



BIONETICS

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MUTAGENIC EVALUATION OF

COMPOUND 000056815

GLYCERINE

(71-89)

Mutagenic Evaluation of Compound FDA 71-89

Glycerine

4/30/75

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Kensington, Maryland
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LBI PROJECT # 2468

MUTAGENIC EVALUATION OF

COMPOUND 000056815

GLYCERINE

(71-89)

SUBMITTED TO

FOOD & DRUG ADMINISTRATION
DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
ROCKVILLE, MARYLAND

SUBMITTED BY

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KENSINGTON, MARYLAND

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EVALUATION SUMMARY

Compound 000056815, Glycerine, did not exhibit genetic activity in any of the in vitro microbial assays employed in this evaluation.



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DATE: 04/30/75

SPONSOR: Food and Drug Administration, Contract Number 223-74-2104

SUBJECT: Evaluation of Test Compound 000056815, Glycerine

I. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

II. MATERIALS

A. Test Compound

1. Date Received: August, 1974
2. Description: Clear viscous liquid

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

Yeast Strain: Saccharomyces cerevisiae, strain D4

Bacteria Strains: Salmonella typhimurium, strains:TA-1535
TA-1537
TA-1538

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 μ M
2. Isocitric acid	49 μ M
3. Tris buffer, pH 7.4	28 μ M
4. MgCl ₂	1.7 μ M
5. Tissue homogenate fraction	72 mg



D. Tissue Homogenates and Supernatant

The tissue homogenates and 9,000 x g supernatants were prepared from tissues of the following mammalian species: Mouse-ICR random bred adult males; rat-Sprague-Dawley adult males; and primate-Macaca mulatta adult males.

E. Positive Control Compounds

Table 1 lists chemicals for positive controls in the direct and activation assays.

TABLE 1
POSITIVE CONTROLS USED IN DIRECT AND ACTIVATION ASSAYS

<u>Assay</u>	<u>Chemical</u> ^a	<u>Solvent</u>	<u>Probable Mutagenic Specificity</u>
Non-activation	Ethyl methanesulfonate	Water or saline	BPS
	2-Nitrofluorene	Dimethylsulfoxide ^c	FS
	Quinacrine mustard	Water or saline	FS
Activation	Dimethylnitrosamine	Water or saline	BPS
	2-Acetylaminofluorene	Dimethylsulfoxide ^c	FS

- ^a Concentrations given in the Results Section
^b BPS = base-pair substitution; FS = frameshift
^c Previously shown to be non-mutagenic

III. METHODS

A. Toxicity

The solubility, toxicity and doses for all chemicals were determined prior to screening.

Each chemical was tested for survival against the specific indicator strains over a range of doses to determine the 50% survival dose. Bacteria were tested in phosphate buffer, pH 7.4, for one hour at 37°C on a shaker. Yeasts were tested in phosphate buffer, pH 7.4, for four hours at 30°C on a shaker. The 50% survival curve and the 1/4 and 1/2 50% doses calculated.

If no toxicity was obtained for a chemical with a given strain, then a maximum dose of 5% (w/v) was used against the strain.

Unless otherwise specified, the doses calculated for the tests in buffer were applied to the activation tests. The solubility of the test chemical under treatment conditions is stated in the Results Section.

B. Plate Tests

In the nonactivation procedure, approximately 10^9 cells of a log-phase culture of the bacterial indicator strains were spread over the surface of a minimal plate, and a measured amount of the test chemical was placed in the center of the test plate. In activation tests, the test chemical was added to the cells, and an aliquot of the mixture was spread on the surface of the test plate. The reaction mixture (0.1 ml) plus tissue extract was then spotted on the surface of the plate. Positive and solvent controls were included. All plates were incubated at 37°C for four days and then scored. Each compound (test, positive control and solvent control) was done in duplicate. Concentrations of the positive control compounds are listed in the Results Section.

