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CORNING Hazleton

MUTAGENICITY TEST ON

T-6564

IN AN *IN VIVO* MOUSE MICRONUCLEUS ASSAY

FINAL REPORT

AUTHOR

Hemalatha Murli, Ph.D.

PERFORMING LABORATORY

Corning Hazleton Inc. (CHV)
9200 Leesburg Pike
Vienna, Virginia 22182

LABORATORY PROJECT IDENTIFICATION

CHV Study No.: 17750-0-455

SUBMITTED TO

3M Corporation
Building 220-2E-02, 3M Center
St. Paul, Minnesota 55144-1000

STUDY COMPLETION DATE

November 1, 1996

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QUALITY ASSURANCE STATEMENT

Project Title: *In Vivo* Mouse Micronucleus Assay

Project No.: 20996 Assay No.: 17750

Protocol No.: 455 Edition No.: 17, Modified for 3M Corporation

Quality Assurance inspections of the study and review of the final report of the above referenced project were conducted according to the Standard Operating Procedures of the Quality Assurance Unit and according to the general requirements of the appropriate Good Laboratory Practice regulations. Findings from the inspections and final report review were reported to management and to the study director on the following dates:

Inspection/Date	Findings Reported	Auditor
Dosing/07/30/1996	07/30/1996	C. Orantes
Harvest/08/1/1996	08/1/1996	C. Smith
Draft Report Review/09/17,18/1996	09/18/1996	C. Orantes/C. Smith
Final Report Review/11/01/1996	11/01/1996	C. Smith

G. T. L. 11/1/96

Quality Assurance Unit Date Released

STUDY COMPLIANCE AND CERTIFICATION

The described study was conducted in compliance with the Good Laboratory Practice regulations as set forth in the Food and Drug Administration (FDA) Title 21 of the U.S. Code of Federal Regulations Part 58, issued December 22, 1978, (effective June 20, 1979) with any applicable amendments. There were no significant deviations from the aforementioned regulations or the signed protocol that would affect the integrity of the study or the interpretation of the test results. The raw data have been reviewed by the Study Director, who certifies that the evaluation of the test article as presented herein represents an appropriate conclusion within the context of the study design and evaluation criteria.

All test and control results in this report are supported by an experimental data record and this record has been reviewed by the Study Director. All raw data, documentation, records, protocol and a copy of the final report generated as a result of this study will be archived in the storage facilities of Corning Hazleton Inc. for at least one year following submission of the final report to the Sponsor. After the one year period, the Sponsor may elect to have the aforementioned materials retained in the storage facilities of Corning Hazleton Inc. for an additional period of time, or sent to a storage facility designated by the Sponsor.

Submitted By:

Study Director:

Hemalatha Murli

Hemalatha Murli, Ph.D.
Mammalian Cytogenetics
Department of Genetic and Cellular Toxicology

11/1/96
Study Completion Date

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SUMMARY

Mutagenicity Test on T-6564 in an *In Vivo* Mouse Micronucleus Assay

The objective of this *in vivo* assay was to evaluate the ability of the test article, T-6564, to induce micronuclei in bone marrow polychromatic erythrocytes of Crl:CD-1®(ICR) BR mice.

In the dose selection study, the test article was solubilized in deionized water and dosed by oral gavage at 1000, 1510, 2010, 2540 and 3010 mg/kg in a first trial and then dosed at 1010, 1520, 2020, and 2530 mg/kg in a second trial. Six animals (three males and three females) were assigned to each dose group. Animals were observed for four days after dosing for toxic signs and/or mortality.

Based on the results of the dose selection study, the maximum tolerated dose was estimated as about 2000 mg/kg. In the micronucleus assay, the test article was solubilized in deionized water and dosed by oral gavage at 498, 995, and 1990 mg/kg. Ten animals (five males and five females) were randomly assigned to each dose/harvest time group. Vehicle and positive control groups, euthanized approximately 24 hours after dosing, were included in the assay. The animals dosed with the test article were euthanized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow.

The test material, T-6564, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse bone marrow micronucleus test.

