



BIONETICS

MUTAGENICITY EVALUATION

OF

FDA 75-98
CHOLIC ACID

FINAL REPORT

Mutagenic Evaluation of Compound FDA 75-98

Cholic Acid

Final Report 9/77

6-7

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CHOLIC ACID

FINAL REPORT

SUBMITTED TO

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BUREAU OF FOODS
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LBI PROJECT NO. 2672

SEPTEMBER 1977



BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-98, Cholic acid, did not exhibit mutagenic activity in any of the assays employed in these studies.



DATE: July, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound: FDA 75-98, Cholic acid

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: December 29, 1976
2. Description: White powder

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
TA-98
TA-100

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)	4 μ moles
2. Glucose-6-phosphate	5 μ moles
3. Sodium phosphate (dibasic)	100 μ moles
4. MgCl ₂	8 μ moles
5. KCl	33 μ moles
6. Homogenate fraction equivalent to 25 mg of wet tissue.	



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 monkey - 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	Methylnitrosoguanidine	Water or saline	BPS ^b
	Ethylmethanesulfonate	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
	8-Aminoquinoline	Dimethylsulfoxide ^c	FS ^b
	2-Aminoanthracene	Dimethylsulfoxide ^c	BPS ^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 the test chemical were determined prior to screening.

The test chemical was tested for toxicity against 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 concentrations and the 1/4 and 1/2 50% doses calculated.

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

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 (Overlay Method)

Approximately 10^8 cells from an overnight culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For nonactivation tests, the three dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests 0.5 ml of a 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Three dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37°C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using positive compounds that are active directly and those that require metabolic activation were run with each assay.

C. Suspension Tests

1. Nonactivation

Bacteria and yeast cultures of the indicator organisms were grown in complete broth, washed and resuspended in 0.9% saline to densities of 1×10^{10} cells/ml and 5×10^9 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, 24-well tissue culture plates (Linbro). 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 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.



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

Male animals (except monkeys) sufficient to provide the necessary quantities of tissues were killed by cranial blow, decapitated and bled. Monkey tissues were obtained from freshly killed and bled male rhesus monkeys. Organs were immediately dissected from the animals using aseptic techniques and placed in ice-cold 0.15M KCl. Upon collection of the desired quantity of organs, they were washed twice with fresh KCl 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. Protein and P-448 determinations were made for each lot of homogenate.

E. Data Recording and Reporting

1. Plate test assays

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were entered into a computer program designed to print out all data by test. The data are presented as revertants per plate for each indicator strain employed in the assay. The positive and solvent controls are provided as reference points.

2. Suspension assays

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. All raw data sheets are dated and signed by the responsible technician.



IV. RESULTS SECTION

A. Solubility Properties of the Test Compound

1. Name or code designation of the test compound: FDA 75-98, Cholic acid
2. Test solvent: *Saline
3. Solubility of the test compound under treatment conditions: Soluble
4. Additional comments: White powder

B. Toxicity and Dosage Determinations for the Test Compound

1. Test date for toxicity determination: April 22, 1977 - Bacteria
April 21, 1977 - Yeast
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>Test Doses</u>	<u>Percent Concentration</u>	
	<u>Bacteria</u>	<u>Yeast</u>
1/4 50% Survival	0.0425	1.25
1/2 50% Survival	0.085	2.5
50% Survival	0.17	5.0

*The concentration of solvent was equal to the highest volume of test material added.

C. Plate Test Results

The plate test results are summarized in the following table. The values presented in this table are the number of revertants per plate.

D. Suspension Assay Results

The suspension test results for the test compound are summarized in the tables following the plate test summary. The values presented in these tables are the calculated mutation frequencies for each control and experimental test point. The first table of the suspension set presents the results for the nonactivation assays, and the second table through the fourth table of the suspension set presents the results for the activation assays. A listing of computer codes and abbreviations is included for reference. Tabulation of all raw data is provided in the Appendix.

