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Mutagenic Evaluation of Compound FDA 73-70
5/30/75

Benzoic Acid, Certified A. C. S.



7315 Wisconsin Avenue
Bethesda, Maryland
20014

LBI PROJECT #2468

MUTAGENIC EVALUATION OF
COMPOUND FDA 73-70

000065850

BENZOIC ACID, CERTIFIED A.C.S.

SUBMITTED TO

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

SUBMITTED BY

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MAY 30, 1975



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EVALUATION SUMMARY

Compound FDA 73-70, Benzoic Acid, did not exhibit genetic activity in any of the in vitro microbial assays employed in this evaluation.



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DATE: May 30, 1975

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

SUBJECT: Evaluation of Test Compound 000065850, Benzoic Acid, Certified A.C.S.,
FDA 73-70

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: White crystalline flakes

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)	6.0 μ M
2. Isocitric acid	49.0 μ M
3. Tris buffer, pH 7.4	28.0 μ M
4. MgCl ₂	1.7 μ M
5. Tissue homogenate fraction	72.0 mg



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D. Tissue Homogenates and Supernatants

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

- ^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. Nonactivation

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 nonactivation 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 nonactivation tests.



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

Male animals (sufficient to provide the necessary quantities of 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. The data were 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: 000065850
Benzoil Acid, Certified A.C.S.
2. Test solvent: DMSO
3. Solubility of the test compound under treatment conditions:
Soluble under treatment conditions.
4. Additional comments: white crystalline flakes

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: February 11, 1975
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	0.25	0.0375
1/2 50% Survival	0.50	0.0750
50% Survival	1.00	0.1500
Plate Tests	0.50	--



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V. SUMMARY OF TEST RESULTS

Plate Tests

A. Name or code designation of the test compound: 000065850

B. Test date: March 29, 1975

C. Concentration of the test compound: 0.5%

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>
<u>1. Nonactivation</u>								
Solvent Control	---	---	11	20	4	5	15	11
Positive Control ^a	---	---	>10 ⁵	>10 ⁵	193	207	145	134
Test Compound	---	---	2	2	1	2	7	3
<u>2. Activation</u>								
Negative Control	---	---	7	10	6	3	7	12
Solvent Control	---	---	20	24	5	9	15	14
Reaction Mixture Control	---	---	23	20	7	8	18	16
Positive Control ^b	Mouse	Liver	>10 ³	>10 ³	39	34	343	357
Positive Control		Lung	12	8	2	1	11	16
Positive Control		Testes	16	17	3	8	7	11
Positive Control	Rat	Liver	>10 ³	>10 ³	89	88	347	341
Positive Control		Lung	14	7	2	3	14	18
Positive Control		Testes	16	13	5	7	10	13
Positive Control	Monkey	Liver	273	356	30	33	123	119
Positive Control		Lung	8	8	2	2	13	14
Positive Control		Testes	14	12	3	6	8	11
Test Compound	Mouse	Liver	6	12	9	10	5	6
Test Compound		Lung	2	0	3	9	8	6
Test Compound		Testes	4	8	10	9	8	13
Test Compound	Rat	Liver	4	8	12	7	8	13
Test Compound		Lung	1	1	3	6	2	3
Test Compound		Testes	5	0	14	12	6	9
Test Compound	Monkey	Liver	5	10	12	10	13	6
Test Compound		Lung	2	8	3	6	2	2
Test Compound		Testes	3	2	7	11	3	5

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



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DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	<p>NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: 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 05/15/75

SPECIES COMPOUND 000065850

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
NAN		10.15	2.10	9.02	1.58	1.69
NAP		273.70	225.89	145.97	113.25	60.11
NA1		8.76	1.53	6.82	2.55	1.13
NA2		11.76	2.13	7.52	1.23	1.48