Mutagenicity Test on T-6564 in an *In Vivo* Mouse Micronucleus Assay

1.0 SPONSOR: 3M Corporation

2.0 MATERIAL (Test Article)

2.1 Client's Identification: T-6564 L-13167 FC1015-X

2.2 Date Received: May 30, 1996

2.3 Physical Description: Clear colorless liquid

2.4 Genetics Assay No.: 17750

3.0 TYPE OF ASSAY: *In Vivo* Mouse Micronucleus Assay

4.0 PROTOCOL NO.: 455, Edition 17, Modified for 3M Corporation

5.0 STUDY DATES

5.1 Initiation Date: June 4, 1996

5.2 Experimental Start Date: July 11, 1996

5.3 Experimental Termination Date: August 22, 1996

6.0 SUPERVISORY PERSONNEL

6.1 Study Director: Hemalatha Murli, Ph.D.

6.2 Laboratory Supervisor: Monica Vegarra, B.S.

7.0 OBJECTIVE

The objective of this *in vivo* assay was to evaluate the ability of the test article, T-6564, to induce micronuclei in bone marrow polychromatic erythrocytes of Crl:CD-1®(ICR) BR mice. This study was conducted using modifications of the procedures suggested by Heddle et al. (1983).

8.0 MATERIALS

Adult male and female mice, strain Crl:CD-1®(ICR) BR, were purchased from Charles River Laboratories, Portage, MI. This healthy, random bred strain was selected to maximize genetic heterogeneity and at the same time assure access to a common source. The protocol for this study was approved by the CHV-IACUC prior to the initiation of dosing.

Animals were housed five per cage during quarantine, and housed five per cage at randomization. The temperature and relative humidity were maintained at 72 ± 6 °F and 55 ± 15%, respectively, except on July 31, 1996, for the definitive study, when the humidity was recorded as 75.1%. A 12-hour light/12-hour dark cycle was maintained. A commercial diet (Purina® Certified Laboratory Pellets ® # 5002) and water were available ad libitum for the duration of the study. The feed was analyzed by the manufacturer for concentrations of specified heavy metals, aflatoxin, chlorinated hydrocarbons, organophosphates, and specified nutrients. The water was analyzed on a retrospective basis for specified microorganisms, pesticides, alkalinity, heavy metals, and halogens. Sanitized caging was used for housing the animals. Personnel handling animals or working within the animal facilities were required to wear suitable protective garments and equipment.

Animals were quarantined for seven days before being placed on study. Animals were randomly assigned to study groups and were individually weighed prior to dosing. All animals were dosed based upon the individual body weights. Animals were uniquely identified by ear tag. Dose or treatment groups were identified by cage card/label.

At the termination of the study all surviving animals were euthanized by CO_2/O_2 inhalation, followed by CO_2 inhalation and penetration of the thorax. Any extra animals not used for the study were saved for training.

9.0 SOLUBILITY AND STABILITY

The test article, T-6564, was supplied as a clear colorless liquid. The specific gravity of the test article was calculated in the testing laboratory as 1.22 g/ml. This specific gravity was used to prepare dosing stocks for the assay. Deionized water was used as the vehicle in this assay. The determination of the test article stability under the dosing conditions of this assay and the test article characteristics as defined in the GLP regulations of FDA (21 CFR 58.105), EPA-TSCA (40 CFR 792.105), and EPA-FIFRA (40 CFR 160.105) is the responsibility of the sponsor.

10.0 DOSE SELECTION STUDY I

10.1 Dose Selection

Dose levels of 1000, 1510, 2010, 2540 and 3010 mg/kg were administered by oral gavage for the first dose selection study.

10.2 Dosing Information

The animals used in the first dose selection assay were dosed on July 11, 1996. The weight range of the animals used in the dose range finding assay was 30.9-35.1 and 22.2-27.6 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 301 mg/ml stock for the high dose (3010 mg/kg). This was prepared by adding deionized water (CHV, Lot # 19) up to a volume of 15.0 ml to 3.7 ml of T-6564, resulting in a clear colorless solution. Dilutions of this stock were prepared for the 2540, 2010, 1510 and 1000 mg/kg dose levels.

