

Report Title: Test For Chemical Induction of Mutation In Mammalian Cells in Culture: The L5178Y TK+/- Mouse Lymphoma Assay

Test Type: Genotoxicity Study

Conducting Laboratory and Location: Microbiological Associates, Bethesda, MD

Test Substance(s): G0539.01 – Octopirox in ethanol

Species: Mammalian cells

Test Conditions: Thymidine kinase locus of L5178Y TK+/- Mouse Lymphoma cells mutation tested in the absence and presence of Aroclor-induced rat liver S-9. Test article concentration from 1.3 to 100 ug/ml for S-9 activated cultures and 0.13 to 10 ug/ml for non-activated cultures.

Results: With S-9 activation: No increase in mutation frequency. Without S-9 activation: The increases in mutant frequency ranged from 2 to 5.8 fold.

Study #: T2982.701

Report Date: 6/25/85

QA report/GLP compliance: Yes

Accession #: 30869

TEST FOR CHEMICAL INDUCTION OF MUTATION
IN MAMMALIAN CELLS IN CULTURE
THE L5178Y TK+/- MOUSE LYMPHOMA ASSAY

THE PROCTER & GAMBLE COMPANY

TEST ARTICLE (TSIN) G0539.01
DRD NO. BYCR 0392

RECEIVED BY

JUL 8 1985

OPERATIONS SECTION

MICROBIOLOGICAL ASSOCIATES INC.

Microbiological Associates Inc.
5221 River Road
Bethesda, Maryland 20816
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A Subsidiary of

Daryl Laboratories Inc

TEST FOR CHEMICAL INDUCTION OF MUTATION
IN MAMMALIAN CELLS IN CULTURE
THE L5178Y TK+/- MOUSE LYMPHOMA ASSAY

Sponsor: The Procter & Gamble Company
Cincinnati, Ohio

Testing Facility: Microbiological Associates, Inc.
5221 River Road
Bethesda, Maryland 20816

Study No.: T2982.701

Test Article I.D. (TSIN): G0539.01

Test Article Lot No.: .01

Test Article DRD No.: BYCR 0392

Test Article Description: White Powder

Storage Conditions: Room Temperature with Desiccation;
Protected from Light

Date Received: 4/11/85

Date Study Initiated: 4/25/85

Completion Date: 6/25/85

Report Date: 6/28/85

Sponsor's Investigator: J. E. Weaver
The Procter & Gamble Company

Study Director: Andrea M. Rogers-Back, Ph.D.
Microbiological Associates, Inc.

Andrea M. Rogers-Back 6/25/85
Andrea M. Rogers-Back, Ph.D. Date
Study Director

Jane J. Clarke 6/25/85
Jane J. Clarke Date
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Charlton H. Blackburne 6/29/85
Charlton H. Blackburne Date
Biologist

Carl M. Hay 6-25-85
Carl M. Hay Date
Biologist

Reginald A. Vogelzon June 25th 1985
Reginald A. Vogelzon Date
Biologist

QUALITY ASSURANCE STATEMENT

Study Title: TEST FOR CHEMICAL INDUCTION OF MUTATION
IN MAMMALIAN CELLS IN CULTURE
THE L5178Y TK+/- MOUSE LYMPHOMA ASSAY

Study Number: T2982.701

Study Director: A. ROGERS-BACK, PH.D.

Initiation Date: APRIL 25, 1985

Review Completed Date: June 25, 1985

This study has been divided into a series of phases. Using a random sampling approach, Quality Assurance monitors each of these phases over a series of studies. Procedures, documentation, equipment, etc., are examined in order to assure that the study is performed in accordance with the Good Laboratory Practice regulations and to assure that the study is conducted according to the protocol.

The following are the inspection dates, phases inspected, and report dates of QA inspections of the study.

INSPECT ON 85/04/22 - 85/04/22, TO STUDY DIR 85/04/22, TO MGMT 85/04/22

PHASES: PROTOCOL REVIEW

INSPECT ON 85/05/20 - 85/05/20, TO STUDY DIR 85/05/20, TO MGMT 85/05/20

PHASES: DILUTION OF THE TEST ARTICLE

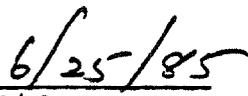
INSPECT ON 85/06/24 - 85/06/24, TO STUDY DIR 85/06/24, TO MGMT 85/06/25

PHASES: FINAL REPORT

This report describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.



Quality Assurance
RA/QA Department


Date

Summary

The Procter & Gamble Company's test article G0539.01 (MA #T2982) was tested in the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay in the presence and absence of Aroclor induced rat liver S-9. Two non-activated assays were conducted on the test article. In the first assay, only one culture that was cloned exhibited more than one 10% Total Growth. The test article induced pronounced delayed toxicity in the non-activated cultures. All of the cultures that were cloned in the first non-activated assay exhibited mutant frequencies which were more than twice the mean mutant frequency of the solvent controls. The Total Growth of the cultures ranged from 1% to 13%. The non-activated assay was repeated over a reduced range of concentrations which produced from 4% to 110% Total Growth. Seven of the ten cultures that were cloned exhibited mutant frequencies which were at least twice the mean mutant frequency of the solvent control cultures. In the S-9 activated assay, the cultures that were cloned were treated with a range of test article concentrations which produced from 12% to 96% Total Growth. None of the cultures that were cloned exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control cultures.

The results indicate that, under the conditions of these tests, test article G0539.01 produced a positive response in the absence of exogenous metabolic activation and a negative response in the presence of metabolic activation.

Introduction

The Procter & Gamble Company's test article G0539.01, DRD #BYCR 0392 (MA #T2982) was received on April 11, 1985 for testing in the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay with and without exogenous metabolic activation by Aroclor induced rat liver microsomes.

Objective

To evaluate the mutagenic potential of the test article using the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay.

Materials and Methods

The experimental protocol (see Appendix I) is based on that described by Clive, D. and Spector, J. F. S. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y TK+/- Mouse Lymphoma cells. Mutation Research 31:17-29, 1975.

A. DETERMINATION OF TOXICITY

A preliminary toxicity test with and without S-9 activation was conducted. Tube cultures were initiated by seeding one Corning polypropylene centrifuge tube per dose level and two per solvent control with 6 ml of a cell suspension from a common pool containing 1×10^6 cells per ml. Ethanol was selected as the solvent of choice. The test article was solubilized and the test article was diluted for testing at 2000, 200, 20, 2, 0.2, 0.02 and 0.002 $\mu\text{g}/\text{ml}$. The test article was added to each appropriately labeled centrifuge tube in amounts at which the final solvent concentration was non-toxic to the cell suspension. Four ml of S-9 activation mixture¹ or 4 ml of F₀P medium was added to each tube depending on whether or not they received activation. Each tube was gassed with 5% CO₂ in air and placed

¹The S-9 activation mixture contained 4.56 mg/ml instead of 4.5 mg/ml as specified in the protocol. This deviation was not significant since the test article toxicity was reproduced from the range finding study to the mutagenesis assay.

on a Bellco roller drum apparatus at approximately 25 rpm for a 4-hour exposure period. The test solutions were prepared under amber lights and kept in darkness during the entire exposure period.

After 4 hours the test article was removed by centrifuging the cells at 1000 rpm for 10 minutes and decanting the supernatant. The cells were washed twice in 10 ml of F₁₀P, resuspended in 20 ml F₁₀P, gassed with 5% CO₂ in air, and replaced on the roller drum apparatus.

Test article toxicity was determined by comparing the cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test article by removing 1 ml samples from each centrifuge tube, making 1:10 dilutions in 0.1% trypsin, incubating at 37°C for 10 minutes, and counting the samples with an electric cell counter.

All calculations were performed using a Hewlett Packard 86 Minicomputer with programs labeled "Cell Culture Adjustment" and "Initial Toxicity".

B. TESTING FOR MUTAGENIC ACTIVITY

1. Cell Preparation

Prior to use in the assay, L5178Y cells which were actively growing in culture were cleansed as described by Clive, et al.² Three ml of THMG stock solution was added to a 100 ml cell suspension containing 0.1×10^6 cells per ml. The culture was gassed with CO₂ in air and placed on an environmental incubator

²Clive, D. and Spector, J. F. S. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. Mutation Research 31:17-29, 1975.

shaker at 125 rpm and 37°C. After 24 hours the THMG was removed by pelletizing the cells and decanting the supernatant. The cells were rinsed in 20 ml F₁₀P and reinstated in culture at 3 x 10⁴ cells per ml in 100 ml of F₁₀P plus 1 ml of THG stock solution.

The cell population density of the prepared cultures was determined by adding 1 ml sample of cells to 9.0 ml of 0.1% trypsin, incubating at 37°C for 10 minutes, and making three counts per sample with an electronic cell counter. Based on the determination of the number of cells per ml, a cell suspension containing 1.0 x 10⁶ cell per ml was prepared, and 6 ml aliquots were dispensed in Corning polypropylene centrifuge tubes.

2. Test Compound Preparation

Based on the data derived from the toxicity test, the test article was prepared so that the highest concentration was 100% toxic. The test article was solubilized and 15 serial eighth log dilutions were carried out. This produced 16 dose levels decreasing approximately 100-fold from highest to lowest. The test article was added to each tube, labeled with the test article T No., test concentration and NA or S-9, in amounts at which the final solvent concentration was non-toxic to the cells. The compound was tested with and without S-9 activation. Four ml of S-9 activation mixture was added to half of the tubes and 4 ml of F₀P was added to the other half. This yielded a final cell suspension of 0.6 x 10⁶ cells per ml.

Two control tubes received solvent only and the positive controls were treated with EMS (1.0 and 0.5 µl/ml) and 7,12-DMBA (7.5 and 5.0 µg/ml). All tubes were gassed with 5% CO₂ in air and placed on a roller drum apparatus for 4 hours at 37°C. The

preparation and addition of the test article was carried out under amber lighting and the cells were incubated in the dark during the 4-hour exposure period.

At the end of exposure period, the cells were washed twice in 10 ml of F₁₀P by centrifuging at 1000 rpm for 10 minutes and decanting the supernatant. The cells were resuspended in 20 ml of F₁₀P, gassed with 5% CO₂ in air, and replaced on the roller drum apparatus at 37°C.

3. Expression Time

After the initial exposure to the test article, the cells were incubated for two days with a cell population adjustment at 24 and 48 hours. The adjustment was made by taking daily cell counts and then replacing a volume of cells with fresh medium which yielded a cell population density of 0.3×10^6 cells per ml.