C. Suspension Tests

1. Non activation

Log-phase bacteria and stationary-phase yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^9 cells/ml and 5×10^7 cells/ml, respectively. This constituted the working stock for tests of a group of test chemicals and their respective controls. Tests were conducted in plastic tissue culture plates. Cells plus appropriate volume(s) of the test chemical were added to the wells to give a final volume of 1.5 ml. The solvent replaced the test chemical in the negative controls. Treatment was at 30°C for four hours for yeast tests and at 37°C for one hour for bacterial tests. All flasks were shaken during treatment. Following treatment, the plates were set on ice. Aliquots of cells were removed, diluted in sterile saline (4°C) and plated on the appropriate complete media. Undiluted samples from flasks containing the bacteria were plated on minimal selective medium in reversion experiments. Samples from a 10^{-1} dilution of treated cells were plated on the selected media for enumeration of gene conversion with strain D4. Bacterial plates were scored after incubation for 48 hours at 37°C . The yeast plates were incubated at 30°C for 3-5 days before scoring.

2. Activation

Bacteria and yeast cells were grown and prepared as described in the non activation tests. Measured amounts of the test and control chemicals plus 0.25 ml of the stock-cell suspension were added to wells of the Linbro plate containing the appropriate tissue fraction and reaction mixture. All flasks (bacteria and yeast) were incubated at 37°C in an oxygen atmosphere with shaking. The treatment times as well as the dilutions, plating procedures and scoring of the plates were the same as described for non activation tests.



D. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities tissues) were killed by cranial blow, decapitated and bled. Organs were immediately dissected from the animal using aseptic techniques and placed in ice-cold 0.25 M sucrose buffered with Tris at pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4°C. The whole organ homogenate obtained from this step was divided into two samples. One sample was frozen at -80°C and the other was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80°C. These two frozen samples were used for the activation studies.

E. Data Recording and Reporting

Following the specified incubation periods all population plates were scored by an automatic colony counter and the results from each plate of a set were recorded, in ink, on data processing forms. All minimal or other types of selective media plates were hand scored and the results recorded along with the respective population data. Other relevant experimental data were recorded on experimental definition forms. For bacteria strains the number of colonies recorded from either the population or selective plates represents that number in 1 ml of test suspension plated. The numbers recorded for the yeast strain D4 represent the number in 0.5 ml of test suspension plated. Data was then processed and printed from a computer program.



IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound:
000056815, Glycerine
2. Test solvent: Saline .
3. Solubility of the test compound under treatment conditions:
Soluble under treatment conditions.
4. Additional comments: Clear viscous liquid

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: 01/28/75
000056815
2. The 50% survival level was determined for bacteria and yeast indicator organisms by conducting survival curves with the test compound at the following concentrations:

Percent Concentration (w/v or v/v)

5.0
0.5
0.05
0.005
0.0005

3. Concentrations of the test compound used in the mutagenicity tests:

<u>Dose</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	2.5	2.5
1/2 50% Survival	2.0	5.0
50% Survival	10.0	10.0
Plate Tests	5.0	--



IV. SUMMARY OF TEST RESULTS

Plate Tests

A. Name or code designation of the test compound: 000056815

B. Test date: 02/14/75

C. Concentration of the test compound: 5.0%

Test	Species	Tissue	TA-1535		TA-1537		TA-1538	
			<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
1. <u>Non-activation</u>								
Solvent Control	---	---	43	78	11	14	26	6
Positive Control ^a	---	---	>10 ³	>10 ³	209	301	47	40
Test Compound	---	---	63	109	18	2	13	10
2. <u>Activation</u>								
Negative Control	---	---	40	90	14	22	14	4
Solvent Control	---	---	27	89	10	33	17	18
Reaction Mixture Control	---	---	47	31	12	34	20	22
Positive Control ^b	Mouse	Liver	>500	>500	83	75	>200	>200
Positive Control		Lung	59	59	10	7	20	10
Positive Control		Testes	42	60	11	10	11	10
Positive Control	Rat	Liver	>300	>300	83	86	>10 ²	>10 ²
Positive Control		Lung	63	77	10	7	19	9
Positive Control		Testes	44	61	10	8	12	9
Positive Control	Monkey	Liver	>100	>100	41	34	>100	93
Positive Control		Lung	71	54	9	8	20	6
Positive Control		Testes	47	61	10	9	13	10
Test Compound	Mouse	Liver	33	29	1	5	6	1
Test Compound		Lung	44	60	2	0	3	0
Test Compound		Testes	25	33	1	2	2	4
Test Compound	Rat	Liver	24	27	2	4	7	1
Test Compound		Lung	47	64	1	2	1	2
Test Compound		Testes	26	33	1	2	1	4
Test Compound	Monkey	Liver	27	33	2	7	6	3
Test Compound		Lung	44	63	1	0	4	0
Test Compound		Testes	32	33	2	2	1	4

