screening to determine the most appropriate testing method. Non-volatile liquids are tested as solids whereas volatile liquids are tested as gases. The results of the spot test (Table I) were negative and a standard toxicity and plate incorporation assay were performed.

1,4-Hexadiene was tested for cytotoxicity in Salmonella typhimurium strain TA98 with and without activation. The results of the cytotoxicity experiment are listed in Table II. 1,4-Hexadiene exhibited toxicity to strain TA98 without and with activation at levels of 1000 ug/plate (Table II). Based on these results, 1000 ug/plate without and with activation was chosen as the highest dose for the mutagenicity assays.

1,4-Hexadiene was tested for mutagenic activity in Salmonella typhimurium strains TA1535, TA97, TA98 and TA100 with and without activation. Results of the mutagenicity trials are shown in Tables III through X. No mutagenic activity was detected in any strain either without or with activation at levels up to 1000 ug/plate. Under the conditions of this assay, 1,4-hexadiene is negative.

SYMBOLS USED IN TABLES III-X

SD Standard deviation.

Table I

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAINS TREATED WITH
1,4-HEXADIENE IN A SPOT TEST IN A CLOSED SYSTEM

Disc*	Chemical**	Activation***	Revertants			
			TA1535	TA97	TA98	TA100
-	Sample	w/o	32	135	33	126
+	Sample	w/o	32	136	31	132
-	Solvent	w/o	31	125	25	171
+	Solvent	w/o	43	128	27	146
-	Sample	w	27	134	39	162
+	Sample	w	28	145	25	159
-	Solvent	w	25	134	40	142
+	Solvent	w	19	145	34	164

* + and - indicate the presence and absence, respectively, of the disc

** Sample = 1,4-Hexadiene; Solvent = DMSO

*** w/o and w indicate the absence and presence, respectively, of S-9 activation

H17328 = 1,4-HEXADIENE
MR 8418-001

TABLE II

Du Pont HLR 418-88

CYTOTOXICITY OF 1,4-HEXADIENE IN SALMONELLA TYPHIMURIUM STRAIN TA98

Concentration (ug/plate)	Colonies/plate Without Activation	Colonies/plate With Activation
0	1574, 1600	1642, 1621
10	1590, 1300	1801, 1757
50	1762, 1620	1771, 1815
100	1663, 1600	1713, 1684
500	1271, 1286	1395, 1369
1000	748, 704	371, 452
5000	105, 18	46, 60

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE III

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA1535
TREATED WITH 1,4-HEXADIENE WITHOUT ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	16, 20	13, 16	16	2	16	-
10	26, 31	15, 24	24	5	24	0.0185
50	16, 25	18, 9	17	6	17	0.3979
100	21, 21	20, 11	18	0	18	0.2497
500	21, 21	23,	22	0	22	0.0453
1000	25, 21	15, 17	20	2	20	0.145
NAAZ-2	631, 684	639, 681	659	33	-	-

o ERROR STANDARD DEVIATION = 3 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = 0.33 +/- 0.63 p < 0.306

95% CONFIDENCE INTERVAL ON SLOPE: -0.82 < 0.33 < 1.5

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE IV

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA1535
TREATED WITH 1,4-HEXADIENE WITH ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	14, 14	18, 18	16	0	16	-
10	14, 23	16, 11	16	5	16	0.5
50	17, 25	10, 22	19	7	19	0.2124
100	14, 14	19, 13	15	0	15	0.3712
500	21, 22	12, 20	19	2	19	0.1917
1000	10, 17	13, 12	13	2	13	0.1726
2AA-2	298, 291	325, 346	315	9	-	-

o ERROR STANDARD DEVIATION = 3 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = -0.29 +/- 0.52 p < 0.294

95% CONFIDENCE INTERVAL ON SLOPE: -1.2 < -0.29 < 0.66

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE V

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA97
TREATED WITH 1,4-HEXADIENE WITHOUT ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	100, 121	98, 112	108	12	108	-
10	119, 106	88, 107	105	11	105	0.2874
50	96, 131	117, 106	113	14	113	0.1739
100	110, 124	124, 118	119	6	119	0.0289
500	127, 121	122, 112	121	5	121	0.0194
1000	90, 122	92, 101	101	12	101	0.1078
ICR-191-2	1995, 2146	2192, 1970	2076	129	-	-

o ERROR STANDARD DEVIATION = 5 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = 0.59 +/- 1.5 p < 0.351