4. Cloning

At the end of the expression period, the cells were placed in cloning medium (C.M.) containing 0.23% granulated agar. TFT at a final concentration of 3 µg/ml was used as the restrictive agent.

a. General Preparation

Two Florence flasks per culture to be cloned were labeled with the compound concentration and whether or not they received S-9 activation. For each pair of flasks one was labeled TFT and one was labeled V.C. (viable count). Each flask was prewarmed to 37°C, filled with 100 ml C.M., and placed on an incubator shaker at 37°C until used.

Six 100 mm petri plates per culture were labeled with the concentration, whether or not activation was used, and the

experiment number. Three of the six were labeled TFT and three were labeled V.C.

b. Cell Plating

Cell counts were made for each tube to determine the volume of each cell population which would yield 3×10^6 cells. This volume was removed, the remainder of the cells were discarded, and the 3×10^6 cells were replaced in the centrifuge tube. The cells were centrifuged at 1000 rpm for 10 minutes, and the supernatant, except for 2 ml, was removed by pipetting. The cells were resuspended in the remaining 2 ml of medium and placed in a TFT flask labeled with the corresponding concentration of the test article.

A 2×10^{-4} dilution was carried out by adding 1.0 ml of the TFT flask suspension to a test tube containing 4 ml of $F_{10}P$, adding 1.0 ml of this to 9 ml of $F_{10}P$, and adding 1.0 ml of that dilution to the appropriate V.C. flask containing 100 ml of C.M. After the dilution 1 ml of stock solution of TFT was added to the TFT flask, and both this flask and the V.C. flask were placed on the shaker at 125 rpm and $37^{\circ}C$.

After 15 minutes the flasks were removed one at a time, and 33 ml of the cell suspension was pipetted into each of three appropriately labeled petri plates. To accelerate the gelling process, the plates were placed in cold storage ($4^{\circ}C$) for 20 minutes. The plates were removed and incubated at $37^{\circ}C$ in a humidified 5% CO_2 atmosphere for 10-12 days.

5. Accumulation of Data

After the incubation period, both the TFT plates and the V.C. plates were scored for the total number of colonies per plate. Three counts per plate were made on an automatic colony

counter, and the median count was recorded. The mutation frequency was determined by dividing the average number of colonies in the three TFT plates by the average number of colonies x 10^4 in the three corresponding V.C. plates and multiplying the quotient by two.

Chemical information on control articles and solvents used for this study:

Control Articles

Ethyl Methansulfonate	$\text{CH}_3\text{SO}_3\text{C}_2\text{H}_5$
CAS Registry Number	62-50-0
Assay (by GLC)	98% min
7,12-Dimethylbenz(a)anthracene	
CAS Registry Number	57-97-6
Assay (by UV-VIS)	95% min
Melting Range	122°C to 123°C

Solvent:

Acetone	CH_3COCH_3
CAS Registry Number	67-64-1
Assay (CH_3COCH_3)	Not less than 99.5%
Isopropyl Alcohol [$(\text{CH}_3)_2\text{CHOH}$]	0.05%
Color (APHA)	10
Density (g/ml) at 25°C	Not above 0.7857
Boiling Range	Not more than 0.8°C
Boiling Point	56.1+0.1°C
Residue after Evaporation	0.001%
Solubility in Water	Clear
Acidity (CH_3COOH)	0.002%
Alkalinity (as NH_3)	0.001%
Aldehyde (HCHO)	0.002%
Methanol (CH_3OH) (by G.C.)	0.05%
Subs Reducing KMnO_4 (Color)	Pass test
Water (H_2O)	0.5%
DMSO	$(\text{CH}_3)_2\text{SO}$
CAS Registry Number	67-68-5
Appearance	Clear, Colorless liquid

Density (gms/ml) at 25°C	1.095 min
Freezing Point	18.0°C min
Residue after Evaporation	0.01%
Color (APHA)	15
Ethanol	C ₂ H ₅ OH
Brand Name	Pharmco
Bottled by	Publicker Industries Co. Linfield, PA
Assay	190 Proof
Molecular Weight	46.07
Density (gms/ml) at 20°C	0.789
Boiling Point	78.5°C
Melting Point	-114.1°C
Solidifies below	-130°C
Flash Point	9-11°C

The following criteria were used as guidelines in judging the significance of the activity of a test article in this system. In evaluating the results, it is considered that increases in mutant frequencies, which occur only at highly toxic concentrations, may be due to epigenetic events. Unfortunately, it is impossible to formulate criteria which could apply to all types of data which may be generated and therefore the scientist's evaluation must be the final endpoint.

Positive - if there is a positive dose response and one or more of the three highest doses exhibit a mutant frequency which is two-fold greater than the background level.

Equivocal - if there is no dose response but any one or more doses exhibit a two-fold increase in mutant frequency over background.

Negative - if there is no dose response and none of the test cultures exhibit mutant frequencies which are two-fold greater than background.

Note: 1. Some of the numbers generated by the test data, whether it is Toxicity, Mutant Frequency, etc., are computed using non-rounded numbers. This may, in some instances, cause what appear to be errors in calculation if only the rounded number are used when checking the data.

2. All of the raw data generated by the assay and appropriate reports will be maintained in Microbiological Associates, Inc.'s archives located at 5221 River Road, Bethesda, Maryland, 20816.

3. The Sponsor has assumed responsibility for the determination of the stability of the test article.

4. All test article stock solutions were freshly prepared immediately before use in each procedure.

Results

The Initial Toxicity Test (Table 1) conducted on test article G0539.01 (MA #T2982) indicated complete toxicity at 20 $\mu\text{g/ml}$ for both the non-activated and the S-9 activated cultures. Based on these data, the test article was tested in a mutagenesis assay over a range of concentrations from 100 $\mu\text{g/ml}$ to 1.3 $\mu\text{g/ml}$ for the S-9 activated cultures and from 10 $\mu\text{g/ml}$ to 0.13 $\mu\text{g/ml}$ for the non-activated cultures.

After a two day expression period, ten non-activated and nine S-9 activated cultures were selected for cloning based on their degree of toxicity. The non-activated cultures that were cloned were treated with 4.2, 2.4, 1.8, 1.3, 1.0, 0.75, 0.56, 0.42, 0.32 or 0.24 $\mu\text{g/ml}$. These concentrations produced a range in Suspension Growth of 10% to 23%. The S-9 activated cultures that were cloned were treated with 13, 10, 7.5, 5.6, 4.2, 3.2, 2.4, 1.8 or 1.3 $\mu\text{g/ml}$. These concentrations produced a range in Suspension Growth of 20% to 97%. The Cloning Data and Total Compound Toxicity Data for the test article are presented in Tables 2 through 5. These data are also presented graphically in Figure 1 (without activation) and Figure 2 (with S-9 activation). The data for the positive controls are presented in Tables 6 and 7. The raw data for the plate counts for this assay are presented in Appendix II.

All non-activated cultures that were cloned and for which complete results are obtained exhibited mutant frequencies which were at least twice the mean mutant frequency of the solvent cultures. The increases in mutant frequency ranged from 15.7 to 3.7 fold. The Total Growth of the cultures ranged from 1% to 13%. None of the S-9 activated cultures that were cloned exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control cultures. The Total Growth of the S-9 activated cultures ranged from 12% to 96%.

The non-activated portion of the assay was repeated in order to generate cultures with greater than 10% Total Growth. The cultures were treated with a range of concentrations from 1.0 $\mu\text{g/ml}$ to 0.013 $\mu\text{g/ml}$. After a two day expression period, ten cultures were selected for cloning based on their degree of toxicity. The cultures that were cloned were treated with 1.0, 0.75, 0.56, 0.42, 0.32, 0.24, 0.18, 0.13, 0.10 or 0.075 $\mu\text{g/ml}$. These concentrations produced a range in Suspension Growth of 16% to 106%. The Cloning Data and Total Compound Toxicity Data for the test article are presented in Tables 8 and 9. These data are also presented graphically in Figure 3. The data for the positive controls are presented in Table 10. The raw data for the plate counts for this assay are presented in Appendix III.

Seven of ten cultures that were cloned exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control cultures. The increases in mutant frequency ranged from 5.8 to 2.0-fold. The Total Growth of these cultures ranged from 4% to 82%. The three remaining cultures that were cloned (0.13, 0.1 and 0.075 $\mu\text{g/ml}$) did not exhibit mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control cultures. The Total Growth of these cultures ranged from 91% to 110%.

TABLE 1

L5178Y TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY
INITIAL COMPOUND TOXICITY TEST

Study Number : T2982.701
 Test Article : G0539.01
 Activation : Induced Rat Liver S-9
 Trial Number : 1
 Dose Range : 2000 TO .002 UG/ML
 Solvent : Ethanol
 Operator : Robert T. Simmons
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 04/27/85 10:52 am

Test Article Concentration (µg/ml)	Cell Concentration (X 10 ⁶)		Suspension Growth % of Control	
	Day 1	Day 2	Total	Control

WITHOUT ACTIVATION

2000	0.013	0.011	0.0	0
200	0.011	0.033	0.0	0
20	0.140	0.098	0.0	0
2	0.419	0.558	2.6	16
.2	0.832	1.080	10.0	60
.02	1.095	1.393	16.9	102
.002	0.961	1.347	14.4	87
Solvent 1	1.123	1.440	18.0	
Solvent 2	1.055	1.284	15.1	

WITH S-9 ACTIVATION

2000	0.033	0.082	0.0	0
200	0.014	0.032	0.0	0
20	0.216	0.210	0.0	0
2	0.974	1.419	15.4	101
.2	0.945	1.459	15.3	100
.02	0.911	+		
.002	0.973	1.324	14.3	97
Solvent 1	0.974	1.377	14.9	
Solvent 2	0.945	1.497	15.7	

+ Culture Lost

Table Prepared By: Robert Simmons 4/27/85
 Signature Date

TABLE 4

L5178Y TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY
CLONING DATA

Study Number : T2982.701
 Test Article : G0539.01
 Activation : Induced Rat Liver S-9
 Trial Number : 1
 Solvent : Ethanol
 Operator : Carl M. Hay
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 06/03/85 12:36 pm