Dosing was achieved using a 10 ml/kg dosing volume. All animals were eight weeks and three days old at the time of dosing. An outline of the dosing scheme is found in the following table.

Dosing Scheme for Dose Selection Assay I

Treatment	Male	Female
T-6564		
1000 mg/kg	3	3
1510 mg/kg	3	3
2010 mg/kg	3	3
2540 mg/kg	3	3
3010 mg/kg	3	3

A total of 30 animals was used in this assay. All doses given were on an acute (one-time only) basis.

10.3 Results and Interpretation

All animals were examined after dosing and daily throughout the duration of the study (four days) for toxic effects and/or mortalities. The animal observations for this assay are summarized in the following table:

Animal Observations for Toxicity for Dose Rangefinding Assay I

Time After Dosing (hours)	Dose Level (mg/kg)	Observations
≈0.2	all	Normal
≈16.8	1000	Normal
	1510	Male # 9761 and Female #s 9776 & 9788 found dead. Remaining males appeared hunched and hypoactive. Remaining female appeared normal.
	2010, 2540, 3010	Normal
≈40.2	1000, 1510, 2010, 2540	Normal
	3010	Male #s 9765 & 9767 found dead. Remaining male and all females appear normal.
≈67.0	1000	Normal
	1510	Male # 9766 found dead. Remaining male and female appeared normal.
	2010, 2540, 3010	Normal
≈88.5	1000	Normal
	1510	Remaining male # 9774 found dead. Remaining female appeared normal.
	2010, 2540	Normal
	3010	Remaining male # 9764 appeared hypoactive and hunched with a rough hair coat. All females appeared normal.

The mortality data for this assay are summarized in the following table:

Summary of Mortalities for Dose Rangefinding I

Treatment	Male	Female
T-6564		
1000 mg/kg	0/3	0/3
1510 mg/kg	3/3	2/3
2010 mg/kg	0/3	0/3
2540 mg/kg	0/3	0/3
3010 mg/kg	2/3	0/3

10.4 Conclusion

Based on these results, the maximum tolerated dose could not be determined.

11.0 DOSE SELECTION STUDY II

11.1 Dose Selection

Dose levels of 1010, 1520, 2020 and 2530 mg/kg were administered by oral gavage for the second dose selection study.

11.2 Dosing Information

The animals used in the second dose selection assay were dosed on July 24, 1996. The weight range of the animals used in the second dose range finding assay was 30.1-35.8 and 23.6-28.4 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 253 mg/ml stock for the high dose (2530 mg/kg). This was prepared by adding deionized water (CHV, Lot # 20) up to a volume of 14.0 ml to 2.9 ml of T-6564, resulting in a clear colorless solution. Dilutions of this stock were prepared for the 2020, 1520 and 1010 mg/kg dose levels.

Dosing was achieved using a 10 ml/kg dosing volume. All animals were eight weeks and two days old at the time of dosing. An outline of the dosing scheme is found in the following table:

Dosing Scheme for Dose Selection Assay II

Treatment	Male	Female
T-6564		
1010 mg/kg	3	3
1520 mg/kg	3	3
2020 mg/kg	3	3
2530 mg/kg	3	3

A total of 24 animals was used in this assay. All doses given were on an acute (one-time only) basis.

11.3 Results and Interpretation

All animals were examined after dosing and daily throughout the duration of the study (three days) for toxic effects and/or mortalities. The animal observations for this assay are summarized in the following table:

Animal Observations for Toxicity for Dose Rangefinding Assay II

Time After Dosing (hours)	Dose Level (mg/kg)	Observations
≈0.1, 1.1 & 15.6	All	Normal
≈17.9	1520	Female # 1417 found dead.
≈21.6	1520	Male # 1405 found dead.
≈39.5	All	Normal
≈66.6	All	Normal
≈90.4	All	Normal

The mortality data for this assay are summarized in the following table:

Summary of Mortalities for Dose Rangefinding II

Treatment	Male	Female
T-6564		
1010 mg/kg	0/3	0/3
1520 mg/kg	1/3	1/3
2020 mg/kg	0/3	0/3
2530 mg/kg	0/3	0/3

11.4 Conclusion

Based on these results, the maximum tolerated dose was estimated as about 2000 mg/kg.