SUMMARY OF TEST RESULTS

PLATE TESTS

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 000081254
 B. TEST DATE: MAY 18, 1977

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE									
			TA-1535		TA-1537		TA-1538		TA-98		TA-100	
			1	2	1	2	1	2	1	2	1	2
1. NON-ACTIVATION												
SOLVENT CONTROL*	---	---	16	12	16	20	25	27	38	31	124	137
POSITIVE CONTROL**	---	---	>1000	934	644	731	>1000	>1000	>1000	>1000	698	677
TEST 1.70000 %	---	---	17	16	15	19	22	24	36	29	120	118
0.85000 %	---	---	14	20	13	12	28	16	33	39	124	115
0.42500 %	---	---	16	15	14	18	24	17	41	28	133	131
2. ACTIVATION												
SOLVENT CONTROL*	MOUSE	LIVER	30	31	22	23	19	10	37	32	222	195
	RAT	LIVER	26	37	20	18	19	17	39	40	147	182
	MONKEY	LIVER	18	15	17	31	23	21	36	37	192	133
POSITIVE CONTROL***	MOUSE	LIVER	502	490	260	256	874	911	>1000	>1000	624	889
	RAT	LIVER	274	374	241	149	938	732	>1000	>1000	>1000	>1000
	MONKEY	LIVER	370	215	173	160	738	901	>1000	937	>1000	>1000
TEST 0.17000 %	MOUSE	LIVER	17	16	20	21	16	22	28	24	187	154
0.08500 %	MOUSE	LIVER	23	18	22	18	19	18	36	34	196	115
0.04250 %	MOUSE	LIVER	15	19	31	22	24	30	34	31	115	132
0.17000 %	RAT	LIVER	21	17	18	23	28	27	27	32	113	109
0.08500 %	RAT	LIVER	13	11	17	19	15	18	35	36	92	108
0.04250 %	RAT	LIVER	14	18	17	18	21	24	39	40	127	106
0.17000 %	MONKEY	LIVER	19	22	19	21	22	20	38	31	124	138
0.08500 %	MONKEY	LIVER	14	19	24	20	16	21	28	39	172	128
0.04250 %	MONKEY	LIVER	17	22	13	19	13	19	31	38	132	109

* NON-ACTIVATION ASSAYS CONSIST OF THE CELLS PLUS THE TEST COMPOUND VEHICLE (SOLVENT). FOR ACTIVATION ASSAYS, THE OVERLAY CONTAINS THE ACTIVATION SYSTEM PLUS THE TEST COMPOUND VEHICLE.

** TA-1535 MNNG 2 UG/PLATE *** TA-1535 ANTH 100 UG/PLATE
 TA-1537 QM 20 UG/PLATE TA-1537 AMQ 100 UG/PLATE
 TA-1538 NF 100 UG/PLATE TA-1538 AAF 100 UG/PLATE
 TA-98 NF 100 UG/PLATE TA-98 AAF 100 UG/PLATE
 TA-100 MNNG 2 UG/PLATE TA-100 ANTH 100 UG/PLATE

NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS (UL) OR MICROGRAMS (UG) PER PLATE.

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

NONACTIVATION COMPOUND 000081254

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
NAN		79.95	1.89	3.90	11.64	5.60	2.39	4.67	CONTROLS
NAP		119.59	87.54	128.54	111.30	142.59	291.13	56.21	
<hr/>									
NA1		47.65	2.42	5.46	11.46	6.80	1.88	4.61	TEST DATA
NA2		30.51	2.39	4.88	9.38	11.11	1.55	4.19	
NA3		29.01	2.47	2.30	5.88	8.10	2.23	4.36	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