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/15/75

SPECIES ICRFLO COMPOUND 000065850

TEST	ORG	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	5.99	2.10	11.20			16.01	4.11
ACT	A+T	12.45	2.10	10.46			15.24	3.26
ACT	A-C	6.65	2.91	10.54	3.19	7.30	19.85	3.33
ACT	PLI	1168.86	10.07	37.43			20.62	4.92
ACT	PLU	8.06	2.19	12.57			39.08	3.22
ACT	PTE	9.32	2.86	12.54			23.53	3.29
ACT	LI1	12.64	2.51	34.86	6.06		16.50	4.31
ACT	LI2	11.44	2.78	29.58	5.85		27.67	6.63
ACT	LU1	12.19	2.50	26.38		3.82	15.51	4.04
ACT	LU2	6.86	3.05	18.56			22.47	4.77
ACT	TE1	13.73	2.78	19.79			22.24	4.92
ACT	TE2	10.50	2.36	12.47			20.15	2.52



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/15/75

SPECIES SPRDAW COMPOUND 000065850

TEST	ORG	TA1535 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	17.47		0.63	5.39		0.58	0.39
ACT	A+T	11.03		1.23	10.55		2.35	2.75
ACT	A-C	11.62	4.98	0.58	5.36	7.30	0.72	0.18
ACT	PLI	1500.00		7.28	59.37		8.75	10.22
ACT	PLU	18.29		0.84	7.31		3.40	1.39
ACT	PTE	22.47		0.33	8.13		3.38	1.93
ACT	LI1	28.63	4.87	0.64	12.60	3.91	3.16	2.07
ACT	LI2	12.04		1.06	9.65	2.94	2.25	0.59
ACT	LU1	14.71		0.69	4.65		0.34	0.68
ACT	LU2	12.28		0.76	3.86		1.73	1.51
ACT	TE1	18.25		0.98	9.73		0.53	0.46
ACT	TE2	9.35		0.86	7.42		0.58	1.50



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LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 05/15/75

SPECIES RHESUS COMPOUND 000065850

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	7.43	7.44	4.49	10.40	1.65
ACT	A+T	14.05	11.84	4.09	15.69	2.55
ACT	A-C	7.01	7.04	5.55	14.76	2.24
ACT	PLI	1352.79	19.31	24.13	14.99	2.71
ACT	PLU	4.62	6.35	2.77	12.58	2.35
ACT	PTE	8.16	8.79	6.29	20.07	2.79
ACT	L11	12.34	21.02	5.41	12.36	4.05
ACT	L12	5.68	9.03	2.48	17.59	2.45
ACT	LU1	7.69	19.81	1.86	15.96	2.32
ACT	LU2	4.68	14.97	2.81	22.43	3.71
ACT	TE1	4.95	17.91	4.83	16.09	2.82
ACT	TE2	6.71	11.54	13.62	12.68	1.75



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VI. INTERPRETATION OF RESULTS AND CONCLUSIONS

Compound FDA 73-70, Benzoic Acid, 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 0.5%, this chemical did not exhibit mutagenic activity for any of the indicator strains used in 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 considered negative. The LI1, LI2 and LU1 doses with TA-1538 using mouse tissue were slightly increased in the initial tests, but were negative in repeat tests. The same was true for the LI1 with TA-1535 and the LI1 and LI2 doses with TA-1538 using rat tissues. Several of the tests with TA-1537 and primate tissues were slightly elevated. Compared to the A+T control, however, the increases were not of sufficient magnitude to be considered positive especially when inspection of the raw data sheets showed relatively low survival at those doses giving higher frequencies.

B. Saccharomyces cerevisiae

1. Nonactivation suspension tests

The results of these tests were negative.

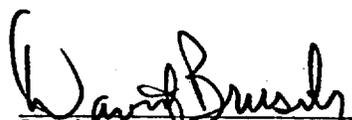
2. Activation suspension tests

The results of these tests were negative. A few dose levels produced slightly increased recombination frequencies, but did not demonstrate a consistent pattern.

C. Conclusions

The test compound, Benzoic Acid, was not considered to be mutagenic in the microbial in vitro assays employed in this evaluation. Some apparent increases in activation tests either proved to be negative in repeat tests or were associated with reduced population counts suggesting that they did not indicate genetic activity.