a TA-1535 EMS 10 µl/plate
 TA-1537 QM 20 µg/plate
 TA-1538 NF 100 µg/plate

b TA-1535 DMNA 50 µm/plate
 TA-1537 AAF 100 µg/plate
 TA-1538 AAF 100 µg/plate

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	<p>NAN = Non Activation: Solvent Control NAP = Non Activation: Positive Control NA1 = Non Activation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s)</p> <p>A+C = Negative Chemical Control A-C = Activation: Solvent Control ACP = Activation: Positive Control ACT = Activation: Test Compound A+T = Activation: Tissue Control</p> <p>LI = Liver Tissue Activation Fraction LU = Lung Tissue Activation Fraction KI = Kidney Tissue Activation Fraction TE = Testes Tissue Activation Fraction 1,2, etc. = Dose Levels</p>
CONCENTRATION	<p>All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units.</p> <p>Example: 0025-2PCT = 0.25 percent concentration</p>
POPU	Total number of viable cells in the plating sample raised to some exponent printed directly below the abbreviation (i.e., EP + 6 = $X 10^6$).
MUT 1	Total number of mutants or convertants obtained from the sample plated raised to some exponent printed directly below the abbreviation (i.e., EP + 0 = $X 10^0$). For strain D4, MUT 1 represents the number of ADE+ convertants.
MUT 2	Only used for strain D4 and represents the number of TRY+ convertants in the plated sample.
FREQ 1	The calculated mutation or gene conversion frequency times the negative exponent written directly below. For strain D4, FREQ 1 represents the ADE+ value.
FREQ 2	Only used for strain D4 and represents the TRY+ conversion frequency.
CONTAM	Presence of contamination on any plates.



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DATA TABLE TERMS AND ABBREVIATIONS (continued)

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
AAF	2-Acetylaminofluorene
DMSO	Dimethylsulfoxide
DMN	Dimethylnitrosamine
EMS	Ethyl Methanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
SPECIES	Animal Strains
SPRDAW	Sprague Dawley Rats
ICRFLO	Flow ICR Random Bred Mice
RHESUS	Rhesus Monkey (<u>Macaca mulatta</u>)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit



LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES COMPOUND 000056815

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
NAN		1.60	5.71	4.89	2.89	46.67
NAP		1158.63	73.09	200.22	24.55	77.84
NA1		2.79	1.43	4.49	2.47	34.85
NA2		3.31	6.21	7.30	3.50	36.83

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES ICRFLO COMPOUND 000056815

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	5.02	7.20	3.61	3.84	19.18
ACT	A+T	16.14	2.38	4.54	6.72	49.80
ACT	A-C	3.28	7.02	8.13	2.60	32.95
ACT	PLI	538.06	17.26	25.91	6.95	106.58
ACT	PLU	5.71	8.54	4.83	6.50	72.42
ACT	PTE	14.00	4.13	3.04	5.84	81.70
ACT	LI1	3.86	4.50	3.36	4.36	49.41
ACT	LI2	2.72	5.26	2.40	6.48	22.22
ACT	LU1	2.80	6.23	7.96	3.70	24.90
ACT	LU2	4.64	4.97	3.53	5.22	17.85
ACT	TE1	5.11	13.47	6.95	4.65	52.43
ACT	TE2	7.36	10.27	10.30	3.61	44.80

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES SPRDAW COMPOUND 000056815

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5
ACT	A+C	1.61	5.41		1.64	3.41	3.32
ACT	A+T		2.25		1.70	1.11	1.01
ACT	A-C	1.49	3.04	1.97	1.65	2.45	3.15
ACT	PLI	19.21	11.93		13.62	6.67	10.95
ACT	PLU	2.07	1.90		1.49	1.75	3.06
ACT	PTE	3.56	2.84		1.74	2.17	2.96
ACT	L11	2.26	385.71	2.86	2.52	1.67	1.21
ACT	L12	23.03	14.52	1.97	2.74	1.59	4.24
ACT	LU1	0.84	700.00	1.11	2.13	2.04	2.35
ACT	LU2	2.61	22.41	1.83	1.72	2.12	2.37
ACT	TE1	2.12	600.00	2.03	1.77	2.60	3.15
ACT	TE2	6.11	26.67	3.05	4.17	2.21	2.87