SPECIES ICRFLO/MOUSE COMPOUND 000081254

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	23.49	6.88	33.33		6.89	4.83	10.51	7.83	NEGATIVE CONTROLS
ACT	A-C	16.62	6.43	3.04		6.90	5.37	18.11	10.30	
ACT	ALI	88.96	7.02	4.71	5.77	9.69	9.66	27.48	14.50	
ACT	ALU	20.22	10.99	6.04	7.71	14.40	11.68	27.30	18.89	

ACT	PLI	103.67	109.55	14.51		105.57	50.00	63.52	45.15	POSITIVE CONTROLS
ACT	PLU	30.25	9.45	49.34		15.68	14.63	36.97	20.73	

ACT	LI1	34.54	2.59	3.57		11.03	5.71	19.59	19.47	TEST COMPOUND
ACT	LI2	33.40	2.79	1.76		9.13	16.67	24.20	14.71	
ACT	LI3	24.60	3.60	5.12		5.40	11.95	17.23	11.27	
ACT	LU1	19.34	4.74	24.79	4.98	9.17	5.86	26.82	24.39	
ACT	LU2	29.19	5.17	7.87	3.82	10.91	7.17	17.14	10.82	
ACT	LU3	21.49	11.22	17.52	2.78	12.97	3.75	17.86	11.05	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

SPECIES SPRDAW/RAT COMPOUND 000081254

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5
ACT	A+C	30.96	4.70	3.79	4.52	5.76	3.17	8.97	5.40
ACT	A-C	9.11	3.48	3.35	5.45	7.75	4.45	8.21	5.43
ACT	ALI	56.33	4.66	7.34	2.22	7.07	14.34	12.82	8.38
ACT	ALU	30.58	6.51	12.93	7.98	7.46	14.09	12.26	8.61
<hr/>									
ACT	PLI	164.44	232.50	116.35	184.29	126.90	148.48	55.13	67.64
ACT	PLU	24.83	3.99	27.25	25.47	8.30	62.16	9.98	6.33
<hr/>									
ACT	LI1	28.91	2.62	6.49	9.30	4.37	7.07	11.01	7.14
ACT	LI2	37.33	5.08	4.72	4.54	6.43	7.55	11.10	6.75
ACT	LI3	33.86	6.53	4.83	4.03	7.83	5.90	9.48	6.87
ACT	LU1	27.33	4.61	15.76	2.84		8.31	9.23	6.02
ACT	LU2	31.58	4.94	14.03	5.15		7.71	9.10	5.76
ACT	LU3	34.02	1.26	8.41	5.27		9.24	11.71	4.28

NEGATIVE CONTROLS

POSITIVE CONTROLS

TEST COMPOUND

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 REPORT EXR34

COMPOUND FREQUENCY SUMMARY REPORT 07/25/77

SPECIES RHESUS/MONKEY COMPOUND 000081254

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	0000D4 ADE EX-5	0000D4 TRY EX-5	
ACT	A+C	78.62	9.70	1.38	7.78	22.78	17.46	8.87	NEGATIVE CONTROLS
ACT	A-C	72.61	13.76	4.74	2.64	21.98	14.29	7.27	
ACT	ALI	91.73	5.86	9.83	20.93	31.64	19.25	10.94	
ACT	ALU	78.99	12.75	27.63	6.97	20.14	19.68	10.96	
ACT	PLI	203.62	67.55	173.66	109.99	190.71	89.69	68.28	POSITIVE CONTROLS
ACT	PLU	96.65	12.74	6.00	11.88	27.05	18.74	7.76	
ACT	L11	79.22	13.60	9.04	10.54	23.33	13.15	7.12	TEST COMPOUND
ACT	L12	78.81	6.11	11.62	8.42	30.11	14.44	9.01	
ACT	L13	72.13	6.30	3.89	11.09	29.34	19.87	9.02	
ACT	LU1	83.88	9.69	10.27	16.82	24.01	13.01	9.80	
ACT	LU2	62.01	8.84	9.60	11.64	30.33	11.97	8.91	
ACT	LU3	65.48	8.19	13.89	12.24	26.45	17.88	7.64	

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 for ACP A-C = Activation: Solvent Control ALI or A+T = Activation: Homogenate Control (Liver) ALU = Activation: Homogenate Control (Lung) ACP = Activation: Positive Control ACT = Activation Test</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 = $\times 10^6$).
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 = 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	Ethylmethanesulfonate
QM	Quinacrine Mustard
NF	Nitrofluorene
ANTH	2-Amino Anthracene
AMQ	8-Amino Quinoline
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
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-98, Cholic 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

The results of these tests were negative.