Test Art Concentr (µg/ml)	Ave #/ TFT Plate	TFT Stand Dev	Ave #/ V.C. Plate	V.C. Stand Dev	Mutant* Frequency	Induced* Mutant Frequency	% Total Growth
13	62/3	± 1	94/3	±11	1.3	0.3	12
10	83/3	± 4	103/3	± 4	1.6	0.6	17
7.5	71/3	±11	113/3	± 3	1.3	0.3	20
5.6	79/3	±12	124/3	± 7	1.3	0.3	30
4.2	93/3	± 4	142/3	± 7	1.3	0.3	67
3.2	79/3	± 5	136/3	± 2	1.2	0.2	76
2.4	75/3	± 6	138/3	± 5	1.1	0.1	86
1.8	78/2	± 1	157/3	± 4	1.0	0.0	96
1.3	56/3	± 6	119/3	± 6	0.9	-0.1	75
Solvent 1	60/3	± 1	159/3	± 6	0.8		
Solvent 2	80/3	± 5	149/3	±11	1.1		

+ Culture Lost † Per 10⁴ surviving cells /# = number of plates counted

Table Prepared By: Carl M. Hay 6-3-85
 Signature Date

TABLE 3

L5178Y TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY

TOTAL COMPOUND TOXICITY DATA

Study Number : T2982.701
 Test Article : G0539.01
 Activation : Non-activated
 Trial Number : 1
 Solvent : Ethanol
 Operator : Carl M. Hay
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 06/03/85 12:34 pm

Test Art Concentr (µg/ml)	Cell Concentr (X 10 ⁶)		Susp Growth		Cloning Growth		% Total Growth
	Day 1	Day 2	Total	%Cntl	Ave VC	%Cntl	
7.5	0.227	0.189	0.0	0		++	
5.6	0.242	0.212	0.0	0		++	
4.2	0.324	0.434	1.6	10	19	11	1
3.2	0.271	0.266	0.0	0		++	
2.4	0.393	0.549	2.4	15	47	28	4
1.8	0.373	0.591	2.4	15	52	31	5
1.3	0.399	0.648	2.9	18	53	31	6
1	0.433	0.638	3.1	19	46	27	5
.75	0.453	0.644	3.2	20	49	29	6
.56	0.438	0.655	3.2	20	57	34	7
.42	0.477	0.664	3.5	22	70	42	9
.32	0.444	0.710	3.5	22		+	
.24	0.483	0.682	3.7	23	96	57	13
Solvent 1	1.031	1.389	15.9		170		
Solvent 2	1.027	1.388	15.8		167		

+ Culture Lost ++ Too Toxic To Clone

Table Prepared By: Carl M. Hay 6-3-85
 Signature Date

FIGURE 1
Study No. T2982.701 (Trial 1)
Test Article G0539.01
Without Activation

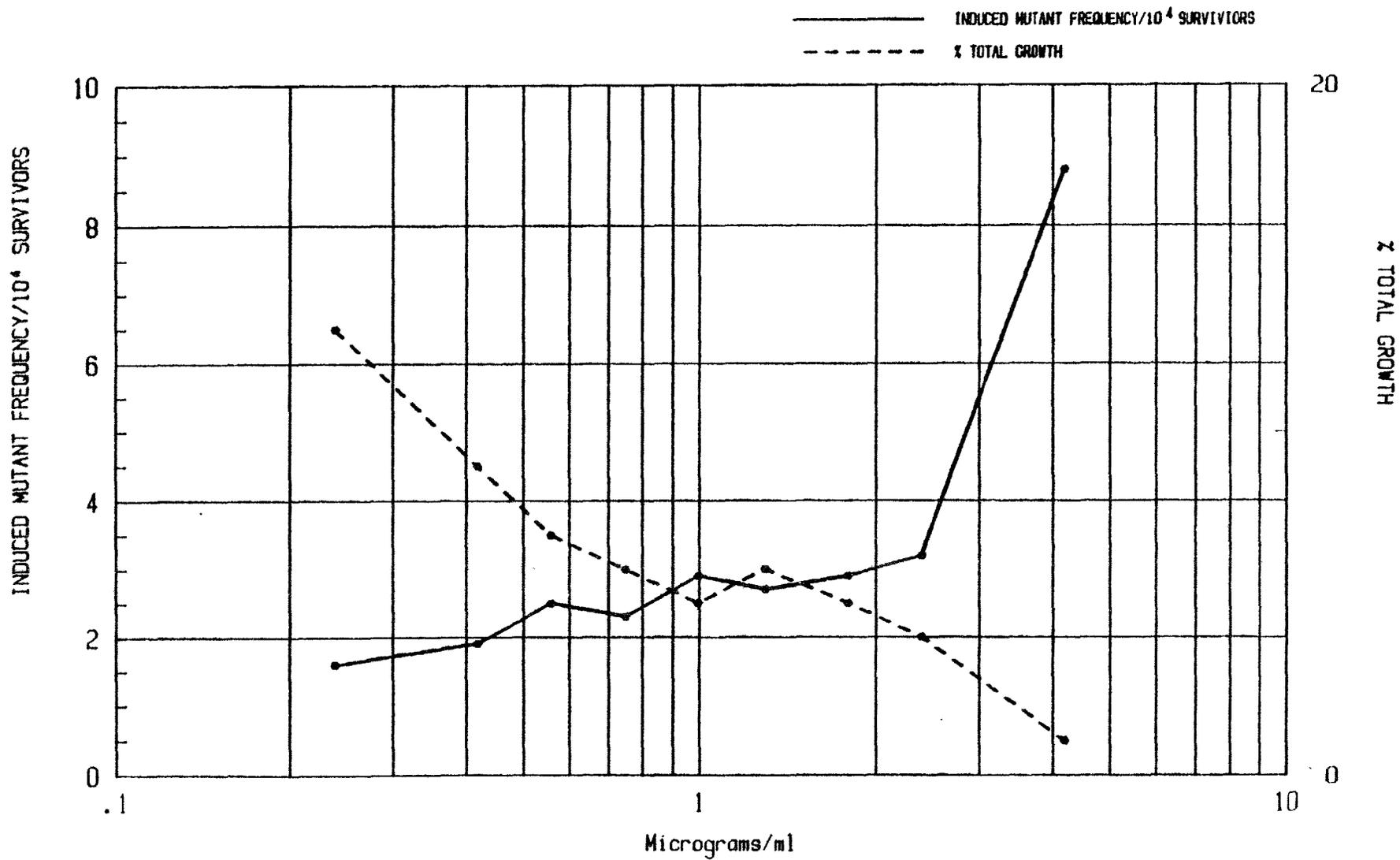


FIGURE 2
Study No. T2982.701 (Trial 1)
Test Article G0539.01
With S-9 Activation

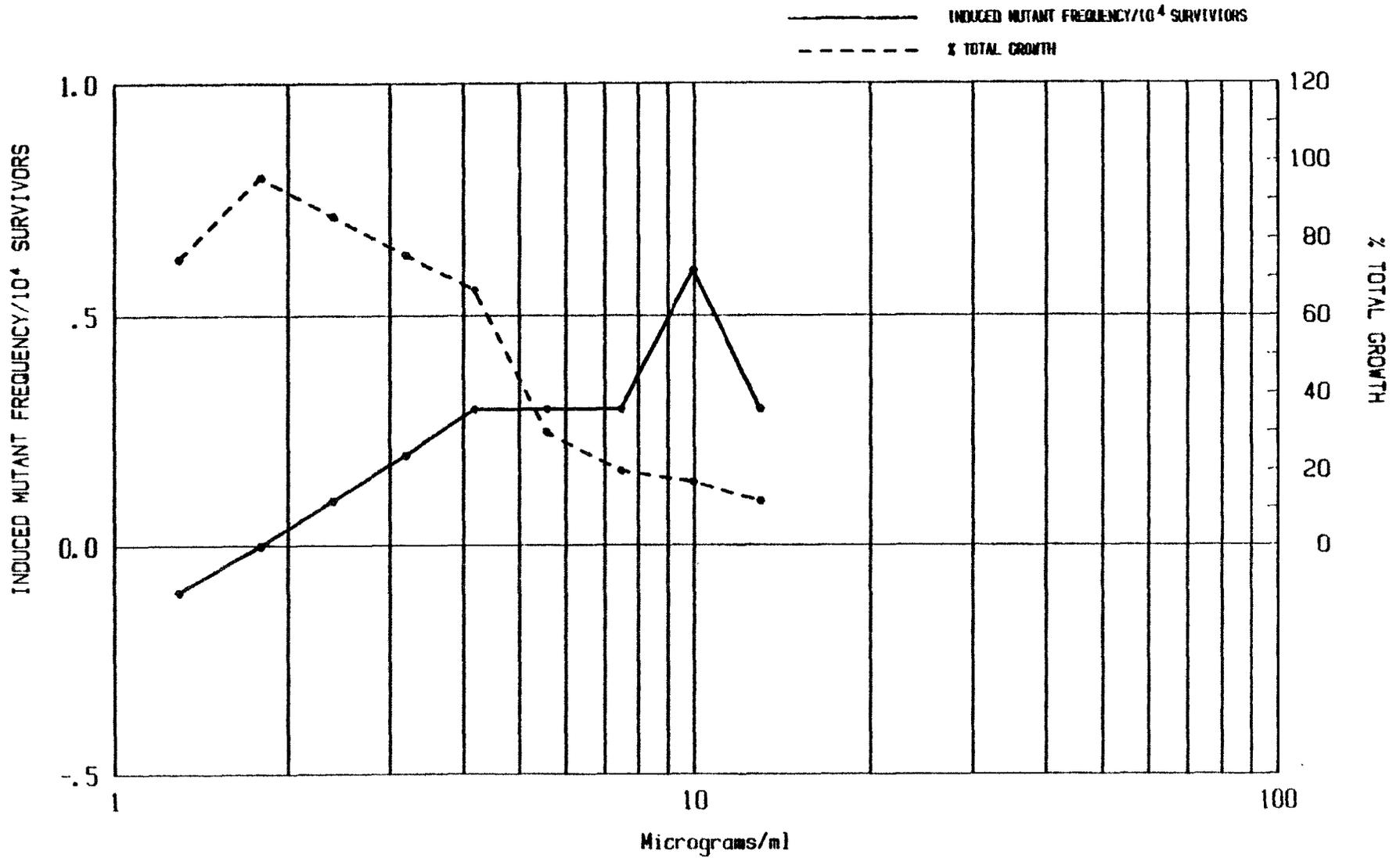


TABLE 6

L5178Y TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY
POSITIVE CONTROL - NO ACTIVATION