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 04/25/75

SPECIES RHESUS COMPOUND 000056815

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	18.56	2.33	5.85	2.22	3.02
ACT	A+T	18.18	2.76	8.85	3.08	2.73
ACT	A-C	13.67	2.66	4.20	3.02	1.99
ACT	PLI	283.75	9.04	86.14	5.98	5.82
ACT	PLU	12.85	2.33	4.58	2.41	2.81
ACT	PTE	15.37	1.56	5.62	2.99	3.95
ACT	LI1	17.40	2.76	11.34	2.14	10.47
ACT	LI2	16.19	3.39	11.04	3.08	3.45
ACT	LU1	12.77	2.82	6.24	1.99	6.16
ACT	LU2	10.46	3.24	7.40	3.21	9.97
ACT	TE1	12.04	2.91	6.93	3.33	3.87
ACT	TE2	12.22	2.67	8.20	1.53	10.40

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound 000056815, Glycerine, was evaluated for genetic activity in a series of in vitro microbial assays with and without metabolic activation. The following results were obtained:

A. Salmonella typhimurium

1. Plate Tests

At a concentration of 5.0%, 000056815 was not mutagenic for the bacterial indicator organisms employed in either the direct or activation plate tests.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. Repeat tests were conducted with several doses of the compound using rat tissues. The unusually high frequencies in the initial test resulted from defective population plates. The repeat tests for these doses were negative. All other results were negative.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

2. Activation suspension tests

The results of these tests were negative.

C. Conclusions

Compound 000056815, Glycerine, did not exhibit genetic activity in the in vitro assays employed in this evaluation.

Submitted by:



David Brusick, Ph.D.
Director of Genetics

Appendix

Tabulation of Data



BIONETICS

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104		PROJECT 02468			
EXPERIMENT 502802		DETECTOR TA1535		SPECIES		DATE - 04/25/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	0312	0005	1.60	0
	NAP		EMS 0.002 %	0394	4565	1158.63	0
000056815	NA1		0005-0 PCT.	0501	0014	2.79	0
000056815	NA2		0025-1 PCT.	0362	0012	3.31	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 504102

DETECTOR TA1537

SPECIES

DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	1435	0082	5.71	0
	NAP		QM 1.0 UG/ML	1293	0945	73.09	0
000056815	NA1		0005-0 PCT.	1327	0019	1.43	0
000056815	NA2		0025-1 PCT.	1223	0076	6.21	1

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104				PROJECT 02468	
EXPERIMENT 507802		DETECTOR TA1538		SPECIES		DATE - 04/25/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		DMSO	0327	0016	4.89	0
	NAP		NF 125 UG-ML	0465	0931	200.22	0
000056815	NA1		0005-0 PCT.	0401	0018	4.49	0
000056815	NA2		0025-1 PCT.	0370	0027	7.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104			PROJECT 02468						
EXPERIMENT 504104		DETECTOR 000004	SPECIES			DATE - 04/25/75			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SALINE	0450	0013	0210	2.89	46.67	4
	NAP		EMS 1.0 %	0501	0123	0390	24.55	77.84	6
000056815	NA1		0005-0 PCT.	0485	0012	0169	2.47	34.85	0
000056815	NA2		0025-1 PCT.	0543	0019	0200	3.50	36.83	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
 EXPERIMENT 503501 DETECTOR TA1535 SPECIES ICRFLO DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0697	0035	5.02	0
	A+T		***NO MATCH***	0694	0112	16.14	0
	A-C		SALINE	0762	0025	3.28	0
	ACP	LI	DMN 50 UM/ML	0762	4100	538.06	0
	ACP	LU	DMN 50 UM/ML	0840	0048	5.71	0
	ACP	TE	DMN 50 UM/ML	0750	0105	14.00	0
000056815	ACT	LI1	0005-0 PCT.	0518	0020	3.86	0
000056815	ACT	LI2	0025-1 PCT.	0625	0017	2.72	0
000056815	ACT	LU1	0005-0 PCT.	0963	0027	2.80	2
000056815	ACT	LU2	0025-1 PCT.	0604	0028	4.64	0
000056815	ACT	TE1	0005-0 PCT.	0744	0038	5.11	3
000056815	ACT	TE2	0025-1 PCT.	0774	0057	7.36	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 503801