2. Nonactivation suspension tests

The results of these tests were negative.

3. Activation suspension tests

The results of these tests were negative. The following tests were repeated:

The initial test with the strain TA-1537 using mouse lung tissue exhibited increased revertant frequency at the LU₁ dose. The repeat test was negative. The test with TA-1538 was repeated with rat liver tissue as this strain exhibited slightly increased revertant frequency in the initial test. The repeat test was 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.

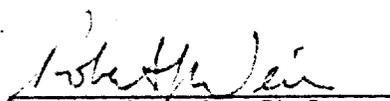
C. Conclusions

The test compound, FDA 75-98, Cholic acid, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

 7/29/77
Date
David J. Brusick, Ph.D.
Director
Department of Molecular
Toxicology

Reviewed by:

 7/29/77
Date
Robert J. Weir, Ph.D.
Vice President



VI. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and cells are incubated in the overlay for 2-3 days, and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test.

- The small number of cell divisions permits potential mutagens to act on replicating DNA which is often more sensitive than non-replicating DNA.
- The combined incubation of the compound and the cells in the overlay permit constant exposure of the indicator cells for 2-3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs dose levels that are selected such that the highest dose will show slight toxicity (as determined by subjective criteria) and several doses ranging down 1 to 2 logs lower.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. Factors which may modify dose response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced and the compound will not appear to be mutagenic.

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.



D. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test chemical are semiquantitative, the criteria used to determine positive effects are inherently subjective and are based primarily on a historical data base. Most data sets are evaluated using the following criteria:

1. Strains TA-1535, TA-1537, and TA-1538

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the lowest increase equal to twice the solvent control value is considered to be mutagenic.

2. Strains TA-98, TA-100, and D4

If the solvent control value is within the normal range, a chemical that produces a positive dose response over three concentrations with the highest increase equal to twice the solvent control value for TA-100 and two to three times the solvent control value for strains TA-98 and D4 is considered to be mutagenic. For these strains, the dose response increase should start at approximately the solvent control values.

3. Pattern

Because TA-1535 and TA-100 were both derived from the same parental strain (G-46) and because TA-1538 and TA-98 were both derived from the same parental strain (D3052), there is a built-in redundancy in the microbial assay. In general the two strains of a set respond to the same mutagen and such a pattern is sought. It is also anticipated that if a given strain, e.g. TA-1537, responds to a mutagen in nonactivation tests it will generally do so in activation tests. (The converse of this relationship is not expected.) While similar response patterns are not required for all mutagens, they can be used to enhance the reliability of an evaluation decision.

4. Reproducibility

If a chemical produces a response in a single test that cannot be reproduced in one or more additional runs, the initial positive test data loses significance.

The preceding criteria are not absolute and other extenuating factors may enter into a final evaluation decision. However, these criteria are applied to the majority of situations and are presented to aid those individuals not familiar with this procedure. As the data base is increased, the criteria for evaluation can be more firmly established.

VII. EXPLANATION OF EVALUATION PROCEDURES FOR SUSPENSION ASSAYS

Data obtained from mutagenicity tests are evaluated on a test by test basis followed by an examination of the total response pattern using all the data. To facilitate this type of evaluation, we have prepared two separate formats in which data are processed. The first is the Compound Summary Backup Detail Sheet, which details the essential raw data from each experiment showing surviving population counts, total mutant or revertant counts, as well as, calculated mutation frequencies. This format permits close examination of each set of test data. The following considerations are part of any assessment.