95% CONFIDENCE INTERVAL ON SLOPE: -2.1 < 0.59 < 3.3

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE VI

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA97
TREATED WITH 1,4-HEXADIENE WITH ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	138, 152	127, 134	138	7	138	-
10	138, 143	126, 132	135	4	135	0.0647
50	157, 127	130, 135	137	9	137	0.3873
100	139, 143	134, 132	137	2	137	0.3345
500	131, 118	113, 118	120	6	120	0.0001
1000	116, 124	111, 110	115	2	115	0.0001
2AA-1	2249, 2165	1558, 1574	1887	26	-	-

o ERROR STANDARD DEVIATION = 2 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = -4.5 +/- 0.99 p < 0.001

95% CONFIDENCE INTERVAL ON SLOPE: -6.3 < -4.5 < -2.7

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE VII

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA98
TREATED WITH 1,4-HEXADIENE WITHOUT ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	16, 22	24, 17	20	5	20	-
10	10, 8	19, 16	13	2	13	0.1224
50	16, 14	15, 12	14	2	14	0.1579
100	22, 23	9, 10	16	1	16	0.2408
500	11, 17	19, 6	13	6	13	0.1224
1000	15, 9	9, 9	11	0	11	0.0599
2NF-25	1778, 1761	1776, 1494	1702	49	-	-

o ERROR STANDARD DEVIATION = 5 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = -1.3 +/- 0.72 p < 0.056

95% CONFIDENCE INTERVAL ON SLOPE: -2.6 < -1.3 < 0.048

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE VIII

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIA STRAIN TA98
TREATED WITH 1,4-HEXADIENE WITH ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	26, 30	28, 33	29	3	29	-
10	25, 18	21, 22	22	2	22	0.0675
50	27, 20	21, 28	24	5	24	0.1408
100	25, 37	21, 24	27	4	27	0.2952
500	18, 31	24, 39	28	10	28	0.3927
1000	22, 19	28, 31	25	2	25	0.1868
2AA-2	2425, 2553	2654, 2543	2544	84	-	-

o ERROR STANDARD DEVIATION = 4 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = 0.029 +/- 0.74 p < 0.485

95% CONFIDENCE INTERVAL ON SLOPE: -1.3 < 0.029 < 1.4

H-17,328 = 1,4-HEXADIENE
MR 8418-001

TABLE IX

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA100
TREATED WITH 1,4-HEXADIENE WITHOUT ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	102, 103	101, 104	103	1	102	-
10	99, 108	77, 88	93	7	93	0.108
50	99, 110	93, 88	98	5	98	0.2449
100	100, 110	97, 99	102	3	102	0.4437
500	95, 108	96, 102	100	6	100	0.3755
1000	104, 101	83, 77	91	3	91	0.0773
NAAZ-2	1017, 847	885, 909	915	45	-	-

o ERROR STANDARD DEVIATION = 7 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = -0.87 +/- 1.1 p < 0.233

95% CONFIDENCE INTERVAL ON SLOPE: -3 < -0.87 < 1.2

H-17,328 - 1,4-HEXADIENE
MR 8418-001

TABLE X

Du Pont HLR 418-88

MUTAGENIC ACTIVITY IN SALMONELLA TYPHIMURIUM STRAIN TA100
TREATED WITH 1,4-HEXADIENE WITH ACTIVATION

Concentration (ug/plate)	Trial 1	Trial 2	Average	SD	Adjusted Mean	P-value
0	112, 130	104, 98	111	7	111	-
10	139, 121	128, 113	125	12	125	0.0497
50	119, 122	121, 109	118	4	118	0.1913
100	98, 121	97, 123	110	17	110	0.4332
500	113, 139	113, 130	124	15	124	0.0653
1000	143, 111	101, 102	114	4	114	0.3322
2AA-1	2079, 2108	2384, 1986	2139	76	-	-

o ERROR STANDARD DEVIATION = 7 (DOSE X TRIAL INTERACTION)

o ANALYSIS FOR LINEARLY INCREASING TREND WITH DOSE RANKING

SLOPE = 0.11 +/- 1.5 p < 0.472

95% CONFIDENCE INTERVAL ON SLOPE: -2.6 < 0.11 < 2.8

H-17,328 = 1,4-HEXADIENE
MR 8418-001