DETECTOR TA1537

SPECIES ICRFLO

DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1860	0134	7.20	0
	A+T		***NO MATCH***	3195	0076	2.38	0
	A-C		DMSO	1653	0116	7.02	0
	ACP	LI	AAF 800 UG/ML	2126	0367	17.26	0
	ACP	LU	AAF 800 UG/ML	0972	0083	8.54	2
	ACP	TE	AAF 800 UG/ML	1452	0060	4.13	0
000056815	ACT	LI1	0005-0 PCT.	0933	0042	4.50	2
000056815	ACT	LI2	0025-1 PCT.	0723	0038	5.26	3
000056815	ACT	LU1	0005-0 PCT.	0337	0021	6.23	3
000056815	ACT	LU2	0025-1 PCT.	0563	0028	4.97	3
000056815	ACT	TE1	0005-0 PCT.	0297	0040	13.47	0
000056815	ACT	TE2	0025-1 PCT.	0409	0042	10.27	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104		PROJECT 02468			
EXPERIMENT 503601		DETECTOR TA1538		SPECIES ICRFLO		DATE - 04/25/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0859	0031	3.61	0
	A+T		***NO MATCH***	1013	0046	4.54	0
	A-C		DMSO	0541	0044	8.13	0
	ACP	LI	AAF 800 UG/ML	0602	0156	25.91	0
	ACP	LU	AAF 800 UG/ML	0766	0037	4.83	0
	ACP	TE	AAF 800 UG/ML	1348	0041	3.04	2
000056815	ACT	LI1	0005-0 PCT.	0951	0032	3.36	2
000056815	ACT	LI2	0025-1 PCT.	1206	0029	2.40	0
000056815	ACT	LU1	0005-0 PCT.	0465	0037	7.96	0
000056815	ACT	LU2	0025-1 PCT.	0879	0031	3.53	2
000056815	ACT	TE1	0005-0 PCT.	0604	0042	6.95	0
000056815	ACT	TE2	0025-1 PCT.	0369	0038	10.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104			PROJECT 02468						
EXPERIMENT 503701		DETECTOR 0000D4	SPECIES ICRFLO			DATE - 04/25/75			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	0417	0016	0080	3.84	19.18	5
	A+T		***NO MATCH***	0506	0034	0252	6.72	49.80	4
	A-C		SALINE	0346	0009	0114	2.60	32.95	0
	ACP	LI	DMN 90 UM/ML	0532	0037	0567	6.95	106.58	0
	ACP	LU	DMN 90 UM/ML	0446	0029	0323	6.50	72.42	6
	ACP	TE	DMN 90 UM/ML	0377	0022	0308	5.84	81.70	0
000056815	ACT	LI1	0005-0 PCT.	0597	0026	0295	4.36	49.41	2
000056815	ACT	LI2	0025-1 PCT.	0540	0035	0120	6.48	22.22	4
000056815	ACT	LU1	0005-0 PCT.	0514	0019	0128	3.70	24.90	4
000056815	ACT	LU2	0025-1 PCT.	0594	0031	0106	5.22	17.85	6
000056815	ACT	TE1	0005-0 PCT.	0452	0021	0237	4.65	52.43	0
000056815	ACT	TE2	0025-1 PCT.	0471	0017	0211	3.61	44.80	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
 EXPERIMENT 502801 DETECTOR TA1535 SPECIES SPRDAW DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0496	0008	1.61	0
	A-C		SALINE	0941	0014	1.49	0
	ACP	LI	DMN 50 UM/ML	0229	0044	19.21	2
	ACP	LU	DMN 50 UM/ML	0242	0005	2.07	0
	ACP	TE	DMN 50 UM/ML	0281	0010	3.56	2
000056815	ACT	LI1	0005-0 PCT.	0531	0012	2.26	0
000056815	ACT	LI2	0025-1 PCT.	0178	0041	23.03	2
000056815	ACT	LU1	0005-0 PCT.	0356	0003	0.84	0
000056815	ACT	LU2	0025-1 PCT.	0306	0008	2.61	0
000056815	ACT	TE1	0005-0 PCT.	0471	0010	2.12	2
000056815	ACT	TE2	0025-1 PCT.	0262	0016	6.11	0