A. Surviving Population Counts

A certain level of chemically-induced toxicity is anticipated, but occasionally isolated tests or groups of tests show very low (<25%) survival compared to the tissue controls. Such isolated decreases may result from improper dilution procedures or defective growth media and decrease confidence in the calculated mutation frequencies especially if the total mutant counts appear unaffected. Data of this type are generally unacceptable and these experiments are routinely repeated at a lower dose level to reduce killing and increase confidence in the nature of the response.

B. Total Mutant Counts

For nonmutagens, the mutant/surviving population ratio should be roughly equivalent for each test point in a given experiment. If the cell number drops in response to killing, the mutant number should decrease proportionately. A mutagenic chemical, however, will produce an altered mutant/surviving population ratio. Mutant numbers as well as calculated frequencies are compared to the negative control data. In certain instances, the mutant frequencies will increase with little or no change in the absolute number of mutants especially where the test chemical is toxic. Data of this type, although not necessarily aberrant, or even rare, must be viewed with special care to ensure that the increased frequencies were not the result of selective toxicity of the test chemical for the his cells. This phenomenon, referred to as selection, can lead to erroneous conclusions. Thus we attempt to keep the surviving population of cells high and look for positive responses that show increases in both numbers of mutants and mutation frequencies. Again, occasional isolated fluctuations in mutant counts are found that can be attributed to improper pipetting or media contamination. These fluctuations are usually easy to identify by inspection of the other data points in the experiment which will be negative.

C. Dose Response Phenomena

Dose-related increases in mutants and mutation frequencies are the most convincing data to have in assessing mutagenic activity of chemicals. In some cases, however, dose-related increases are not observed for mutagens. This depends considerably on the dose levels selected. The figure on the following page illustrates how one might obtain various types of dose-related responses by a mutagen based solely on dose selection. It also emphasizes the need to keep dose levels within a relatively low range of toxicity so that data are consistently on the uphill side of the hypothetical curve.

