CORNING Hazleton

MUTAGENICITY TEST ON

T-6357

IN AN *IN VIVO* MOUSE MICRONUCLEUS ASSAY

FINAL REPORT

AUTHOR

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LABORATORY PROJECT IDENTIFICATION

CHV Study No.: 17387-0-455

SUBMITTED TO

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

STUDY COMPLETION DATE

April 23, 1996

QUALITY ASSURANCE STATEMENT

Project Title: *In Vivo* Mouse Micronucleus Assay

Project No.: 20996 Assay No.: 17387

Protocol No.: 455 Edition No.: 17

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
Harvest/02/28/1996	02/28/1996	C. Orantes
Draft Report Review/04/18,19/1996	04/19/1996	C. Orantes
Final Report Review/04/23/1996	04/23/1996	C. Orantes

Quality Assurance Unit 4/23/96 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:

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4/23/96

Study Completion Date

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SUMMARY

Mutagenicity Test on T-6357 in an *In Vivo* Mouse Micronucleus Assay

The objective of this *in vivo* assay was to evaluate the ability of the test article, T-6357, 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 200, 400, 610, 810, and 1010 mg/kg in Trial I and no toxic signs were observed in any of the animals. In Trial II, animals were dosed by oral gavage at 1500, 2375, 3250, 4125 and 5000 mg/kg. Six animals (three males and three females) were assigned to each dose group. Animals were observed for three 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 2000 mg/kg. In the micronucleus assay, the test article was solubilized in deionized water and dosed oral gavage at 500, 1000, and 2000 mg/kg. Ten animals (five males and five females) were randomly assigned to each dose/harvest time group. Vehicle and positive control groups euthanatized approximately 24 hours after dosing were included in the assay. The animals dosed with the test article were euthanatized approximately 24, 48 and 72 hours after dosing for extraction of the bone marrow.

The test material, T-6357, 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-6357 in an *in vivo* Mouse Micronucleus Assay

1.0 SPONSOR: 3M

2.0 MATERIAL (Test Article)

2.1 Client's Identification: T-6357

2.2 Date Received: January 16, 1996

2.3 Physical Description: Clear, amber liquid

2.4 Genetics Assay No.: 17387

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

4.0 PROTOCOL NO.: 455, Edition 17

5.0 STUDY DATES

5.1 Initiation Date: January 18, 1996

5.2 Experimental Start Date: February 14, 1996

5.3 Experimental Termination Date: March 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-6357, 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-ACUC prior to the initiation of dosing.

Animals were housed seven per cage during quarantine, and housed five at randomization. The temperature and relative humidity were maintained at 72±6°F and 55±15%, respectively, except on February 19, 1996, for the dose selection studies, when the relative humidity was recorded at 31.1% and on the following dates for the micronucleus assay: February 17, 18, 19, 24, 24, and 25, 1996 when the relative humidity was recorded at 39.1%, 37.3%, 34.5%, 32.5, 34.4%, and 20.8%, respectively. 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 at least 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 euthanatized by CO_2 followed by penetration of the thorax. Any extra animals not used for the study were used for training purposes.

9.0 SOLUBILITY AND STABILITY:

The test article, T-6357, was supplied as a clear amber liquid. The solubility of the test article was evaluated in deionized water. A clear, light yellow solution was obtained at a concentration of about 391.6 mg/ml. Deionized water was the vehicle of choice for this assay. The stability of the test material under the dosing conditions of this assay is the responsibility of the sponsor.

10.0 DOSE SELECTION STUDY

TRIAL I

10.1 Dose Selection

Dose levels of 200, 400, 610,810 and 1010 mg/kg were administered by oral gavage for the dose selection study.

10.2 Dosing Information

The animals used in the dose selection assay were dosed on February 14, 1996. The weight range of the animals used in the dose range finding assay was 28.5-36.6 and 22.9-27.6 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 100 mg/ml stock for the high dose (1000 mg/kg). This was prepared by adding 10.98ml of deionized water (Lot # 19, prepared at CHV) to 1.2081 g of T-6357, resulting in a clear pale yellow solution with a final volume of 12.0 ml. Dilutions of this stock were prepared for the remaining dose levels.

Dosing was achieved using a 10.0 ml/kg dosing volume. All animals were nine weeks old at the time of dosing. An outline of the dosing scheme is found in the following table.