REPEAT TEST TA-1535

<u>Compound</u>	<u>Test</u>	<u>Population</u>	<u>Mutants</u>	<u>Frequency (X10⁻⁸)</u>
000056815	A-C DMSO	0649	42	6.47
000056815	LI2 0025-1 PCT.	0289	17	5.88

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
 EXPERIMENT 504501 DETECTOR TA1537 SPECIES SPRDAW DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1219	0066	5.41	0
	A+T		***NO MATCH***	1245	0028	2.25	0
	A-C		DMSO	1448	0044	3.04	0
	ACP	LI	AAF 800 UG/ML	0989	0118	11.93	0
	ACP	LU	AAF 800 UG/ML	1319	0025	1.90	0
	ACP	TE	AAF 800 UG/ML	0952	0027	2.84	2
000056815	ACT	LI1	0005-0 PCT.	0007	0027	385.71	3
000056815	ACT	LI2	0025-1 PCT.	0124	0018	14.52	3
000056815	ACT	LU1	0005-0 PCT.	0001	0007	700.00	3
000056815	ACT	LU2	0025-1 PCT.	0058	0013	22.41	3
000056815	ACT	TE1	0005-0 PCT.	0004	0024	600.00	3
000056815	ACT	TE2	0025-1 PCT.	0105	0028	26.67	2

REPORT EXR33 LITTON BIOMETRICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 506210 DETECTOR TA1537 SPECIES SPRDAW DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMSO	1523	0030	1.97	0
000056815	ACT	LI1	0005-0 PCT.	1644	0047	2.86	0
000056815	ACT	LI2	0025-1 PCT.	1832	0036	1.97	2
000056815	ACT	LU1	0005-0 PCT.	2064	0023	1.11	2
000056815	ACT	LU2	0025-1 PCT.	1527	0028	1.83	2
000056815	ACT	TE1	0005-0 PCT.	1624	0033	2.03	2
000056815	ACT	TE2	0025-1 PCT.	1411	0043	3.05	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
 EXPERIMENT 504301 DETECTOR TA1538 SPECIES SPRDAW DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	3528	0058	1.64	0
	A+T		***NO MATCH***	4002	0068	1.70	0
	A-C		DMSO	3028	0050	1.65	0
	ACP	LI	AAF 800 UG/ML	2158	0294	13.62	0
	ACP	LU	AAF 800 UG/ML	3414	0051	1.49	0
	ACP	TE	AAF 800 UG/ML	4136	0072	1.74	0
000056815	ACT	LI1	0005-0 PCT.	1947	0049	2.52	0
000056815	ACT	LI2	0025-1 PCT.	2411	0066	2.74	0
000056815	ACT	LU1	0005-0 PCT.	2396	0051	2.13	0
000056815	ACT	LU2	0025-1 PCT.	2329	0040	1.72	0
000056815	ACT	TE1	0005-0 PCT.	2431	0043	1.77	2
000056815	ACT	TE2	0025-1 PCT.	2228	0093	4.17	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104		PROJECT 02468					
EXPERIMENT 504401		DETECTOR 0000D4		SPECIES SPRDAW		DATE - 04/25/75			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1085	0037	0036	3.41	3.32	0
	A+T		***NO MATCH***	4064	0045	0041	1.11	1.01	0
	A-C		SALINE	1144	0028	0036	2.45	3.15	0
	ACP	LI	DMN 90 UM/ML	1005	0067	0110	6.67	10.95	0
	ACP	LU	DMN 90 UM/ML	1142	0020	0035	1.75	3.06	0
	ACP	TE	DMN 90 UM/ML	1150	0025	0034	2.17	2.96	0
000056815	ACT	LI1	0005-0 PCT.	1320	0022	0016	1.67	1.21	3
000056815	ACT	LI2	0025-1 PCT.	1131	0018	0048	1.59	4.24	1
000056815	ACT	LU1	0005-0 PCT.	0978	0020	0023	2.04	2.35	0
000056815	ACT	LU2	0025-1 PCT.	1180	0025	0028	2.12	2.37	1
000056815	ACT	TE1	0005-0 PCT.	0922	0024	0029	2.60	3.15	2
000056815	ACT	TE2	0025-1 PCT.	0906	0020	0026	2.21	2.87	6