Study Number : T2982.701
 Test Article : Ethyl Methanesulfonate
 Activation : No activation
 Trial Number : 1
 Solvent : Dimethylsulfoxide
 Operator : Carl M. Hay
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 06/03/85 2:20 pm

CLONING DATA

Test Art	Ave #/ Concentr (µl/ml)	TFT TFT Plate	TFT Stand Dev	Ave #/ V.C. Plate	V.C. Stand Dev	Mutant* Frequency	Induced* Mutant Frequency	% Total Growth
1	.5	++ 331/3	±23	++ 78/3	± 4	8.5	7.8	10
Solvent 1		53/3	± 3	176/3	±14	0.6		
Solvent 2		50/3	± 2	147/3	±11	0.7		

TOTAL COMPOUND TOXICITY DATA

Test Art	Cell Concentr (X 10 ⁶)	Susp Growth Total	%Cntl	Cloning Growth Ave VC	%Cntl	% Total Growth
Concentr (µl/ml)	Day 1 Day 2					
1	0.051 0.101	0.0	0	++		
.5	0.259 0.901	3.0	20	78	48	10
Solvent 1	1.040 1.285	14.8		176		
Solvent 2	.997 1.395	15.5		147		

+ Culture Lost ‡ Per 10⁴ surviving cells

++ too toxic to clone

/# = number of plates counted

Table Prepared By: Carl M. Hay 6-3-85
 Signature Date

TABLE 7

L5178Y TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY
POSITIVE CONTROL - WITH ACTIVATION

Study Number : T2982.701
 Test Article : 7,12 Dimethylbenz(a)anthracene
 Activation : With activation
 Trial Number : 1
 Solvent : Acetone
 Operator : Carl M. Hay
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 06/03/85 2:22 pm

CLONING DATA

Test Art	Ave #/ Concentr (µg/ml)	TFT TFT Plate	TFT Stand Dev	Ave #/ V.C. Plate	V.C. Stand Dev	Mutant* Frequency	Induced* Mutant Frequency	% Total Growth
7.5	247/3	±14	111/3	±12	4.5	3.5	28	
5	200/3	± 5	125/3	± 6	3.2	2.2	58	
Solvent 1	80/3	± 7	160/3	±10	1.0			
Solvent 2	69/3	± 7	153/3	± 2	0.9			

TOTAL COMPOUND TOXICITY DATA

Test Art	Cell Concentr (X 10 ⁶)	Susp Growth Total	%Cnt1	Cloning Growth Ave VC	%Cnt1	% Total Growth	
7.5	0.399	1.158	5.1	40	111	71	28
5	0.588	1.405	9.2	72	125	80	58
Solvent 1	.861	1.409	13.5		160		
Solvent 2	.849	1.287	12.1		153		

* Culture Lost † Per 10⁴ surviving cells /# = number of plates counted

Table Prepared By: Carl M. Hay 6-3-85
 Signature _____ Date _____

FIGURE 3
Study No. T2982.701 (Trial 2)
Test Article G0539.01
Without Activation

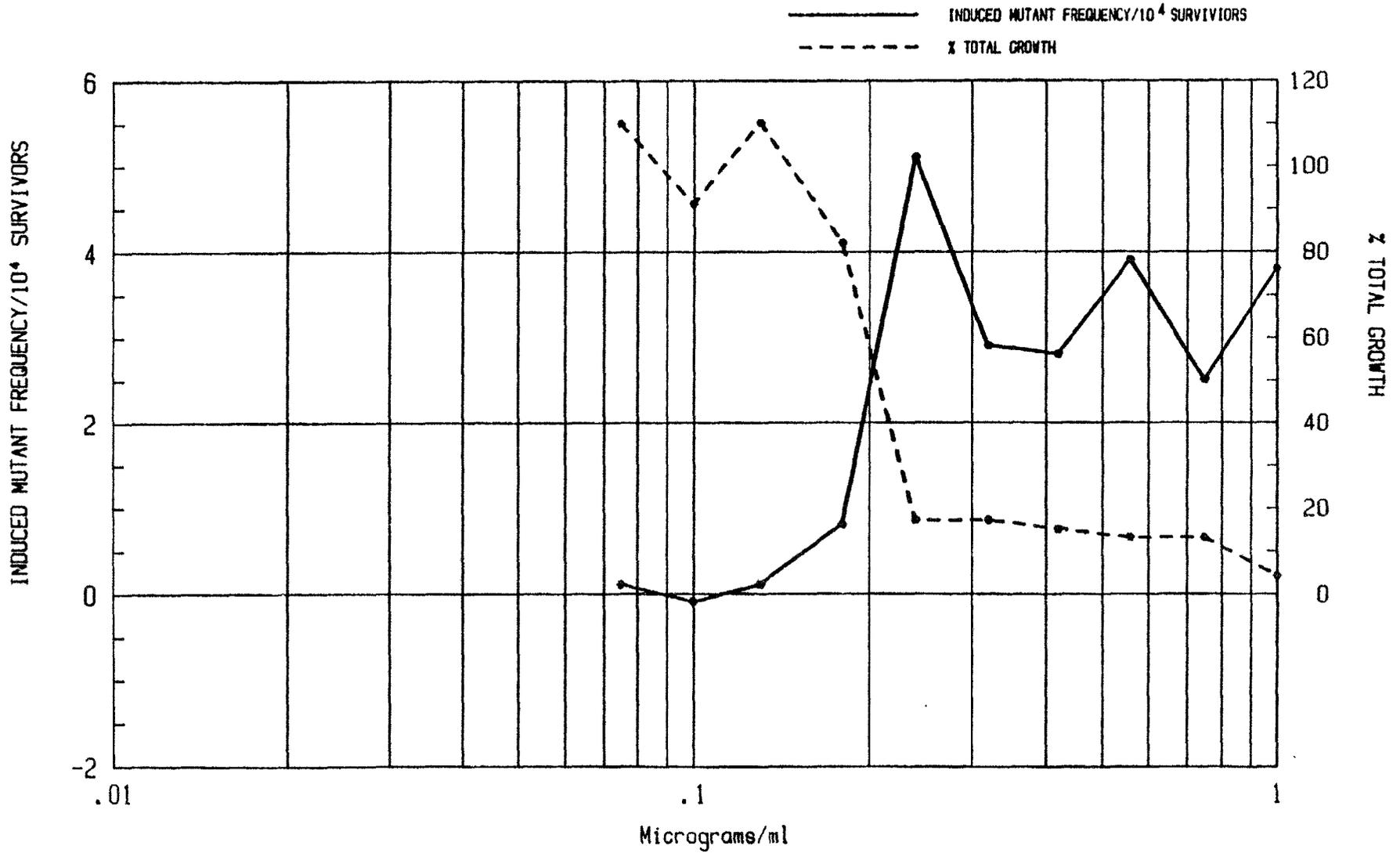


TABLE 10

L5178Y TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY
 POSITIVE CONTROL - NO ACTIVATION

Study Number : T2982.701
 Test Article : Ethyl Methanesulfonate
 Activation : No activation
 Trial Number : 2
 Solvent : Dimethylsulfoxide
 Operator : Carl M. Hay
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 06/17/85 1:16 pm

CLONING DATA

Test Art	Ave #/ Concentr (µl/ml)	TFT TFT Plate	TFT Stand Dev	Ave #/ V.C. Plate	V.C. Stand Dev	Mutant* Frequency	Induced* Mutant Frequency	% Total Growth
1	125/3	±14	7/3	± 3	35.7	35.0	2	
.5	292/3	± 7	59/3	± 3	9.9	9.2	39	
Solvent 1	24/3	± 3	99/3	± 5	0.5			
Solvent 2	42/3	± 4	105/3	± 3	0.8			

TOTAL COMPOUND TOXICITY DATA

Test Art	Cell Concentr (X 10 ⁶)	Susp Growth Total	%Cntl	Cloning Growth Ave VC	%Cntl	% Total Growth
1	0.497	0.663	3.7	32	7	2
.5	0.740	0.944	7.8	67	59	39
Solvent 1	.890	1.165	11.5	99		
Solvent 2	.954	1.119	11.9	105		

* Culture Lost † Per 10⁴ surviving cells /# = number of plates counted

Table Prepared By:

Carl M. Hay
 Signature

6-17-85
 Date

APPENDIX I

APPENDICES

Conclusion

The Procter & Gamble Company's test article G0539.01, (MA #T2982) was tested in the L5178Y TK+/- Mouse Lymphoma Mutagenesis Assay in the presence and absence of Aroclor induced rat liver S-9. Two non-activated assays were conducted. In the first assay, only one culture that was cloned exhibited greater than 10% Total Growth. The test article induced pronounced delayed toxicity in the non-activated cultures. All of the cultures that were cloned in the first non-activated assay exhibited mutant frequencies which were more than twice the mean mutant frequency of the solvent control cultures. The non-activated assay was repeated over a reduced range of test article concentrations. Seven of ten cultures that were cloned in the second assay exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control cultures. In the S-9 activated portion of the assay, none of the cultures that were cloned exhibited mutant frequencies which were significantly greater than the mean mutant frequency of the solvent control cultures.

The results indicate that, under the conditions of these tests, test article G0539.01 produced a positive response in the absence of exogenous metabolic activation and a negative response in the presence of metabolic activation.