Submitted by:


David Brusick, Ph.D.
Director of Genetics



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APPENDIX
Tabulation of Data



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104		PROJECT 02468			
EXPERIMENT 505803		DETECTOR TA1535		SPECIES			
				DATE - 05/15/75			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	1340	0136	10.15	0
	NAP		EMS 0.002 %	1327	3632	273.70	0
000065850	NA1		0005-1 PCT.	1084	0095	8.76	0
000065850	NA2		0025-2 PCT.	0910	0107	11.76	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104		PROJECT 02468			
EXPERIMENT 508301		DETECTOR TA1537		SPECIES			
					DATE - 05/15/75		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SALINE	0713	0015	2.10	0
	NAP		QM 1.0 UG/ML	0421	0951	225.89	0
000065850	NA1		0005-1 PCT.	1173	0018	1.53	0
000065850	NA2		0025-2 PCT.	0892	0019	2.13	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104			PROJECT 02468		
EXPERIMENT 505802		DETECTOR TA1538			SPECIES		
					DATE - 05/15/75		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
		NAN	DMSO	0599	0054	9.02	0
		NAP	NF 125 UG-ML	0546	0797	145.97	0
000065850	NA1		0005-1 PCT.	0557	0038	6.82	0
000065850	NA2		0025-2 PCT.	0625	0047	7.52	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104			PROJECT 02468				
EXPERIMENT 505804		DETECTOR 0000D4			SPECIES		DATE - 05/15/75		
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SALINE	0947	0015	0016	1.58	1.69	0
	NAP		EMS 1.0 %	0702	0795	0422	113.25	60.11	7
000065850	NA1		0075-3 PCT.	0706	0018	0008	2.55	1.13	1
000065850	NA2		0375-4 PCT.	0405	0005	0006	1.23	1.48	1



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 506301 DETECTOR TA1535 SPECIES ICRFLO DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0734	0044	5.99	0
	A+T		***NO MATCH***	0819	0102	12.45	0
	A-C		SALINE	0917	0061	6.65	0
	ACP	LI	DMN 50 UM/ML	0456	5330	1168.86	1
	ACP	LU	DMN 50 UM/ML	0608	0049	8.06	0
	ACP	TE	DMN 50 UM/ML	0590	0055	9.32	0
000065850	ACT	LI1	0005-1 PCT.	0443	0056	12.64	0
000065850	ACT	LI2	0025-2 PCT.	0857	0098	11.44	0
000065850	ACT	LU1	0005-1 PCT.	0443	0054	12.19	0
000065850	ACT	LU2	0025-2 PCT.	0845	0058	6.86	0
000065850	ACT	TE1	0005-1 PCT.	0335	0046	13.73	0
000065850	ACT	TE2	0025-2 PCT.	0724	0076	10.50	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 505901 DETECTOR TA1537 SPECIES ICRFLO DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1476	0031	2.10	0
	A+T		***NO MATCH***	1524	0032	2.10	0
	A-C		DMSO	1445	0042	2.91	0
	ACP	LI	AAF 800 UG/ML	1489	0150	10.07	0
	ACP	LU	AAF 800 UG/ML	1463	0032	2.19	0
	ACP	TE	AAF 800 UG/ML	1293	0037	2.86	0
000065850	ACT	LI1	0005-1 PCT.	1273	0032	2.51	0
000065850	ACT	LI2	0025-2 PCT.	1294	0036	2.78	0
000065850	ACT	LU1	0005-1 PCT.	1320	0033	2.50	0
000065850	ACT	LU2	0025-2 PCT.	1343	0041	3.05	0
000065850	ACT	TE1	0005-1 PCT.	1222	0034	2.78	0
000065850	ACT	TE2	0025-2 PCT.	1482	0035	2.36	0