12.0 MICRONUCLEUS STUDY

12.1 Dose Selection

Based on results from the dose selection study, dose levels of 498, 995, and 1990 mg/kg were tested in this study.

12.2 Micronucleus Assay Dosing Information

The animals used in the micronucleus assay were dosed on July 30, 1996. Cyclophosphamide (CP), (CAS # 6055-19-2; Sigma, Lot # 26H0473), the positive control, was solubilized in sterile deionized water (Lot # 19, prepared at CHV) and was administered by oral gavage at 80.0 mg/kg. The vehicle control, deionized water (CHV, Lot # 20), was administered concurrently with the test article at a volume of 10 ml/kg. The weight range of the animals used in the micronucleus assay was 31.0-37.7 and 22.4-28.3 grams for the males and females, respectively. The dosing solutions for the assay were prepared by making a 199 mg/ml stock for the high dose (1990 mg/kg). This was prepared by adding deionized water (CHV, Lot # 20) up to a volume of 30.0 ml to 4.9 ml of T-6564, resulting in a clear colorless solution. Dilutions of this stock were prepared for the 995 and 498 mg/kg dose levels. A second group of animals (designated Secondary Dose Group) was also assigned to the study and was dosed with the high dose of the test article. These animals were only used in the assay as replacements for any which died in the primary dose group.

Ten animals (five males and five females) were randomly assigned to each dose/-harvest time group. Vehicle and positive control groups, euthanized approximately 24 hours after dosing, were included in the assay. The animals dosed with the test article were euthanized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow. An outline of the dosing scheme is found in the following table:

Dosing Scheme for Micronucleus Assay

Treatment	Number of Animals Assigned: Primary Dose Groups 24 Hr M	24 Hr F	48 Hr M	48 Hr F	72 Hr M	72 Hr F	Secondary Dose Group [a] M	Secondary Dose Group [a] F
T-6564								
498 mg/kg	5	5	5	5	5	5	-	-
995 mg/kg	5	5	5	5	5	5	-	-
1990 mg/kg	5	5	5	5	5	5	10	10
Vehicle Control, deionized water, 10 ml/kg	5	5	-	-	-	-	-	-
Positive Control, Cyclophosphamide, 80.0 mg/kg	5	5	-	-	-	-	-	-

[a] The animals assigned to the secondary dose groups were dosed and were only used to replace animals which died in the primary dose group at the high dose level. All extra animals not used as replacements were euthanized at the completion of the trial.

A total of 130 animals was used in this assay. The age of the animals at the time of dosing was eight weeks and one day.

Volumes dosed were 10 ml/kg and were based upon individual animal weights.

13.0 BONE MARROW HARVEST, SLIDE PREPARATION AND ANALYSIS

At the appropriate harvest time, the animals were euthanized by CO_2/O_2 inhalation, followed by CO_2 inhalation and penetration of the thorax. The adhering soft tissue and epiphyses of both femora were removed. The marrow was flushed from the bone and transferred to centrifuge tubes containing 3 - 5 ml bovine serum (one tube for each animal). Following centrifugation to pellet the tissue, the supernatant was removed by aspiration and portions of the pellet were spread on slides and air dried. The slides were fixed in methanol, and stained in May-Grunwald solution followed by Giemsa (Schmid, 1975). The air-dried slides were coverslipped.

The slides were coded for analysis, and scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. Standard forms were used to record these data. One thousand PCEs per animal were scored. The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field. The normal frequency of micronuclei in this Crl:CD-1®(ICR) BR strain is about 0.0 - 0.4%.

The frequency of PCEs versus NCEs was determined by scoring the number of PCEs and NCEs observed in the optic fields while scoring the first 1000 erythrocytes.