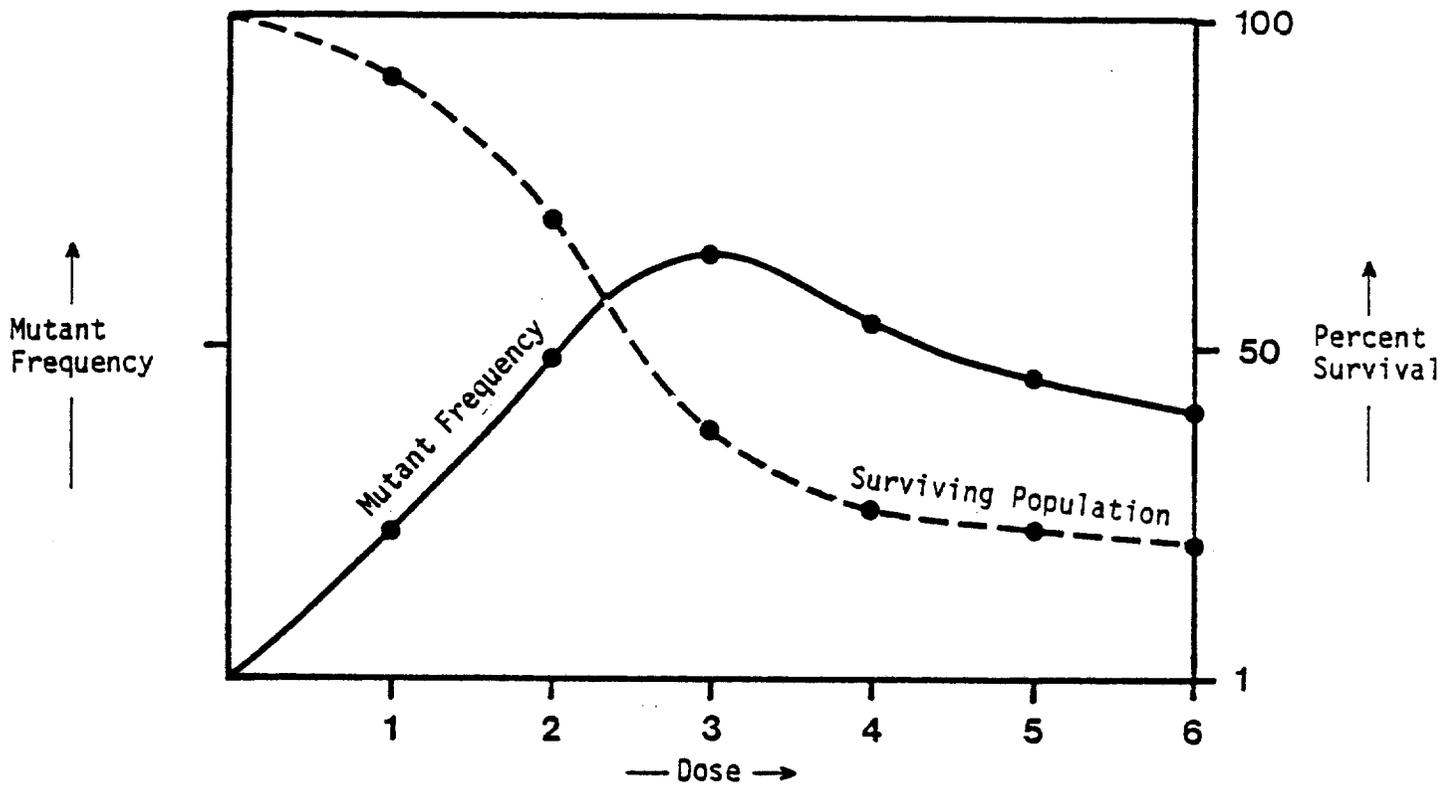
D. Control Tests

Positive and negative control tests are conducted with each experiment and consist of direct acting positive agents for nonactivation assays and chemicals that require metabolic transformation for activation assays. In nonactivation assays, the NAN control contain the test chemical solvent plus cells, but no chemical, and is used as a reference to assess the level of response obtained in the various tests. It is not possible at this time to put precise cut-off points where negative responses become positive responses. A statistical component for our computer program is under development and will be included when available. Positive controls are only used as relative reference points and to demonstrate that the system is functioning with known mutagens. In activation assays, three types of negative controls are run: (1) A solvent control minus the chemical and minus the activation system (A-C); (2) a control plus the positive control chemical minus the activation system (A+C); and (3) a control containing the activation system and the test chemical solvent (ALI or ALU). All three controls are used collectively to assess the level of response in the various activation tests. A chemical may appear positive when compared to an A-C control but not when compared to an A+T control. The value of each of the above controls with respect to their weight in evaluation is $ALI \text{ or } ALU > A-C > A+C$.

The other data format is the Compound Frequency Summary Report sheet in which all the calculated frequencies obtained for a given compound are displayed in a table. This format permits an overview of all data. The points form a matrix of information that should present a consistent pattern. Nonmutagens should produce a matrix with data frequencies clustered around the negative control values. Occasional random high or low fluctuations are not uncommon and seldom indicate true genetic activity. Mutagenic chemicals should, on the other hand, produce a set of consistent responses that demonstrate a logical pattern. The patterns depend on the mutagenic specificity of the chemical but can be easily recognized in the Compound Frequency Summary Report format.

These mutagenicity assays are designed to optimize the probability of recognizing mutagens from nonmutagens and, in most cases, they work well. Occasionally, the data points are such that a definitive conclusion cannot be made without additional data.