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COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 506401 DETECTOR TA1538 SPECIES ICRFLO DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0598	0067	11.20	0
	A+T		***NO MATCH***	0841	0088	10.46	0
	A-C		DMSO	0446	0047	10.54	0
	ACP	LI	AAF 800 UG/ML	0505	0189	37.43	0
	ACP	LU	AAF 800 UG/ML	0565	0071	12.57	2
	ACP	TE	AAF 800 UG/ML	0606	0076	12.54	0
000065850	ACT	LI1	0005-1 PCT.	0175	0061	34.86	1
000065850	ACT	LI2	0025-2 PCT.	0284	0084	29.58	2
000065850	ACT	LU1	0005-1 PCT.	0163	0043	26.38	0
000065850	ACT	LU2	0025-2 PCT.	0291	0054	18.56	2
000065850	ACT	TE1	0005-1 PCT.	0187	0037	19.79	0
000065850	ACT	TE2	0025-2 PCT.	0401	0050	12.47	0



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REPORT .EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104		PROJECT 02468			
EXPERIMENT 509302		DETECTOR TA1538		SPECIES ICRFLD		DATE - 05/15/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMSO	0595	0019	3.19	0
000065850	ACT	LI1	0005-1 PCT.	0297	0018	6.06	2
000065850	ACT	LI2	0025-2 PCT.	0479	0028	5.85	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104			PROJECT 02468		
EXPERIMENT 511205		DETECTOR TA1538		SPECIES ICRFLO		DATE - 05/15/75	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMSO	0794	0058	7.30	0
000065850	ACT	LU1	0005-1 PCT.	0629	0024	3.82	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104			PROJECT 02468				
EXPERIMENT 510401		DETECTOR 0000D4			SPECIES ICRFLO			DATE - 05/15/75	
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	0706	0113	0029	16.01	4.11	0
	A+T		***NO MATCH***	1319	0201	0043	15.24	3.26	6
	A-C		SALINE	0660	0131	0022	19.85	3.33	2
	ACP	LI	DMN 90 UM/ML	0325	0067	0016	20.62	4.92	6
	ACP	LU	DMN 90 UM/ML	0870	0340	0028	39.08	3.22	2
	ACP	TE	DMN 90 UM/ML	0850	0200	0028	23.53	3.29	4
000065850	ACT	LI1	0075-3 PCT.	0394	0065	0017	16.50	4.31	0
000065850	ACT	LI2	0375-4 PCT.	0347	0096	0023	27.67	6.63	6
000065850	ACT	LU1	0075-3 PCT.	0445	0069	0018	15.51	4.04	0
000065850	ACT	LU2	0375-4 PCT.	0503	0113	0024	22.47	4.77	0
000065850	ACT	TE1	0075-3 PCT.	0508	0113	0025	22.24	4.92	6
000065850	ACT	TE2	0375-4 PCT.	0397	0080	0010	20.15	2.52	6



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 506501 DETECTOR TA1535 SPECIES SPRDAW DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0435	0076	17.47	0
	A+T		***NO MATCH***	0571	0063	11.03	0
	A-C		SALINE	0697	0081	11.62	0
	ACP	LI	DMN 50 UM/ML	0388	5820	1500.00	0
	ACP	LU	DMN 50 UM/ML	0410	0075	18.29	0
	ACP	TE	DMN 50 UM/ML	0356	0080	22.47	2
000065850	ACT	LI1	0005-1 PCT.	0255	0073	28.63	0
000065850	ACT	LI2	0025-2 PCT.	0739	0089	12.04	2
000065850	ACT	LU1	0005-1 PCT.	0442	0065	14.71	0
000065850	ACT	LU2	0025-2 PCT.	0505	0062	12.28	2
000065850	ACT	TE1	0005-1 PCT.	0378	0069	18.25	2
000065850	ACT	TE2	0025-2 PCT.	0706	0066	9.35	2



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 509303 DETECTOR TA1535 SPECIES SPRDAW DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		SALINE	0824	0041	4.98	0
000065850	ACT	L11	0005-1 PCT.	0493	0024	4.87	2