A total of 30 animals was used in this assay.

	DOSE GROUPS	
TREATMENT	M	F
T-6357		
200 mg/kg	3	3
400 mg/kg	3	3
610 mg/kg	3	3
810 mg/kg	3	3
1010 mg/kg	3	3

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 (three days) for toxic effects and/or mortalities. Immediately following dosing all animals appeared normal and remained healthy until the end of the observation period.

10.4 Conclusion

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

TRIAL II

10.5 Dose Selection

Dose levels of 1500, 2375, 3250, 4125 and 5000 mg/kg were administered by oral gavage for the dose selection study.

10.6 Dosing Information

The animals used in the dose selection assay were dosed on February 15, 1996. The weight range of the animals used in the dose range finding assay was 28.5-36.4 and 23.6-27.5 grams, for the males and females, respectively. Dosing solutions were prepared just prior to dosing and were prepared by making a 500 mg/ml stock for the high dose (5000 mg/kg). This was prepared by adding deionized water (Lot # 19, prepared at CHV) to 7.4993 g of T-6357 up to a volume of 15.0 ml, resulting in a clear pale yellow solution. Dilutions of this stock were prepared for the 1500, 2375, 3250, and 4125 mg/kg dose levels.

Dosing was achieved using a 10.0 ml/kg dosing volume. All animals were nine weeks and one day old at the time of dosing. An outline of the dosing scheme is found in the following table.

A total of 30 animals was used in this assay.

TREATMENT	DOSE GROUPS M	F
T-6357		
1500 mg/kg	3	3
2375 mg/kg	3	3
3250 mg/kg	3	3
4125 mg/kg	3	3
5000 mg/kg	3	3

All doses given were on an acute (one-time only) basis.

10.7 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.

Immediately after dosing, in the 1500 mg/kg dose group, 4 animals were hypoactive, and the other 2 were normal. In the 2375 mg/kg dose group, 2 males were languid and ataxic, and the remaining animals were normal and healthy. In the 3250 mg/kg dose group, 2 males and 1 female were languid and ataxic, and the remaining animals were normal and healthy. In the 4125 mg/kg dose group, 1 male was prostrate with labored breathing, and the remaining animals were hypoactive. In the 5000 mg/kg dose group, 1 male and 1 female were prostrate with labored breathing, and the remaining animals were slightly hypoactive.

Approximately 1 hour after dosing, all animals from the 1500 mg/kg dose group were normal and healthy. In the 2375 mg/kg dose group, 1 male and 1 female were hypoactive, and the remaining animals were normal and healthy. In the 3250 mg/kg dose group, 1 male and 2 females were hypoactive and the remaining animals were normal and healthy. All animals from the 4125 mg/kg dose group were hypoactive. In the 5000 mg/kg dose group, 1 male and 2 females were prostrate, and the remaining animals were hypoactive.

Approximately 17 hours after dosing, all animals from the 1500 mg/kg dose group were normal and healthy. All animals from the 2375 mg/kg were hypoactive and hunched, and 2 males (#'s 6336, 6334) also had squinted eyes with blood colored staining on eyes. All 3250 mg/kg dose level animals were hypoactive and

hunched, and 2 males (#'s 6333, 6337) also had blood colored staining on eyelids. All 4125 mg/kg animals were hypoactive with dyspnea and blood colored staining on eyelids. All 5000 mg/kg animals were hypoactive with dyspnea; all males also had tremors, chromodacryorrhea, and were cold to the touch. Their cage also had several blood colored spots in it. One female (# 6340) had urine colored stains, profound foot splay, and was cold to the touch. A second female also had a blood colored glaze on the eyelids and was cold to the touch.

Approximately 42.5 hours after dosing, all animals from the 1500 mg/kg dose group and all females from the 2375 mg/kg and 3250 mg/kg dose groups were normal and healthy. One 2375 mg/kg dose group male (# 6336) was found dead and the remaining males were hypoactive. One 3250 mg/kg dose group male (# 6337) was found dead and the remaining males were hypoactive. In the 4125 mg/kg dose group, 2 males (# 6332, 6338) and one female (# 6355) were found dead; the remaining male were hypoactive, hunched with dyspnea; the remaining female was hypoactive. In the 5000 mg/kg dose group, 1 male (# 6340) and 1 female (# 6360) were found dead; the remaining males were hypoactive; the remaining females were hypoactive and had tremors, squinted eyes and dyspnea.