14.0 EVALUATION CRITERIA

14.1 General

The criteria for the identification of micronuclei were those of Schmid (1976). Micronuclei were darkly stained and generally round, although almond and ringshaped micronuclei occasionally occurred. Micronuclei had sharp borders and were generally between 1/20 and 1/5 the size of the PCE. The unit of scoring was the micronucleated cell, not the micronucleus; thus the occasional cell with more than one micronucleus was counted as one micronucleated PCE, not two (or more) micronuclei. The staining procedure permitted the differentiation by color of PCEs and NCEs (bluish-grey and red, respectively).

14.2 Data Presentation and Interpretation

Data are summarized by sex and dose groups for the different time points. Individual animal data are also presented. The analysis of these data was performed using an analysis of variance (Winer, 1971) on either untransformed (when variances are homogeneous) and rank transformed (when variances are heterogeneous) proportions of cells with micronuclei per animal. If the analysis of variance was significant ($p<0.05$), a Dunnett's t-test (Dunnett, 1955; 1964) was used to determine which dose groups, if any, were significantly different from the negative control. Analyses were performed separately for each harvest time and sex combination. The criteria for determining a positive response involved a statistically significant dose-related increase in micronucleated PCEs, or the detection of a reproducible and statistically significant positive response for at least one dose level. A test article that induced neither a statistically significant dose response nor a statistically significant and reproducible increase at one dose level was considered negative. In either case, the final decision was based on scientific judgment.

15.0 RESULTS AND INTERPRETATION

All animals were observed immediately after dosing and periodically throughout the duration of the assay for toxic symptoms and/or mortalities. All animals in the vehicle and positive control groups appeared normal after dosing and remained healthy until the appropriate harvest times. All test article dosed groups appeared normal immediately after dosing. Approximately 22 hours after dosing, one male (#1605) from the secondary dose group was found dead. All other animals appeared normal at this time and remained healthy until the appropriate harvest times.

The test article, T-6564, induced no significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls in either sex or at any of the harvest times. The positive control, Cyclophosphamide, induced significant increases in micronucleated PCEs in both sexes as compared to the vehicle controls, with means and standard errors of 3.86% ± 0.55% and 3.58% ± 0.79% for the males and females, respectively. The data summarized by dose group are presented in Table 1 and individual animal data are found in Tables 2 through 7. Historical control data are presented in Table 8.

16.0 CONCLUSION

The test material, T-6564, did not induce a significant increase in micronuclei in bone marrow polychromatic erythrocytes under the conditions of this assay and is considered negative in the mouse micronucleus assay.

17.0 REFERENCES

Dunnett, C.W.: A multiple comparisons procedure for comparing several treatments with a control. J. Am. Statist. Assoc., 50:1096-1121, 1955.

Dunnett, C.W.: New tables for multiple comparisons with a control. Biometrics, 20:482-491, 1964.

Heddle, J.A., Hite, M., Kirkhart, B., Larsen, K., MacGregor, J.T., Newell, G.W. and Salamone, M.F.: The induction of micronuclei as a measure of genotoxicity. Mutation Res., 123:61-118, 1983.

Schmid, W.: The micronucleus test. Mutation Res., 31:9-15, 1975.

Schmid, W.: The micronucleus test for cytogenetic analysis. Chemical Mutagens: Principles and Methods for Their Detection, Vol. 4 (A. Hollaender, ed.). Plenum, pp. 31-53, 1976.

Winer, B.J.: Statistical Principles in Experimental Design, McGraw-Hill, New York, Second Edition, 1971.

18.0 DEVIATIONS FROM THE SIGNED PROTOCOL

1. In both trials of the dose rangefinding studies, animals were observed for four days and not three days after dosing. This had no impact on study integrity.

2. Due to a technical oversight, more than 1000 erythrocytes were analyzed for animal #'s 1576 and 1628. This had no impact on the integrity of the study.

3. On July 31, 1996, for the definitive study, the humidity was recorded as 75.1%. This had no impact on the integrity of the study.