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 507001 DETECTOR TA1537 SPECIES SPRDAW DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	1906	0012	0.63	0
	A+T		***NO MATCH***	2198	0027	1.23	0
	A-C		DMSO	1900	0011	0.58	0
	ACP	LI	AAF 800 UG/ML	1594	0016	7.28	0
	ACP	LU	AAF 800 UG/ML	1657	0014	0.84	2
	ACP	TE	AAF 800 UG/ML	1841	0006	0.33	2
000065850	ACT	LI1	0005-1 PCT.	1415	0009	0.64	0
000065850	ACT	LI2	0025-2 PCT.	1611	0017	1.06	0
000065850	ACT	LU1	0005-1 PCT.	1736	0012	0.69	0
000065850	ACT	LU2	0025-2 PCT.	1711	0013	0.76	0
000065850	ACT	TE1	0005-1 PCT.	1844	0018	0.98	2
000065850	ACT	TE2	0025-2 PCT.	1740	0015	0.86	2



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 507901 DETECTOR TA1538 SPECIES SPRDAW DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0445	0024	5.39	0
	A+T		***NO MATCH***	0275	0029	10.55	2
	A-C		DMSO	0392	0021	5.36	0
	ACP	LI	AAF 800 UG/ML	0507	0301	59.37	0
	ACP	LU	AAF 800 UG/ML	0588	0043	7.31	0
	ACP	TE	AAF 800 UG/ML	0541	0044	8.13	0
000065850	ACT	LI1	0005-1 PCT.	0127	0016	12.60	0
000065850	ACT	LI2	0025-2 PCT.	0311	0030	9.65	0
000065850	ACT	LU1	0005-1 PCT.	0215	0010	4.65	0
000065850	ACT	LU2	0025-2 PCT.	0492	0019	3.86	0
000065850	ACT	TE1	0005-1 PCT.	0226	0022	9.73	2
000065850	ACT	TE2	0025-2 PCT.	0283	0021	7.42	2



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 511206 DETECTOR TA1538 SPECIES SPRDAW DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		DMSO	0794	0058	7.30	0
000065850	ACT	LI1	0005-1 PCT.	0435	0017	3.91	2
000065850	ACT	LI2	0025-2 PCT.	0613	0018	2.94	2



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 506601 DETECTOR 0000D4 SPECIES SPRDAW DATE - 05/15/75

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	1037	0006	0004	0.58	0.39	4
	A+T		***NO MATCH***	1020	0024	0028	2.35	2.75	4
	A-C		SALINE	1104	0008	0002	0.72	0.18	4
	ACP	LI	DMN 90 UM/ML	0949	0083	0097	8.75	10.22	4
	ACP	LU	DMN 90 UM/ML	0794	0027	0011	3.40	1.39	6
	ACP	TE	DMN 90 UM/ML	1035	0035	0020	3.38	1.93	4
000065850	ACT	LI1	0075-3 PCT.	0917	0029	0019	3.16	2.07	4
000065850	ACT	LI2	0375-4 PCT.	0845	0019	0005	2.25	0.59	4
000065850	ACT	LU1	0075-3 PCT.	0877	0003	0006	0.34	0.68	4
000065850	ACT	LU2	0375-4 PCT.	0925	0016	0014	1.73	1.51	5
000065850	ACT	TE1	0075-3 PCT.	1318	0007	0006	0.53	0.46	4
000065850	ACT	TE2	0375-4 PCT.	0865	0005	0013	0.58	1.50	4