Approximately 66.5 hours after dosing, all animals from the 1500 mg/kg, and all surviving animals from the 2375 mg/kg and 3250 mg/kg dose groups were normal and healthy. All surviving animals in the 4125 mg/kg dose group were hypoactive with dyspnea. In the 5000 mg/kg dose group, 2 females (#'s 6346, 6352) were found dead and the surviving males were hypoactive, hunched and had dyspnea.

Approximately 71.5 hours after dosing, 1 male from the 5000 mg/kg dose group (# 6342) was found dead.

Approximately 91 hours after dosing, 1 male (# 6344) from the 2375 mg/kg dose group, 1 male from the 3250 mg/kg dose group and 1 male (# 6339) from the 4125 mg/kg dose group were found dead. All remaining animals from the 1500, 2375 and 3250 mg/kg and 4125 mg/kg female dose groups were normal and healthy. The surviving male (# 6343) from the 5000 mg/kg dose group appeared pale, hunched and hypoactive.

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

Summary of Mortalities Within 3 Days in Mice Dosed Acutely with T-6357

	Observations	
Treatment	Male	Female
1500 mg/kg	0/3	0/3
2375 mg/kg	2/3	0/3
3250 mg/kg	2/3	0/3
4125 mg/kg	3/3	1/3
5000 mg/kg	2/3	3/3

10.8 Conclusion

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

11.0 MICRONUCLEUS STUDY

11.1 Dose Selection

Based on results from the dose selection study, dose levels of 500, 1,000, and 2,000 mg/kg were selected for testing in this study.

11.2 Micronucleus Assay Dosing Information

The animals used in the micronucleus assay were dosed on February 27, 1996. Cyclophosphamide (CAS # 6055-19-2; Sigma, Lot # 44H0486), the positive control, was solubilized in deionized water (Lot # 19, prepared at CHV) and was administered by oral gavage at 80.0 mg/kg. The vehicle control, deionized water (Lot # 19, prepared at CHV), was administered concurrently with the test article at a volume of 10.0 ml/kg. The weight range of the animals used in the micronucleus assay was 29.2-39.5 and 23.0-32.2 grams for the males and females, respectively. The dosing solutions for the assay were prepared by making a 200 mg/ml stock for the high dose (2000 mg/kg). This was prepared by adding deionized water to 5.0088 g of T-6357 up to a volume of 25 ml. A clear pale

yellow solution was obtained at a concentration of 200 mg/ml. Dilutions of this stock were prepared for the remaining dose levels. A second group of animals (designated Secondary Dose Group) was also assigned to the study and was dosed at the high dose selected. 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, euthanatized approximately 24 hours after dosing, were included in the assay. The animals dosed with the test article were euthanatized 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

A total of 120 animals was used in this assay

	Number of Animals Assigned							
	Primary Dose Groups						Secondary Dose Groups[a]	
	24 Hr		48 Hr		72 Hr			
Treatment	M	F	M	F	M	F	Male	Female
T-6357								
500 mg/kg	5	5	5	5	5	5	-	-
1000 mg/kg	5	5	5	5	5	5	-	-
2000 mg/kg	5	5	5	5	5	5	5	5
Vehicle Control, deionized water, 10.0 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 euthanatized at the completion of the trial.

The age of the animals at the time of dosing was nine weeks and one day.

Volumes dosed were 10.0 ml/kg and were based upon individual animal weights.

12.0 BONE MARROW HARVEST, SLIDE PREPARATION AND ANALYSIS

At the appropriate harvest time, the animals were euthanatized with CO_2 followed by penetration of the thorax and 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 using Depex® mounting medium.

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.

13.0 EVALUATION CRITERIA:

13.1 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).

13.2 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.

14.0 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 and remained healthy until the appropriate harvest times, except for one 72 hour male at the 1000 mg/kg dose level (# 6562), which was found dead about 72 hours post dosing.