TABLE 9

L517BY TK+/- MOUSE LYMPHOMA MUTAGENESIS ASSAY

TOTAL COMPOUND TOXICITY DATA

Study Number : T2982.701
 Test Article : G0539.01
 Activation : Non-activated
 Trial Number : 2
 Solvent : Ethanol
 Operator : Carl M. Hay
 Study Director : Andrea M. Back, Ph.D.
 Date & Time : 06/17/85 1:14 pm

Test Art Concentr (µg/ml)	Cell Concentr (X 10 ⁶)		Susp Growth		Cloning Growth		% Total Growth
	Day 1	Day 2	Total	%Cntl	Ave VC	%Cntl	
1	0.363	0.450	1.8	16	27	26	4
.75	0.412	0.643	2.9	25	54	51	13
.56	0.411	0.649	3.0	26	54	51	13
.42	0.387	0.678	2.9	25	61	58	15
.32	0.449	0.659	3.3	29	60	57	17
.24	0.460	0.635	3.2	28	62	59	17
.18	0.779	1.015	8.8	77	113	107	82
.13	0.921	1.089	11.1	97	119	113	110
.1	0.868	1.093	10.5	91	105	100	91
.075	0.979	1.119	12.2	106	110	104	110
Solvent 1	.908	1.122	11.3		110		
Solvent 2	.946	1.116	11.7		101		

+ Culture Lost ++ Too Toxic To Clone

Table Prepared By: _____

Carl M. Hay
 Signature

6-17-85
 Date

Received by RA/QA 4/25/85
[Signature]

PROTOCOL NO. C29

Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK⁺ Mouse Lymphoma Assay

Issue Date: March 1, 1982
Supersedes Issue Dated: September 15, 1980

Test Substance Identification Number (TSIN) # G0539.01

Divisional Request Document Number (DRD) # ndRYCR0392

Sponsor: The Procter & Gamble Company
Cincinnati, Ohio

Testing Facility:
(To be filled in by
Operations Section)

Microbiological Associates, Inc.
5221 River Road
Bethesda, MD 20016

Study # T2982.701
(To be filled in by
Testing Facility)

Purpose: To determine the potential of a chemical compound to induce mutations at the thymidine kinase (TK) locus of cultured L5178Y TK⁺ mouse lymphoma cells.^{1, 2, 3}

Justification for Selection of Test System:

The L5178Y/TK⁺ mouse lymphoma cells clone 3.7.2C is the system of choice due to the amount of background data available.

Route of Administration of Test Substance and Reason for Choice:

IN VITRO with and without metabolic activation.
Route specified by test procedure.

Records to be Maintained:

Receipt of and/or preparation and storage of L5178Y/TK⁺ mouse lymphoma cell cultures, S-9, and restrictive medium. Documentation of test substance preparation, preparation of cells, preparation of S-9 mix, dosing, washing, cell count, cloning, colony counts, and sizing. Include any other records that would be required to reconstruct the study and demonstrate adherence to protocol.

PROTOCOL NO. C29 (Cont'd)

Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK Mouse Lymphoma Assay

Issue Date: March 1, 1982

<u>Test Substance(s)</u>	<u>DRD</u>	<u>Description</u>		<u>Expiration</u>
<u>TSIN #</u>	<u>Number</u>	<u>Color</u>	<u>Physical Form</u>	<u>Date</u>
G0539.01	BYCR0392	White	Powder	12/7/85

Storage Conditions: (Check one)

- Room temp. Refrigerator Freezer
 Other Ambient (50°-90°F)

Hazards: (Check one)

- None known. Take ordinary precautions in handling.
 As follows: Irritant.

Special Instructions: (Check one)

- None
 As follows: Avoid undue contact with the skin and eye contact. Flush with water.

Dose Preparation:

Vehicles in order of preference

- [1] Water or F_{OP}*
 DMSO
 [2] EtOH — use EtOH ^{car} _{wholes-}
 [3] Acetone
 Other

Solubility Water = [<] 1%, Ethanol & Acetone = [>] 10%

Hand
 4/2/85

Unless the solubility properties of the test substance are provided by the Sponsor or the solubility properties are available from another source, a suitable solvent must be found for the test substance prior to testing using the Standard Operating Procedures of the Test Facility.

*See Appendix 1 for abbreviations and glossary of terms.

PROTOCOL NO. C29 (Cont'd)Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK⁺ Mouse Lymphoma Assay

Issue Date: March 1, 1982

Dose Preparation
(Cont'd):

The preferred solvents, in order of preference, are water (or F_{OP}), dimethyl-sulfoxide, ethanol, and acetone. Any other solvent which shows no toxic effect to the L5178Y cells and no significant increase in background mutation frequency at the levels used is acceptable subject to approval by the Sponsor.

Chemicals:

Positive controls and other chemicals to be used for testing will be purchased from a commercial source or obtained from the Sponsor. Chemicals are stored according to the recommendations of the commercial supplier or Sponsor. After completion of the assay, unused commercially obtained chemicals may be saved for future use. Excess chemicals obtained from a Sponsor, however, will be either returned or discarded at the discretion of the Sponsor.

Dosage Level:

All solutions of the test substance are prepared fresh on the day of the test. Doses are chosen on the basis of the toxicity test described in Toxicity Test Section. A complete mutagenicity assay consists of at least five cloned doses of the test substance (see mutagenicity test below for criteria used for selection of doses to be cloned) and a solvent control all tested with and without activation, a positive control of ethyl methanesulfonate (EMS), a mutagen that does not require activation, and a positive control either of 2-acetylaminofluorene (2-AAF), 7,12 dimethyl benzanthracene (DMBA), or benzo(a)pyrene (Bap), mutagens that require metabolic activation with an S-9 fraction obtained from the livers of rodents induced with a chemical such as Aroclor. In some special cases the S-9 fraction used will be obtained from the livers of uninduced rodents. In these cases, the positive control used will be dimethylnitrosamine (DMN). All dosing solutions will be prepared as 100X concentrated stock solutions.

[] Other, specify:

Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK⁺ Mouse Lymphoma Assay

Issue Date: March 1, 1982

Dosage Level (Cont'd): Note

A concentration analysis of the test substance - vehicle mixture(s) will ~~xxx~~; will not [] be required.

If a concentration analysis is required:

~~xxx~~ Prepare a sufficient quantity of the most concentrated test substance - vehicle mixture(s) so that a portion can be returned to the Sponsor's Divisional Toxicologist.

Shipping Instructions

Send approximately 50-100ml. Send [] frozen; ~~xxx~~ under ambient conditions; [] other _____

[] Analyze the test substance - vehicle mixture(s) for test substance concentration using the analytical method in Appendix _____.

Test System

Identification:

Individual cultures and cloning plates are to be identified according to the Standard Operating Procedures of the Test Facility.

Test System:

L5178Y/TK⁺ clone 3.7.2C mouse lymphoma cells obtained from D. Clive, Research Triangle Park, N.C., Burroughs-Wellcome Co.

Test System Storage:

Frozen stocks of the L5178Y clone 3.7.2C cells are prepared and maintained in a liquid nitrogen freezer according to the Standard Operating Procedures of the Test Facility.

Methods:

Cell Line

The TK⁺ clone 3.7.2C L5178Y cell line is maintained as growing suspension cultures according to the Standard Operating Procedures of the Test Facility. The medium used is Fischer's Medium for Leukemic Cells of Mice containing approximately 10% (v/v) horse serum and supplemented according to the Standard Operating Procedures of the Test Facility. Medium may be obtained from a suitable commercial supplier as a powder, or 1X or 10X liquid.

PROTOCOL NO. C29 (Cont'd)Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK⁺ Mouse Lymphoma Assay

Issue Date: March 1, 1982

Methods (Cont'd):Cell Line (Cont'd)

Cells are periodically cleansed free of spontaneous TK^{-/-} mutants by treatment of stock cultures with THMG according to the Standard Operating Procedures of the Test Facility. TK^{-/-} cells are sensitive to the toxic effects of methotrexate in the presence of exogenous thymidine, hypoxanthine and glycine (THMG treatment). TK^{+/-} cells, however, are not killed by THMG treatment since they can use the exogenous thymidine, hypoxanthine, and glycine (THG) to overcome the block to folate metabolism imposed by the methotrexate. Cultures used for the assay are cleansed within the two week period prior to initiation of the project.

Preparation of the Microsomal Enzyme (S-9)
Metabolic Activation SystemNon-Induced S-9 Fraction

A liver microsomal enzyme (S-9) activation system is employed in this assay to detect promutagens.^{2,4} S-9 is prepared by the homogenization of minced livers from commercially obtained male, Sprague-Dawley rats (200-275 gms) according to the Standard Operating Procedures of the Test Facility. The liver is homogenized in a mixture consisting of one part liver in gms wet weight to three parts solvent in ml. (either 0.15N KCl or 0.25 M sucrose according to the Standard Operating Procedures of the Test Facility).

Aliquots of the S-9 are stored frozen below -70°C until used.

Induced S-9 Fraction

Induced S-9 fraction is prepared from rats given a single intraperitoneal injection of a polychlorinated biphenyl (Aroclor) in corn oil five days prior to sacrifice. The standard dose of Aroclor is 500 mg/kg body weight. The Aroclor used for injection may be either a 2:1 mixture of Aroclor 1242:Aroclor 1254 or Aroclor 1254 alone according to the Standard Operating Procedures of the Test Facility.

Toxicity Test:

In addition to limitations imposed by the solubility of a substance, the levels at which it can be tested for mutagenicity are determined by its toxic effect on L5178Y cells. As a result the toxicity of a compound is first tested over a wide range of concentrations.

PROTOCOL NO. C29 (Cont'd)

Chemical Induction of Mutation
In Mammalian Cells in Culture
Line L5178Y TK⁺ Mouse Lymphoma Assay

Issue Date: March 1, 1982

Toxicity Test (Cont'd): Toxicity is measured by the ability of a given dose of substance to inhibit the suspension growth of treated cultures. The method and length of exposure of cells to chemical and incubation conditions are similar to those used in the Mutagenicity Test Section. The exact procedure is conducted according to the Standard Operating Procedures of the Test Facility.

From the information obtained in the toxicity test, the doses of substance to be tested in the mutagenicity test are determined. Based on the results of the toxicity test, the highest dose of test chemical to be used in the mutagenicity test is chosen to give substantial or complete toxicity relative to the solvent control. Within the limits of predictability of the toxicity test, subsequent doses are chosen to span the range of relative toxicity to a level where little or no relative toxic effect is observed.

Mutagenicity Test: S-9 Mix (Metabolic Activation System)

Prior to dosing the cells S-9 mix will be prepared by combining S-9 fraction with a neutralized solution of NADP and sodium isocitrate. The final concentrations of each component in the cultures during treatment are 100 μ l/ml S-9, 2.4 mg/ml NADP, and 4.5 mg/ml sodium isocitrate in F_{Op} . Alternatively, the amount of any given batch of S-9 liver fraction used may be determined from its protein concentration, or the amount required to elicit a standard response in a mutagenicity test with a positive control chemical according to the Standard Operating Procedures of the Test Facility. The S-9 mix will be prepared shortly before use from freshly thawed S-9 fraction. Unused portions should be discarded at the end of the day.