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 504901

DETECTOR TA1535

SPECIES RHESUS

DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	1546	0287	18.56	0
	A+T		***NO MATCH***	2718	0494	18.18	0
	A-C		SALINE	2187	0299	13.67	0
	ACP	LI	DMN 50 UM/ML	2080	5902	283.75	0
	ACP	LU	DMN 50 UM/ML	1666	0214	12.85	0
	ACP	TE	DMN 50 UM/ML	1971	0303	15.37	0
000056815	ACT	LI1	0005-0 PCT.	1649	0287	17.40	0
000056815	ACT	LI2	0025-1 PCT.	2113	0342	16.19	0
000056815	ACT	LU1	0005-0 PCT.	2083	0266	12.77	0
000056815	ACT	LU2	0025-1 PCT.	2314	0242	10.46	0
000056815	ACT	TE1	0005-0 PCT.	2442	0294	12.04	2
000056815	ACT	TE2	0025-1 PCT.	2119	0259	12.22	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
 EXPERIMENT 505001 DETECTOR TA1537 SPECIES RHESUS DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	2665	0062	2.33	0
	A+T		***NO MATCH***	3079	0085	2.76	0
	A-C		DMSO	2597	0069	2.66	0
	ACP	LI	AAF 800 UG/ML	2899	0262	9.04	0
	ACP	LU	AAF 800 UG/ML	2577	0060	2.33	0
	ACP	TE	AAF 800 UG/ML	3459	0054	1.56	2
000056815	ACT	LI1	0005-0 PCT.	2207	0061	2.76	0
000056815	ACT	LI2	0025-1 PCT.	2127	0072	3.39	0
000056815	ACT	LU1	0005-0 PCT.	2018	0057	2.82	0
000056815	ACT	LU2	0025-1 PCT.	1947	0063	3.24	0
000056815	ACT	TE1	0005-0 PCT.	2025	0059	2.91	0
000056815	ACT	TE2	0025-1 PCT.	2211	0059	2.67	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 505101

DETECTOR TA1538

SPECIES RHESUS

DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0855	0050	5.85	0
	A+T		***NO MATCH***	0599	0053	8.85	0
	A-C		DMSO	0834	0035	4.20	0
	ACP	LI	AAF 800 UG/ML	0866	0746	86.14	0
	ACP	LU	AAF 800 UG/ML	0896	0041	4.58	0
	ACP	TE	AAF 800 UG/ML	0836	0047	5.62	0
000056815	ACT	LI1	0005-0 PCT.	0335	0038	11.34	0
000056815	ACT	LI2	0025-1 PCT.	0299	0033	11.04	0
000056815	ACT	LU1	0005-0 PCT.	0417	0026	6.24	2
000056815	ACT	LU2	0025-1 PCT.	0419	0031	7.40	0
000056815	ACT	TE1	0005-0 PCT.	0462	0032	6.93	0
000056815	ACT	TE2	0025-1 PCT.	0488	0040	8.20	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 504802

DETECTOR 0000D4

SPECIES RHESUS

DATE - 04/25/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMN 90 UM/ML	1127	0025	0034	2.22	3.02	0
	A+T		***NO MATCH***	1427	0044	0039	3.08	2.73	0
	A-C		SALINE	1257	0038	0025	3.02	1.99	0
	ACP	LI	DMN 90 UM/ML	1322	0079	0077	5.98	5.82	0
	ACP	LU	DMN 90 UM/ML	1493	0036	0042	2.41	2.81	0
	ACP	TE	DMN 90 UM/ML	1139	0034	0045	2.99	3.95	0
000056815	ACT	LI1	0005-0 PCT.	0936	0020	0098	2.14	10.47	0
000056815	ACT	LI2	0025-1 PCT.	0811	0025	0028	3.08	3.45	2
000056815	ACT	LU1	0005-0 PCT.	1006	0020	0062	1.99	6.16	1
000056815	ACT	LU2	0025-1 PCT.	0903	0029	0090	3.21	9.97	4
000056815	ACT	TE1	0005-0 PCT.	0930	0031	0036	3.33	3.87	1
000056815	ACT	TE2	0025-1 PCT.	0981	0015	0102	1.53	10.40	0