HYPOTHETICAL MUTATION AND TOXICITY KINETICS



HYPOTHETICAL EXPERIMENT

- (1) Dose levels
1, 2 & 3 were used
- (2) Dose levels
2, 3 & 4 were used
- (3) Dose levels
3, 4 & 5 were used

OBSERVED DOSE RESPONSE

A typical positive dose response set of data would be obtained.

The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.

Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX
Tabulation of Data



BIONETICS

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT		CONTRACT	DETECTOR	SPECIES	PROJECT	DATE	
713005		223-76-2102	TA100		2672	07/25/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1237	0989	79.95	0
	NAP		EMS 0.066%	1429	1709	119.59	0
000081254	NA1		0017-2 PCT.	1020	0486	47.65	0
000081254	NA2		0085-3 PCT.	1108	0338	30.51	0
000081254	NA3		0425-4 PCT.	1041	0302	29.01	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/25/77			
EXPERIMENT 712506	DETECTOR TA1535	SPECIES	/				
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0634	0012	1.89	0
	NAP		EMS 0.2%	0658	0576	87.54	0
000081254	NA1		0017-2 PCT.	0703	0017	2.42	0
000081254	NA2		0085-3 PCT.	0711	0017	2.39	0
000081254	NA3		0425-4 PCT.	0648	0016	2.47	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102		PROJECT 2672		DATE - 07/25/77			
EXPERIMENT 711301		DETECTOR TA1537	SPECIES	/			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0487	0019	3.90	0
	NAP		QM 13 UG/ML	0827	1063	128.54	0
000081254	NA1		0017-2 PCT.	0293	0016	5.46	0
000081254	NA2		0085-3 PCT.	0574	0028	4.88	0
000081254	NA3		0425-4 PCT.	0608	0014	2.30	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT		CONTRACT	223-76-2102	SPECIES		PROJECT	2672	DATE	- 07/25/77
		713006	DETECTOR TA1538			/			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM		
		NAN	SOLVENT	0730	0085	11.64	0		
		NAP	NF 667 UG/ML	0460	0512	111.30	0		
000081254	NA1		0017-2 PCT.	0637	0073	11.46	0		
000081254	NA2		0085-3 PCT.	0832	0078	9.38	0		
000081254	NA3		0425-4 PCT.	0680	0040	5.88	0		

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT		CONTRACT	223-76-2102	SPECIES		PROJECT	2672	DATE	- 07/25/77
		712508	DETECTOR TA98			/			
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8		CONTAM	
		NAN	SOLVENT	1071	0060	5.60		0	
		NAP	NF 667 UG/ML	0918	1309	142.59		0	
000081254	NA1		0017- 2 PCT.	1059	0072	6.80		0	
000081254	NA2		0085- 3 PCT.	0657	0073	11.11		0	
000081254	NA3		0425-4 PCT.	0753	0061	8.10		0	