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 507201 DETECTOR TA1535 SPECIES RHESUS DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 50 UM/ML	0888	0066	7.43	2
	A+T		***NO MATCH***	0541	0076	14.05	0
	A-C		SALINE	0913	0064	7.01	0
	ACP	LI	DMN 50 UM/ML	0502	6791	1352.79	0
	ACP	LU	DMN 50 UM/ML	0823	0038	4.62	0
	ACP	TE	DMN 50 UM/ML	0625	0051	8.16	0
000065850	ACT	LI1	0005-1 PCT.	0235	0029	12.34	0
000065850	ACT	LI2	0025-2 PCT.	0774	0044	5.68	2
000065850	ACT	LU1	0005-1 PCT.	0234	0018	7.69	0
000065850	ACT	LU2	0025-2 PCT.	0556	0026	4.68	0
000065850	ACT	TE1	0005-1 PCT.	0404	0020	4.95	0
000065850	ACT	TE2	0025-2 PCT.	0656	0044	6.71	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104 PROJECT 02468
EXPERIMENT 511401 DETECTOR TA1537 SPECIES RHESUS DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0847	0063	7.44	2
	A+T		***NO MATCH***	0515	0061	11.84	0
	A-C		DMSO	0710	0050	7.04	0
	ACP	LI	AAF 800 UG/ML	0844	0163	19.31	0
	ACP	LU	AAF 800 UG/ML	0914	0058	6.35	0
	ACP	TE	AAF 800 UG/ML	0842	0074	8.79	0
000065850	ACT	LI1	0005-1 PCT.	0314	0066	21.02	0
000065850	ACT	LI2	0025-2 PCT.	0642	0058	9.03	2
000065850	ACT	LU1	0005-1 PCT.	0212	0042	19.81	0
000065850	ACT	LU2	0025-2 PCT.	0441	0066	14.97	0
000065850	ACT	TE1	0005-1 PCT.	0268	0048	17.91	0
000065850	ACT	TE2	0025-2 PCT.	0589	0068	11.54	2



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2104

PROJECT 02468

EXPERIMENT 507601

DETECTOR TA1538

SPECIES RHESUS

DATE - 05/15/75

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AAF 800 UG/ML	0802	0036	4.49	0
	A+T		***NO MATCH***	0464	0019	4.09	0
	A-C		DMSO	0721	0040	5.55	0
	ACP	LI	AAF 800 UG/ML	0601	0145	24.13	2
	ACP	LU	AAF 800 UG/ML	0649	0018	2.77	0
	ACP	TE	AAF 800 UG/ML	0572	0036	6.29	0
000065850	ACT	LI1	0005-1 PCT.	0222	0012	5.41	2
000065850	ACT	LI2	0025-2 PCT.	0404	0010	2.48	0
000065850	ACT	LU1	0005-1 PCT.	0269	0005	1.86	2
000065850	ACT	LU2	0025-2 PCT.	0499	0014	2.81	0
000065850	ACT	TE1	0005-1 PCT.	0352	0017	4.83	0
000065850	ACT	TE2	0025-2 PCT.	0367	0050	13.62	0



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REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

		CONTRACT 22374-2104			PROJECT 02468				
EXPERIMENT 510501		DETECTOR 0000D4			SPECIES RHESUS			DATE - 05/15/75	
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	0423	0044	0007	10.40	1.65	0
	A+T		***NO MATCH***	0548	0086	0014	15.69	2.55	0
	A-C		SALINE	0759	0112	0017	14.76	2.24	0
	ACP	LI	DMN 90 UM/ML	0774	0116	0021	14.99	2.71	0
	ACP	LU	DMN 90 UM/ML	0469	0059	0011	12.58	2.35	0
	ACP	TE	DMN 90 UM/ML	0538	0108	0015	20.07	2.79	0
000065850	ACT	LI1	0075-3 PCT.	0518	0064	0021	12.36	4.05	0
000065850	ACT	LI2	0375-4 PCT.	0449	0079	0011	17.59	2.45	0
000065850	ACT	LU1	0075-3 PCT.	0733	0117	0017	15.96	2.32	0
000065850	ACT	LU2	0375-4 PCT.	0593	0133	0022	22.43	3.71	0
000065850	ACT	TE1	0075-3 PCT.	0603	0097	0017	16.09	2.82	0
000065850	ACT	TE2	0375-4 PCT.	0686	0087	0012	12.68	1.75	0