19.0 EXPERIMENT DATA TABLES

TABLE 1

MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY: 17750

TREATMENT	DOSE	HARVEST TIME (HR)	% MICRONUCLEATED PCEs MEAN OF 1000 PER ANIMAL ± S.E. MALES	FEMALES	TOTAL	RATIO PCE:NCE MEAN ± S.E. MALES	FEMALES
CONTROLS							
VEHICLE	Water	24 hr	0.08 ± 0.06	0.08 ± 0.04	0.08 ± 0.03	0.53 ± 0.08	0.73 ± 0.07
POSITIVE	CP 80.0 mg/kg	24 hr	3.86 ± 0.55*	3.58 ± 0.79*	3.72 ± 0.46*	0.61 ± 0.05	0.96 ± 0.17
TEST ARTICLE	498 mg/kg	24 hr	0.20 ± 0.06	0.06 ± 0.06	0.13 ± 0.05	0.71 ± 0.08	0.92 ± 0.05
		48 hr	0.02 ± 0.02	0.08 ± 0.04	0.05 ± 0.02	0.59 ± 0.05	0.53 ± 0.04
		72 hr	0.08 ± 0.06	0.12 ± 0.07	0.10 ± 0.04	0.52 ± 0.08	1.12 ± 0.43
	995 mg/kg	24 hr	0.12 ± 0.06	0.18 ± 0.16	0.15 ± 0.08	0.69 ± 0.12	1.03 ± 0.13
		48 hr	0.12 ± 0.04	0.06 ± 0.04	0.09 ± 0.03	0.63 ± 0.03	0.54 ± 0.06
		72 hr	0.10 ± 0.08	0.10 ± 0.06	0.10 ± 0.05	0.45 ± 0.08	0.85 ± 0.21
	1990 mg/kg	24 hr	0.30 ± 0.13	0.08 ± 0.06	0.19 ± 0.08	0.69 ± 0.07	0.75 ± 0.07
		48 hr	0.00 ± 0.00	0.04 ± 0.02	0.02 ± 0.01	0.60 ± 0.06	0.56 ± 0.07
		72 hr	0.08 ± 0.06	0.12 ± 0.04	0.10 ± 0.03	0.49 ± 0.11	0.78 ± 0.21

* Significantly greater than the corresponding vehicle control, $p<0.05$.
CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte

TABLE 2

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY NO.: 17750

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
24 HOUR HARVEST	MALE			
VEHICLE CONTROL	Water	1557	1	0.52
		1574	0	0.79*
		1576	0	0.62
		1585	3	0.39
		1614	0	0.35
POSITIVE CONTROL	CP 80.0 mg/kg	1564	46	0.62
		1566	32	0.49
		1569	25	0.77
		1607	56	0.64
		1612	34	0.53
TEST ARTICLE	498 mg/kg	1562	3	0.73
		1570	1	0.73
		1581	3	0.97
		1586	3	0.61
		1587	0	0.48
	995 mg/kg	1568	1	0.69
		1571	0	0.64
		1591	3	1.04
		1598	2	0.80
		1602	0	0.29
	1990 mg/kg	1558	2	0.72
		1567	0	0.61
		1580	8	0.96
		1594	3	0.55
		1611	2	0.63

CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte
* 1059 erythrocytes scored

TABLE 3

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY NO.: 17750

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
24 HOUR HARVEST	FEMALE			
VEHICLE CONTROL	Water	1669	1	0.65
		1671	2	0.81
		1678	0	0.82
		1679	1	0.88
		1683	0	0.50
POSITIVE CONTROL	CP 80.0 mg/kg	1641	51	0.72
		1653	33	1.06
		1663	56	0.57
		1664	25	0.89
		1667	14	1.56
TEST ARTICLE	498 mg/kg	1627	0	0.97
		1630	0	1.00
		1635	0	0.74
		1644	3	0.90
		1652	0	1.01
	995 mg/kg	1633	0	0.78
		1637	0	0.77
		1660	0	0.96
		1666	8	1.26
		1672	1	1.39
	1990 mg/kg	1643	0	0.89
		1650	0	0.67
		1668	1	0.88
		1681	3	0.53
		1682	0	0.78