Dosing, Expression Growth and Cloning of Cells

Each sample will be prepared for dosing by transferring 6 ml L5178Y/TK⁺ clone 3.7.2C cells at a concentration of 1×10^6 cells/ml to a labeled, sterile 50 ml centrifuge tube or T25 tissue culture flask. Four ml of F_{Op} are then added to each sample to be tested without metabolic activation, and 4 ml of the S-9 mix to each sample to be tested with metabolic activation. Each sample is mixed gently and 0.1 ml of the 100X concentrated dose of test substance, solvent, or positive control chemical is added. Any major change in pH (by color change of phenol red in culture medium) caused by addition of the test substance is noted, and

Test for Chemical Induction of Mutation
In Mammalian Cells in Culture
the L5178Y TK⁻ Mouse Lymphoma Assay

Issue Date: March 1, 1982

Mutagenicity Test
(Cont'd):

Dosing, Expression Growth and Cloning of Cells (Cont'd)

the pH is quickly brought back to approximate neutrality (normal red-orange color of medium) by dropwise addition of 0.5-1.5N NaOH or HCl. Each sample vessel is then gassed with 5% CO₂-in-air, sealed and incubated at 37 ± 0.5°C on a roller drum (centrifuge tube) or gyrotory shaker (tissue culture flask) for four hours. The cell samples are then centrifuged, the supernatant discarded and the cells washed twice with fresh F_{10p}. The cells are then resuspended in F_{10p} at a concentration of approximately 3 X 10⁵ cells/ml, based on the original cell number of 6 X 10⁶ per culture prior to treatment with chemical, and all samples incubated as described above for a two or three day expression period according to the Standard Operating Procedures of the Test Facility. During the expression period, the cell concentration is determined daily and all cultures are diluted to 3 X 10⁵ cells/ml, if necessary, in order to keep the cells in an active state of growth.

At the end of the expression period, doses are chosen for cloning based on the relative toxicity shown during the expression period. In general, dose levels which exhibit from 10 to 90% relative growth inhibition during the expression period are chosen for cloning. However, if that level of toxicity is not achieved within the solubility limits of the compound, then dose(s) showing less than 10% inhibition may be cloned. Dose levels showing greater than 90% growth inhibition will not be cloned. A portion of each culture is centrifuged and resuspended in F_{10p}. The appropriate dilutions are then made and a portion of each sample is plated on petri dishes in soft agar medium with and without the selective agent, TFT according to the Standard Operating Procedures of the Test Facility. Three dishes of each sample at 1 x 10⁶ cells/plate are prepared in TFT medium. Three dishes of each sample at an estimated cell number from 100-200 cells/plate are prepared in cloning medium without selective agent. All petri dishes are then incubated at 37.0 ± 0.5°C for 10-14 days to allow colonies to form from individual cells. At the end of this time, the number of colonies on each plate is counted. The number of viable cells (survivors) originally placed on the plates containing the TFT medium is determined from the number of colonies in dishes containing the non-selective medium. The number of TK⁻ mutants is determined from the number of colonies in dishes containing the TFT medium.

PROTOCOL NO. C29 (Cont'd)

Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK Mouse Lymphoma Assay

Issue Date: March 1, 1982

Protocol Changes:

If it becomes necessary to change the approved protocol, verbal agreement to make this change should be made between the Study Director and the Sponsor. As soon as practical, this change and the reasons for it should be put in writing and signed by both the Study Director and the Sponsor's Divisional Toxicologist. This document is then attached to the protocol as an addendum.

Results:

The raw data are reported for each negative and positive control and each dose of substance. Raw data consist of dose preparation information, the daily cell concentrations, the number of viable, colony-forming cells on each petri dish containing non-selective medium, and the number of TFT-resistant colony-forming cells on each dish. A mutation frequency (the number of TFT-resistant colony-forming cells per unit survivor) and the fold increase in mutation frequency relative to the solvent control is determined for each sample. The induced mutation frequency, the mutation frequency of each sample minus the spontaneous mutation frequency shown in the solvent controls may also be determined. In addition to the mutation frequencies, the percent survival relative to the control is reported for each sample for both the expression period growth in suspension and the overall growth (the relative suspension growth corrected for viability as determined by the plating efficiency in non-selective medium).

Results of each test are considered independently, but in order to be considered a valid test, the spontaneous mutation frequencies observed for the negative controls should be no higher than 150 mutants per 10^6 survivors. In addition, the mutation frequencies observed for the positive controls should fall within acceptable ranges as determined by a developing historical base.

Report:

Final Report

A report of the results will be prepared for this study by the contract laboratory within 30 days from the completion of the study. The report will include, but not be limited to, the following:

1. Name and address of the facility performing the study and the dates on which the study was initiated and completed.
2. Objectives as stated in the approved protocol, and any changes to the original protocol.

PROTOCOL NO. 329 (Cont'd)

Test for Chemical Induction of Mutation
in Mammalian Cells in Culture
the L5178Y TK⁺ Mouse Lymphoma Assay

Issue Date: March 1, 1982

Report (Cont'd):

Final Report (Cont'd)

3. A detailed description of all methods used.
4. Statistical methods employed for analysis of the data, if any.
5. Deviations from the Test Facility's Standard Operating Procedures or the approved protocol.
6. A summary of the results as they relate to the study's objective.
7. The location where all raw data will be stored.
8. The daily cell concentrations, all colony counts, and results of calculations.

This report shall conform to all requirements outlined in Section 58.185, Subpart J, Good Laboratory Practices Regulations.

Sponsor: James E. Weaver *James E. Weaver*
Divisional Toxicologist

Date Approved by Sponsor's Divisional Toxicologist 3-7-85
5 Feb 6/24/85

Proposed Starting Date: April 24, 1985)

Defined as Initial Toxicity Test)

Proposed Completion Date: June 7, 1985)

Defined as Submission of Final Report) To be completed
) by the Test
) Facility

Study Director: Ante V. Boyer-Bach)

Date: April 22, 1985)

Study Cost: \$6,500.00)

Abbreviations and Glossary of Terms:

1. 2-AAF - 2-acetylaminofluorene
2. BUdR - bromodeoxyuridine
3. Cloning medium- Fischer's Medium for Leukemic Cells of Mice supplemented as described below for F_{OP} and with approximately 20% (v/v) horse serum and 0.32-0.37% noble agar according to the Standard Operating Procedures of the Test Facility
4. DMN - dimethylnitrosamine
5. DMSO - dimethylsulfoxide
6. EMS - ethyl methanesulfonate
7. F_{OP} - Fischer's Medium for Leukemic Cells of Mice supplemented with sodium pyruvate, Pluronic F68, and penicillin-streptomycin according to the Standard Operating Procedures of the Test Facility
8. F_{10P} - F_{OP} plus approximately 10% (v/v) horse serum
9. Gassing - Replacement of the air in a culture vessel with 5% CO₂-in-air by purging with CO₂-air mixture
10. NADP - β-nicotinamide adenine dinucleotide phosphate
11. Selective cloning medium - Cloning medium containing TFT according to the Standard Operating Procedures of the Test Facility
12. S-9 - The supernatant obtained by centrifugation of a homogenate of liver at 9000 X g according to the Standard Operating Procedures of the Test Facility
13. TFT - trifluorothymidine
14. TK - thymidine kinase

REFERENCES

- ¹Clive, D. and J. F. S. Spector. Laboratory procedure for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells. *Mutation Res.*, 31: 17-29 (1975).
- ²Clive, D., K. O. Johnson, J. F. S. Spector, A. G. Batson, and M. M. M. Validation and characterization of the L5178Y/TK^{+/-} mouse lymphoma mutagen assay system. *Mutation Res.* 59: 61-108 (1979).
- ³Clive, D., W. G. Flamm, and J. B. Patterson. Specific locus mutational assay systems for mouse lymphoma cells. In A. Hollaender (ed.), *Chemical Mutagens: Principles and Methods for their Detection*. Volume 3, Plenum Press, New York, 1973, pp. 79-103.
- ⁴Ames, B. N., W. E. Durston, E. Yamasaki, and F. D. Lee. Carcinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. *Proc. Nat. Acad. Sci. USA* 70: 2281-2285 (1973).

APPENDIX II

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2982
 Trial No. : 1
 Test Antigen : Test Article
 Activation: Non-activated
 TFI/V.C. : TFT
 Solvent :

Operator : Carl M. Hay
 Date : 06-03-85
 Time : 10:40 am
 Cult Unit: µg/ml
 Subsets : 1
 Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
4.2		86	90	90	89
2.4		81	75	94	84
1.8		92	96	81	90
1.3		97	64	60	88
1		89	71	81	80
.75		62	70	79	71
.56		88	90	90	89
.42		98	90	75	88
.24		103	102	117	107
Solvent	1	44	51	56	50
Solvent	2	52	57	44	51
.32		+	+	+	

+ = culture lost.

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2962 Operator: Carl M. Hay
 Trial No.: 1 Date: 06/03/85
 Test Art/+: Test Article Time: 10:59 am
 Activation: Non-activated Cult Unit: pg/ml
 TFT/V.C.: TFT Subsets: 1
 Solvent: Ethanol Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
4.2		86	90	90	89
2.4		81	95	94	90
1.8		92	96	81	90
1.3		97	84	83	88
1		89	71	81	80
.75		62	73	79	71
.56		88	90	90	89
.42		98	90	75	88
.32		+	-	+	
.24		103	102	117	107
Solvent	1	44	51	56	50
Solvent	2	52	57	44	51

+ = culture lost

Carl M. Hay
6-3-85

MOUSE LYMPHOMA

Cloning Data

Audit Trail

V 1.4

Experiment: LRBC
 Trial No. : 1
 Test Ant/+ : EMS
 Activation: Non-activated
 TFT/V.C. : TFT
 Solvent :

Operator : Carl M. Hay
 Date : 06/03/85
 Time : 11:01 am
 Cult Unit: pl/ml
 Subsets : 1
 Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
.5		303	360	300	331
Solvent	1	56	54	49	53
Solvent	2	52	47	51	50

Carl M. Hay
 6-3-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 0982 Operator : Carl M. Hay
Trial No. : 1 Date : 06.03.85
Tst Art/+ : EMS Time : 2:08 pm
Activation: Non-activated Cult Unit: pl/ml
TFT/V.C. : TFT Subsets : 1
Solvent : Dimethylsulfoxide Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
------------	----------------	----------	----------	----------	---------------

1		++	++	++	
---	--	----	----	----	--

++ too toxic to clone

Carl M. Hay

6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2682
 Trial No. : 1
 Tst Ant/+: EMS
 Activation: Non-activated
 TFT/V.C. : TFT
 Solvent : Dimethylsulfoxide
 Operator : Carl M. Hay
 Date : 06/03/85
 Time : 11:00 am
 Cult Unit: pl/ml
 Subsets : 1
 Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		++	++	++	
.5		300	300	300	301
Solvent	1	50	54	49	51
Solvent	2	52	47	51	50