The test article, T-6357, 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. Due to toxicity, a significant reduction was observed in the PCE/NCE ratios of the males from the 500, 1000, and 2000 mg/kg dose groups at the 72 hour harvest time. The positive control, CP, induced significant increases in micronucleated PCEs in both sexes as compared to the vehicle controls, with means and standard errors of 7.98% ± 1.30% and 4.16% ± 0.92% 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.

15.0 CONCLUSION:

The test material, T-6357, 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.

16.0 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.

17.0 DEVIATIONS FROM THE SIGNED PROTOCOL

The following deviations were made from the signed protocol.

1. On February 19, 1996, for the dose selection studies, the relative humidity was recorded at 31.1%. For the micronucleus assay: on February 17, 18, 19, 24, 24, and 25, 1996, the relative humidity was recorded at 39.1%, 37.3%, 34.5%, 32.5%, 34.4%, and 20.8%, respectively. This did not affect the animals and there was no impact on the integrity of this study.

2. Due to a technical error in the preparation of dosing stocks for Trial I of the dose selection study, the actual dose levels used were 610, 810, and 1010 mg/ml. The difference is <2% and had no impact on the integrity of this study.

18.0 EXPERIMENT DATA TABLES

TABLE 1
MICRONUCLEUS DATA SUMMARY TABLE

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY: 17387

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	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.67 ± 0.05	0.71 ± 0.08
POSITIVE	CP 80.0 mg/kg	24 hr	7.98 ± 1.30*	4.16 ± 0.92*	6.07 ± 0.98*	0.78 ± 0.08	0.71 ± 0.05
TEST ARTICLE	500 mg/kg	24 hr	0.02 ± 0.02	0.10 ± 0.03	0.06 ± 0.02	0.72 ± 0.09	0.73 ± 0.02
		48 hr	0.04 ± 0.04	0.02 ± 0.02	0.03 ± 0.02	0.54 ± 0.12	0.62 ± 0.03
		72 hr	0.00 ± 0.00	0.02 ± 0.02	0.01 ± 0.01	0.43 ± 0.06**	0.52 ± 0.03
	1000 mg/kg	24 hr	0.10 ± 0.03	0.04 ± 0.02	0.07 ± 0.02	0.63 ± 0.05	0.77 ± 0.04
		48 hr	0.10 ± 0.05	0.04 ± 0.02	0.07 ± 0.03	0.65 ± 0.06	0.56 ± 0.03
		72 hr	0.03 ± 0.03	0.00 ± 0.00	0.01 ± 0.01	0.29 ± 0.04**	0.58 ± 0.06
	2000 mg/kg	24 hr	0.06 ± 0.06	0.10 ± 0.04	0.08 ± 0.04	0.61 ± 0.07	0.70 ± 0.07
		48 hr	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.67 ± 0.04	0.69 ± 0.04
		72 hr	0.02 ± 0.02	0.04 ± 0.02	0.03 ± 0.02	0.36 ± 0.03**	0.53 ± 0.04

* Significantly greater than the corresponding vehicle control, $p<0.05$.
** Significantly lower than the corresponding vehicle control, $p<0.05$.
CP = Cyclophosphamide

TABLE 2
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
24 HOUR HARVEST	MALE				
VEHICLE CONTROL	Water		6556	0	0.47
			6560	0	0.65
			6589	1	0.72
			6595	0	0.71
			6605	0	0.78
POSITIVE CONTROL	CP 80.0	mg/kg	6548	125	0.58
			6553	63	1.06
			6573	90	0.63
			6579	71	0.78
			6580	50	0.83
TEST ARTICLE	500	mg/kg	6551	0	1.01
			6581	0	0.54
			6585	1	0.86
			6597	0	0.57
			6598	0	0.65
	1000	mg/kg	6550	0	0.72
			6561	1	0.66
			6569	1	0.56
			6572	1	0.72
			6574	2	0.48
	2000	mg/kg	6547	0	0.78
			6549	3	0.63
			6587	0	0.55
			6599	0	0.40
			6602	0	0.70