CP = Cyclophosphamide
PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 4

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY NO.: 17750

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
48 HOUR HARVEST	MALE			
TEST ARTICLE	498 mg/kg	1563	0	0.60
		1582	0	0.75
		1593	1	0.43
		1603	0	0.54
		1618	0	0.62
	995 mg/kg	1572	1	0.67
		1589	1	0.66
		1596	2	0.52
		1604	2	0.66
		1615	0	0.62
	1990 mg/kg	1577	0	0.75
		1579	0	0.68
		1583	0	0.60
		1595	0	0.41
		1606	0	0.53

PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 5

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY NO.: 17750

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
48 HOUR HARVEST	FEMALE			
TEST ARTICLE	498 mg/kg	1628	2	0.58*
		1636	1	0.66
		1638	0	0.39
		1654	1	0.48
		1662	0	0.54
	995 mg/kg	1621	1	0.36
		1649	0	0.44
		1651	0	0.57
		1657	0	0.71
		1665	2	0.59
	1990 mg/kg	1626	0	0.40
		1632	1	0.64
		1634	0	0.48
		1639	1	0.77
		1677	0	0.51

PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte
* 1005 erythrocytes scored

TABLE 6

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY NO.: 17750

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
72 HOUR HARVEST	MALE			
TEST ARTICLE	498 mg/kg	1561	0	0.65
		1565	0	0.74
		1599	0	0.44
		1600	3	0.34
		1609	1	0.42
	995 mg/kg	1555	1	0.48
		1556	0	0.17
		1559	0	0.58
		1590	4	0.45
		1597	0	0.57
	1990 mg/kg	1554	3	0.21
		1560	1	0.78
		1573	0	0.63
		1613	0	0.24
		1617	0	0.58

PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 7

MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M Corporation

TEST ARTICLE: T-6564

ASSAY NO.: 17750

TREATMENT		ANIMAL NUMBER	# MN PCEs/ 1000 PCEs	RATIO PCE:NCE
72 HOUR HARVEST	**FEMALE**			
TEST ARTICLE	498 mg/kg	1619	0	0.60
		1620	1	0.73
		1624	0	0.45
		1656	4	2.82
		1680	1	0.99
	995 mg/kg	1645	2	1.60
		1648	3	0.79
		1658	0	0.66
		1674	0	0.33
		1675	0	0.88
	1990 mg/kg	1622	1	0.67
		1625	2	0.73
		1629	0	0.34
		1640	2	1.58
		1642	1	0.56

PCE = Polychromatic erythrocyte
# MN PCEs = Micronucleated PCEs
NCE = Normochromatic erythrocyte

TABLE 8

MOUSE MICRONUCLEUS HISTORICAL CONTROL DATA 7/95 THROUGH 12/95

		% MICRONUCLEATED PCEs PER 1000 PCE MEAN OF 1000 PER ANIMAL ± S.E.			RATIO PCE:NCE MEAN ± S.E.	
		MALES	FEMALES	TOTAL	MALES	FEMALES
POOLED VEHICLE CONTROLS						
	MIN	0.00	0.00	0.01	0.31	0.24
	MAX	0.22	0.24	0.17	0.85	1.03
	AVG	0.087 ± 0.007	0.081 ± 0.008	0.084 ± 0.005	0.550 ± 0.021	0.587 ± 0.025
	N	47	47	47	47	47
POSITIVE CONTROLS Cyclophosphamide, 80.0 mg/kg						
	MIN	2.00	1.50	2.41	0.41	0.40
	MAX	5.68	6.36	5.38	0.72	0.79
	AVG	3.682 ± 0.240	3.170 ± 0.245	3.426 ± 0.184	0.577 ± 0.020	0.588 ± 0.026
	N	19	19	19	19	19

PCE = Polychromatic erythrocyte
NCE = Normochromatic erythrocyte
N = Number of harvests