++ too toxic to clone

Carl M. Hay
6-3-85

MOUSE LYMFHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2982	Operator: Carl M. Hay
Trial No.: 1	Date: 06/03/85
Test Art/+: Test Article	Time: 11:07 am
Activation: Induced Rat Liver S-9	Cult Unit: pc/ml
TFT/V.C.: TFT	Subsets: 1
Solvent:	Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
13		62	61	63	62
10		77	84	87	83
7.5		81	56	75	71
5.6		90	71	71	78
4.2		93	88	96	93
3.2		81	84	73	79
2.4		84	71	70	75
1.8		+	76	77	76
1.3		47	59	62	56
Solvent	1	60	61	58	60
Solvent	2	76	77	87	80

+ = culture lost

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2952 Operator: Carl M. Hay
 Trial No.: 1 Date: 06/03/85
 Test Art/+: Test Article Time: 11:20 am
 Activation: Induced Rat Liver S-9 Cult Unit: µg/ml
 TFT/V.C.: TFT Subsets: 1
 Solvent: Ethanol Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
13		62	61	63	62
10		77	84	87	83
7.5		81	56	75	71
5.6		96	71	71	79
4.2		93	88	96	93
3.2		81	84	73	79
2.4		84	71	70	75
1.8		+	78	77	78
1.3		47	59	62	56
Solvent	1	60	61	56	60
Solvent	2	76	77	97	80

+ = culture lost

Carl M. Hay

6-3-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2980	Operator : Carl M. Hay
Trial No. : 1	Date : 06/03/85
Tet Ant/+ : 7.12 DNEA	Time : 11:23 am
Activation: Induced Rat Liver S-9	Cult Unit: µg/ml
TFT/V.C. : TFT	Subsets : 1
Solvent :	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
7.5		267	235	238	247
S		207	197	174	200
Solvent	1	70	85	86	80
Solvent	2	63	79	65	69

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2981	Operator : Carl M. Hay
Trial No. : 1	Date : 06/03/85
Tst. Ant.+ : 7.12 DMBA	Time : 11:30 am
Activation: Induced Rat Liver S-9	Cult Unit: µg/ml
TFT/V.C. : TFT	Subsets : 1
Solvent : Acetone	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
7.5		267	235	236	247
5		207	199	194	200
Solvent	1	70	85	86	80
Solvent	2	63	75	65	69

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2F82
Trial No. : 1
Test Art/+ : Test Article
Activation: Non-activated
TFT/V.C. : V.C.
Solvent :

Operator : Carl M. Hay
Date : 06/03/85
Time : 11:34 am
Cult Unit: µg/ml
Subsets : 1
Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
4.2		21	15	21	19
2.4		44	44	52	47
1.6		57	43	55	52
1.3		58	43	59	53
1		56	43	38	46
.75		58	46	44	49
.56		62	61	47	57
.42		72	71	66	70
.32		+	+	+	
.24		93	95	100	96
Solvent	1	166	180	165	170
Solvent	2	174	165	163	167

+ = culture test

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2782
Trial No. : 1
Test Art/+ : Test Article
Activation: Non-activated
TFT/V.C. : V.C.
Solvent : Ethanol

Operator : Carl M. Hay
Date : 06/03/85
Time : 11:50 am
Cult Unit: µg/ml
Subsets : 1
Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
4.2		21	15	21	19
2.4		44	44	52	47
1.8		57	43	55	52
1.3		56	43	59	53
1		56	43	56	46
.75		58	46	44	49
.56		62	61	47	57
.42		72	71	66	70
.32		+	+	+	
.24		93	95	100	96
Solvent	1	166	180	165	170
Solvent	2	174	165	163	167

+ = culture lost

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2982	Operator : Carl M. Hay
Trial No. : 1	Date : 06/03/85
Tst Ant/+: EMS	Time : 11:53 am
Activation: Non-activated	Cult Unit: pl/ml
TFT/V.C. : V.C.	Subsets : 1
Solvent :	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
.5		72	74	62	78
Solvent	1	156	167	165	176
Solvent	2	162	135	143	147

Carl M. Hay
6-3-85

MOUSE LYMPHOMA

Cloning Data

Audit Trail

V 1.4

Experiment: 2532
Trial No. : 1
Test Art/rt : EMS
Activation: Non-activated
TFT/V.C. : V.C.
Solvent : Dimethylsulfoxide

Operator : Carl M. Hay
Date : 06/01/85
Time : 2:11 pm
Cult Unit: pl. ml
Subsets : 1
Readings : 7

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		++	++	++	

++ too toxic to clone

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 1982
 Trial No. : 1
 Test Ant. + : EMB
 Activation: Non-activated
 TTT, V.C. : V.C.
 Solvent : Dimethylsulfoxide

Operator : Carl M. Hay
 Date : 6-3-85
 Time : 11:30 am
 Cult. Unit: plasm
 Substrate :
 Readings : 5

Culture ID	Culture Subsect	Count #1	Count #2	Count #3	Average Count
1		++	++	++	
.5		72	79	82	78
Solvent	1	156	167	168	175
Solvent	2	162	135	140	147

++ too toxic to clone

Carl M. Hay

6-3-85

MOUSE LYMPHOMA

Cloning Data

Audit Trail

V 1.4

Experiment: 2982	Operator : Carl M. Hay
Trial No. : 1	Date : 06/03/85
Test Article : Test Article	Time : 11:58 am
Activation: Induced Fat Liver 8-9	Cult Unit: µg/ml
TPT/V.C. : V.C.	Subsets : 1
Solvent :	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
13		90	106	97	94
10		105	106	98	103
7.5		111	118	111	113
5.6		115	130	128	124
4.2		136	137	152	142
3.2		138	136	133	136
2.4		140	142	131	138
1.8		151	159	161	157
1.3		127	116	113	119
Solvent	1	167	157	154	159
Solvent	2	141	164	142	149

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2962	Operator : Carl M. Hay
Total No. : 1	Date : 06 03/85
Test Art/+ : Test Article	Time : 12:11 pm
Activation: Induced Rat Liver S-9	Cult Unit: ug/ml
TFT/V.C. : V.C.	Subsets : 1
Solvent : Ethanol	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
17		89	106	97	94
10		105	106	98	103
7.5		111	118	111	113
5.6		115	130	126	124
4.2		136	137	152	142
3.2		138	136	133	136
2.4		140	142	131	138
1.8		151	159	161	157
1.3		127	116	113	119
Solvent	1	167	157	154	159
Solvent	2	141	164	142	149

Carl M. Hay
6-3-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2982	Operator : Carl M. Herz
Trial No. : 1	Date : 06/03/85
Test Art. + : 7.12 DMBA	Time : 12:14 pm
Activation: Induced Rat Liver S-9	Cult Unit: µg/ml
TFT/V.C. : V.C.	Subsets : 1
Solvent :	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
7.5		99	106	127	111
5		117	130	127	125
Solvent	1	166	163	146	160
Solvent	2	153	150	156	153

Carl M. Herz
6-3-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2582	Operator : Carl M. Hay
Trial No. : 1	Date : 06-03/85
Tst Ant/4 : 7,12 DMBA	Time : 12:18 pm
Activations: Induced Rat Liver S-9	Cult Unit: ug/ml
TFT/V.C. : V.C.	Subsets : 1
Solvent : Acetone	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
7.5		99	106	127	111
5		117	130	127	125
Solvent	1	166	168	146	160
Solvent	2	153	150	150	151

Carl M. Hay
6-3-85

APPENDIX III

MOUSE LYMPHOMA

Cloning Data

V 1.4

Audit Trail

Experiment: 1-62	Operator: Carl M. Hay
Trial No.: 2	Date: 6-17-85
Test Art.: Test Article	Time: 10:11 am
Activation: Non-activated	Cult Unit: µg/ml
TFT/V.C.: TFT	Subsets: 1
Solvent:	Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		59	58	70	62
.75		70	94	80	86
.5e		102	109	107	112
.41		105	11e	92	111
.32		10e	115	111	111
.24		184	181	167	184
.18		92	81	101	91
.13		47	52	60	53
.1		35	38	40	38
.075		56	53	40	51
Solvent	1	35	35	47	38
Solvent	2	40	4e	41	41

Carl M. Hay
6-17-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 1351
Trial No.: 2
Test Article: Test Article
Activation: Non-activated
TFT M.C.: TFT
Solvent: Ethanol

Operator: Carl M. Hay
Date: 06-17-85
Time: 10:28 am
Cell Count: 40 ml
Subjects: 1
Residual: 0

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		59	58	70	62
.75		90	94	80	88
.56		132	109	137	126
.42		106	116	91	111
.32		106	115	111	111
.24		184	181	187	184
.18		92	81	101	91
.13		47	52	60	53
.1		38	38	40	39
.075		59	50	40	51
Solvent	1	35	35	43	38
Solvent	2	40	40	41	40

Carl M Hay
6-17-85

MOUSE LYMPHOMA

Cloning Data
Audit Trail

V 1.4

Experiment: 2982
T-tel No. : 2
Tet Ant. + : EMS
Activation: Non-activated
TFT/V.C. : TFT
Solvent :

Operator : Carl M. Hay
Date : 06-17-85
Time : 10:31 am
Cult Unit: p1001
Subsets : 1
Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		143	123	110	125
.5		285	289	302	292
Solvent	1	24	26	27	24
Solvent	2	37	41	27	42

Carl M Hay
6-17-85

MOUSE LYMFHOMA
Cloning Data

V 1.4

Experiment: IP21	Operator: Carl M. Hay
Trial No.: 2	Date: 6/17/85
Test Ant +: EMS	Time: 10:35 am
Activation: Non-activated	Cult Unit: μ l/ml
TFT/V.D.: TFT	Subsets: 1
Solvent: Dimethylsulfoxide	Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		147	127	110	125
.5		285	289	302	292
Solvent	1	24	20	27	24
Solvent	2	37	41	47	42

Carl M. Hay
6-17-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: D781
Trial No.: 1
Test Article: Test Article
Activation: Non-activated
TFT/V.C.: V.C.
Solvent:

Operator: Carl M. Han
Date: 06/17/85
Time: 10:29 am
Cult Unit: µg/ml
Subsets: 1
Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		20	25	37	27
.75		46	54	61	54
.56		57	55	49	54
.42		58	66	57	61
.32		64	56	61	60
.24		56	71	59	62
.18		114	111	113	113
.17		116	123	117	119
.1		100	115	100	105
.075		101	101	127	110
Solvent	1	108	112	109	110
Solvent	2	+	100	101	101

+ = culture lost

Carl M. Han
6-17-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 2982	Operator : Carl M. Hay
Trial No. : 2	Date : 06/17/85
Test Art. + : Test Article	Time : 10:55 am
Activation: Non-activated	Cult Unit: µg. ml
TFT-V.C. : V.C.	Subsets : 3
Solvent : Ethanol	Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		20	25	37	27
.75		48	54	61	54
.56		57	55	49	54
.42		59	60	57	61
.32		64	56	61	60
.24		56	71	59	62
.16		114	111	113	113
.13		118	123	117	119
.1		100	115	100	105
.075		101	101	127	110
Solvent	1	108	112	109	110
Solvent	2	+	100	101	101

+ = culture test

Carl M. Hay
6-17-85

MOUSE LYMPHOMA
Cloning Data
Audit Trail

V 1.4

Experiment: 2982
 Trial No. : 1
 Test Agent : EMS
 Activation: Non-activated
 TFI V.C. : V.C.
 Solvent :

Operator : Carl M. Hay
 Date : 6-17-85
 Time : 10:56 am
 Cult Unit: μ l/ml
 Subsets : 1
 Readings : 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		3	10	9	7
.5		58	56	63	59
Solvent	1	92	105	100	99
Solvent	2	106	106	102	105

Carl M. Hay
6-17-85

MOUSE LYMPHOMA
Cloning Data

V 1.4

Experiment: 1982	Operator: Carl M. Hay
Trial No.: 1	Date: 06/17/85
Tet Ant/+: EMS	Time: 11:00 am
Activation: Non-activated	Cult Unit: μ l/ml
TFT V.C.: V.C.	Subsets: 1
Solvent: Dimethylsulfoxide	Readings: 3

Culture ID	Culture Subset	Count #1	Count #2	Count #3	Average Count
1		3	10	9	7
.5		58	56	63	59
Solvent	1	92	105	100	99
Solvent	2	106	108	102	105

Carl M. Hay
6-17-85



THE PROCTER & GAMBLE COMPANY

MIAMI VALLEY LABORATORIES

P. O. BOX 39175
CINCINNATI, OHIO 45247

April 11, 1985

Dr. Steve Haworth
Microbiological Associates, Inc.
5221 River Road
Bethesda, MD 20016

Dear Dr. Haworth:

This is to authorize you to carry out the following study according to the attached protocol, and in conformance with the stipulations of our current Laboratory Services Agreement.

Notice: 1) This study is expected to be submitted to the following regulatory agency: FDA. The study should be listed on the Test Facility's Master list of regulated studies. The stipulations of the protocol are to be implemented in complete conformance with Good Laboratory Practices Regulations (21 CFR, Part 58) for nonclinical laboratory studies with the following exceptions:

If two or more test substances appear on the protocol, it may be conducted as a single study, resulting in a single final report.

2) Quality Assurance Inspections:

The final report will be inspected by the Test Facility's QAU. The Test Facility's Standard Operating Procedure for randomly inspecting all operations should be used to assure study validity and sufficient data should be made a part of each report to allow the Sponsor to check the reported results against the raw data.

3) Documentation of the derivation, characterization, and stability testing of the test substance(s) will be the responsibility of the Sponsor.

Test: Mouse Lymphoma Assay
Protocol No.: C29
Test Substance No.: G0539.01
Physical Form: Powder

Issue Date: March 1, 1982
Doc. Req. No.: BYCR 0392

Three copies of the final report are needed as soon as possible, and are to be sent to my attention at the above address.

Dr. Steve Haworth
Microbiological Associates, Inc.
April 11, 1985
Page 2

Matters involving the scientific aspects of the work can be handled directly with the Sponsor's Divisional Toxicologist or E. D. Thompson. All unused samples are to be returned to the Divisional Toxicologist at the following address (the cost of shipment should be included in the study cost):

Mr. J. E. Weaver Telephone No. (513) 530-2302
The Procter & Gamble Company
Sharon Woods Technical Center
11511 Reed Hartman Hwy. - Room HB-2D31
Cincinnati, OH 45241

Complete both copies of the attached protocol by adding your study number, proposed start and completion dates, and have the Study Director sign and date them. The Study Director should define the start and completion dates on the protocol. Retain one copy and return one copy (which includes the study cost) to me along with a letter stating that you agree to do the work specified in the attached protocol. In addition, if you cannot meet the report dates, please let me know.

An invoice for 80% of the amount should be sent to:

Mr. R. T. Lyons
The Procter & Gamble Company
11511 Reed Hartman Highway - Room No. HB-2D31
Cincinnati, OH 45241

An invoice for 20% of the amount should be sent to:

Mr. Detlef Müller
The Procter & Gamble Company
European Technical Center
Temselaan 100, B 1820-Grimbergen
(Strombeek-Bever)
BELGIUM

Sincerely,

THE PROCTER & GAMBLE COMPANY
Research & Development Department



H. A. Derner
Human & Environmental Safety Division

Approved:



G. S. Hassing, Ph.D
Director, Human & Environmental Safety Division

bg

Attachments

cc: Study File

J. E. Weaver

E. D. Thompson

D. Müller

TEST SUBSTANCE CHARACTERIZATION REPORT
(TSCR)

For Tox Office
Use Only:
DRD # B4420392
TSIN # 60539.01

1. Characterization, Microbial and Properties Information:

	<u>Date Submitted</u>	<u>Submitter Code (if exists) or Lab Notebook #</u>	<u>Component or Property</u>	<u>(✓)</u>	<u>Measured Value</u>	<u>Limits</u>	<u>Testing Lab or Data Source</u>
1	<u>2/27/85</u>	<u>JDM 0108</u>	<u>MCT</u>	<u>✓</u>	<u>Pass</u>	<u>Must Pass</u>	<u>Micro</u>
2	<u>11/8/84</u>	<u>HC-0173-46</u>	<u>% Octopirox</u>	<u>_____</u>	<u>100.26%</u>	<u>98% Min.</u>	<u>1B21</u>
3	<u>12/05/84</u>	<u>84312004</u>	<u>Assay</u>	<u>_____</u>	<u>99.4</u>	<u>97% Min.</u>	<u>Analytical</u>
4	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
5	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
6	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
7	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
8	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
9	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
10	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
11	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
12	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
13	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
14	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>
15	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>	<u>_____</u>

12. Approvals:

The test substance as made and characterized is a representative example of the intended formulation. Making records for plant-made product should be obtained and evaluated by Products Research.

a. Process Development: *JDM* (Signature) John Mellison (Name) 3/14/85 (Date)

b. Products Research: *T. Johnson* (Signature) _____ (Name) 2/27/85 (Date)

_____ finished product samples will be retained by Quality Assurance.
samples

c. GMP-Quality Assur.: *T. Johnson* (Signature) T. Johnson (Name) 3/4/85 (Date)

13. The characterization tests requested are appropriate and the test substance is acceptable for: acute animal test; subchronic animal test; chronic animal test; human safety test; in vitro test; environmental safety test.

James E. Cooper (Toxicologist's Signature) _____ (Name) 3/25/85 (Date)

TSCR Distribution: Original - Tox Office; Copies - Toxicologist, GMP/QA, Products REsearch and Process Dev.

SUBJECT NONCLINICAL STUDY - REGULATORY
STATUS

ATTENTION

Notifications pertaining to:

DED # BYCRO392
TSIN G0539.01

1. Studies requested on the above document:

- are expected to be submitted to the following regulatory agencies as a GLP regulated study: FDA

- are expected to be submitted to the following regulatory agencies but is not a GLP regulated study: _____

Metabolism, Pharmacological Screen, Other: _____

- are not expected to be submitted to a regulatory agency. (Boxes #3 and #4 below need not be checked).

2. - The test substance has been characterized and results are shown on the test substance characterization report which accompanies the DED.
3. - The method of synthesis fabrication or derivation of the test related substances has been documented. (Required for regulated studies).
4. - Stability testing has been done or will be done on the test substance. (Required for regulated studies).

Sponsor's Divisional Toxicologist: JAMES E. WEAVER

Date: 3/7/85

lg:KIQAUZ

**** RESTRICTED DATA ****

[View Report](#)

WTDS STUDY SUMMARY

<i>TSIN</i>	<i>Test Material Name</i>
G0539.01	OCTOPIROX
<i>WTDS Accession Number:</i> 30869 <i>Archive Location:</i> 1294 and WAC0031n	
<i>DRD:</i> BYCR 0392	
<i>Sector:</i> Beauty Care	
<i>Category:</i> Unavailable	
<i>Site:</i> Unavailable	
<i>Division:</i> BEAUTY CARE PRODUCT DIVISION	
<i>Toxicologist:</i> WEAVER, J.E.	
<i>Test Type:</i> MOUSE LYMPHOMA ASSAY	
<i>Test System:</i> L5178Y MOUSE LYMPHOMA CELLS	
<i>TSCA 8(e) Status:</i> A -- REVIEWED - NO REPORTABLE INFORMATION	

<i>Study Title:</i> MOUSE LYMPHOMA ASSAY	
<i>Lab Study Number:</i> T2982-701	<i>Report Date:</i> 06/25/1985
<i>Study Dates:</i>	
<i>Performing Laboratory:</i> MICROBIOLOGICAL ASSOCIATES	
<i>Text Summary:</i>	G0539.01 IS NOT A MUTAGEN WHEN TESTED IN THIS BIOASSAY WITH METABOLIC ACTIVATION. WITHOUT ACTIVATION, THIS MATERIAL PRODUCED SUBSTANTIAL TOXICITY SUCH THAT ONLY THE RESULTS OF THE LOWEST DOSE FULFILLED THE CRITERIA FOR A VALID ASSAY. AT THIS LOWEST DOSE, THE TEST MATERIAL WAS POSITIVE. THE POSSIBILITY THAT DIFFERENTIAL TOXICITY WAS RESPONSIBLE FOR THIS APPARENT POSITIVE MUTAGENIC EFFECT IS BEING CONSIDERED.

<i>TSIN:</i>	G0539.01
<i>Test Material:</i>	OCTOPIROX

Original summary was prepared as a text document and subsequently reformatted to the tabular summary format.