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

TABLE 3
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
24 HOUR HARVEST	FEMALE				
VEHICLE CONTROL	Water		6612	0	0.57
			6617	0	0.96
			6625	1	0.75
			6637	1	0.75
			6647	0	0.52
POSITIVE CONTROL	CP 80.0	mg/kg	6616	27	0.81
			6633	29	0.75
			6641	24	0.78
			6656	64	0.69
			6658	64	0.53
TEST ARTICLE	500	mg/kg	6607	2	0.73
			6610	1	0.79
			6626	1	0.60
			6642	1	0.72
			6646	0	0.79
	1000	mg/kg	6611	1	0.76
			6615	0	0.92
			6634	1	0.65
			6649	0	0.78
			6666	0	0.75
	2000	mg/kg	6621	1	0.54
			6627	0	0.76
			6644	0	0.88
			6653	2	0.56
			6665	2	0.77

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

TABLE 4
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
48 HOUR HARVEST	**MALE**				
TEST ARTICLE	500	mg/kg	6554	0	0.77
			6557	0	0.51
			6568	0	0.72
			6583	0	0.60
			6594	2	0.12
	1000	mg/kg	6566	0	0.58
			6570	1	0.49
			6582	3	0.77
			6601	1	0.81
			6603	0	0.61
	2000	mg/kg	6552	1	0.78
			6584	0	0.64
			6591	0	0.66
			6596	0	0.56
			6604	0	0.71

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

TABLE 5
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
48 HOUR HARVEST	**FEMALE**				
TEST ARTICLE	500	mg/kg	6613	0	0.60
			6628	1	0.72
			6648	0	0.56
			6651	0	0.61
			6661	0	0.61
	1000	mg/kg	6638	0	0.55
			6639	1	0.47
			6640	0	0.53
			6645	0	0.59
			6652	1	0.64
	2000	mg/kg	6608	1	0.78
			6620	0	0.68
			6624	1	0.78
			6629	0	0.59
			6664	0	0.63

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

TABLE 6
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
72 HOUR HARVEST	**MALE**				
TEST ARTICLE	500	mg/kg	6563	0	0.35
			6571	0	0.30
			6577	0	0.51
			6586	0	0.39
			6606	0	0.61
	1000	mg/kg	6558	1	0.27
			6559	0	0.19
			6562*		
			6565	0	0.31
			6588	0	0.40
	2000	mg/kg	6564	0	0.39
			6567	0	0.37
			6576	1	0.46
			6578	0	0.35
			6592	0	0.24

* Animal found dead

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

TABLE 7
MICRONUCLEUS TEST - INDIVIDUAL ANIMAL DATA

SPONSOR: 3M

TEST ARTICLE: T-6357

ASSAY NO.: 17387

TREATMENT			ANIMAL NUMBER	NO.MN PCEs (1000)	RATIO PCE:NCE
72 HOUR HARVEST	**FEMALE**				
TEST ARTICLE	500	mg/kg	6622	0	0.56
			6631	0	0.58
			6636	0	0.53
			6657	0	0.45
			6659	1	0.46
	1000	mg/kg	6609	0	0.76
			6614	0	0.64
			6618	0	0.59
			6619	0	0.51
			6643	0	0.41
	2000	mg/kg	6623	1	0.66
			6635	0	0.41
			6654	0	0.54
			6655	1	0.57
			6662	0	0.45

MN = Micronucleus
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

Corning Hazleton Inc.
9200 Leesburg Pike
Vienna, Virginia 22182-1699
703 893.5400
703 759.6947 Fax

April 19, 1996

CORNING Hazleton

Steven C. Gordon, Ph.D., DABT
3M Medical Department
Building 220-2E-02, 3M Center
St. Paul, MN 55144-1000

RE: DRAFT REPORT AND PROTOCOL AMENDMENTS
In Vivo Micronucleus Assay
Protocol No.: 455CO, Ed. No.: 4, Modified for Sponsor
Genetics Assay No.: 17387, 17387-1
Test Material: T-6357

Dear Dr. Gordon:

Enclosed please find two (2) copies of the above referenced report. The report includes an unsigned Quality Assurance Statement and Compliance and Certification Statement, which will be signed upon issuance of the Final Report.

The final report will be issued after your review and notification to us. Please contact the undersigned with any questions, comments, or necessary revisions at your earliest convenience. The report will be finalized after 1 year if no notification is received.

Thank you for giving us this opportunity to work with you.

Sincerely,

CORNING Hazleton

Hema Murli

Hema Murli, Ph.D.
Mammlian Cytogenetics
Department of Genetic and
Cellular Toxicology

HM/paj
enclosures