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 714303 DETECTOR 000004 SPECIES / DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0878	0021	0041	2.39	4.67	0
	NAP		EMS 1.0 %	0733	2134	0412	291.13	56.21	0
000081254	NA1		0005-0 PCT.	0955	0018	0044	1.88	4.61	0
000081254	NA2		0025-1 PCT.	1099	0017	0046	1.55	4.19	0
000081254	NA3		0125-2 PCT.	1032	0023	0045	2.23	4.36	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713002 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1788	0420	23.49	0
	A-C		SOLVENT	1691	0281	16.62	0
	ALI		TISSUE	1866	1660	88.96	0
	ALU		TISSUE	1924	0389	20.22	0
	ACP	LI	DMN 90 UM/ML	1415	1467	103.67	0
	ACP	LU	DMN 90 UM/ML	1534	0464	30.25	0
000081254	ACT	LI1	0017-2 PCT.	1413	0488	34.54	0
000081254	ACT	LI2	0085-3 PCT.	1584	0529	33.40	0
000081254	ACT	LI3	0425-4 PCT.	1671	0411	24.60	0
000081254	ACT	LU1	0017- 2 PCT.	1758	0340	19.34	0
000081254	ACT	LU2	0085-3 PCT.	1151	0336	29.19	0
000081254	ACT	LU3	0425-4 PCT.	1740	0374	21.49	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 714010 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0770	0053	6.88	0
	A-C		SOLVENT	1136	0073	6.43	0
	ALI		TISSUE	0299	0021	7.02	0
	ALU		TISSUE	0737	0081	10.99	0
	ACP	LI	DMN 90 UM/ML	0398	0436	109.55	0
	ACP	LU	DMN 90 UM/ML	0730	0069	9.45	0
000081254	ACT	LI1	0017-2 PCT.	0771	0020	2.59	2
000081254	ACT	LI2	0085-3 PCT.	1217	0034	2.79	2
000081254	ACT	LI3	0425-4 PCT.	1416	0051	3.60	2
000081254	ACT	LU1	0017- 2PCT.	1456	0069	4.74	2
000081254	ACT	LU2	0085-3 PCT.	1411	0073	5.17	2
000081254	ACT	LU3	0425-4 PCT.	1756	0197	11.22	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 711202 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A-C		AMQ 333 UG/ML	0492	0164	33.33	0
	A-C		SOLVENT	0559	0017	3.04	0
	ALI		TISSUE	0552	0026	4.71	0
	ALU		TISSUE	0447	0027	6.04	0
	ACP	LI	AMQ 333 UG/ML	0586	0085	14.51	0
	ACP	LU	AMQ 333 UG/ML	0302	0149	49.34	0
000081254	ACT	LI1	0017-2 PCT.	0308	0011	3.57	0
000081254	ACT	LI2	0085-3 PCT.	0454	0008	1.76	0
000081254	ACT	LI3	0425-4 PCT.	0293	0015	5.12	0
000081254	ACT	LU1	0017-2 PCT.	0117	0029	24.79	0
000081254	ACT	LU2	0085-3 PCT.	0178	0014	7.87	0
000081254	ACT	LU3	0425-4 PCT.	0137	0024	17.52	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
EXPERIMENT 715770 DETECTOR TA1537 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	ALI		TISSUE	0572	0033	5.77	0
	ALU		TISSUE	0506	0039	7.71	0
000081254	ACT	LU1	0017-2 PCT.	0502	0025	4.98	0
000081254	ACT	LU2	0085-3 PCT.	0497	0019	3.82	0
000081254	ACT	LU3	0425-4 PCT.	0467	0013	2.78	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713004 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0639	0044	6.89	0
	A-C		SOLVENT	0623	0043	6.90	0
	ALI		TISSUE	0578	0056	9.69	0
	ALU		TISSUE	0389	0056	14.40	0
	ACP	LI	ANTH 67 UG/ML	0610	0644	105.57	0
	ACP	LU	ANTH 67 UG/ML	0440	0069	15.68	0
000081254	ACT	LI1	0017-1 PCT.	0390	0043	11.03	1
000081254	ACT	LI2	0085-3 PCT.	0438	0040	9.13	0
000081254	ACT	LI3	0425-4 PCT.	0537	0029	5.40	1
000081254	ACT	LU1	0017-2 PCT.	0545	0050	9.17	0
000081254	ACT	LU2	0085-3 PCT.	0486	0053	10.91	0
000081254	ACT	LU3	0425-4 PCT.	0663	0086	12.97	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 713001		CONTRACT 223-76-2102 DETECTOR TA98		PROJECT 2672 SPECIES ICRFLO/MOUSE		DATE - 07/25/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	2276	0110	4.83	0
	A-C		SOLVENT	1526	0082	5.37	0
	ALI		TISSUE	1325	0128	9.66	0
	ALU		TISSUE	1113	0130	11.68	0
	ACP	LI	ANTH 67 UG/ML	0830	0415	50.00	0
	ACP	LU	ANTH 67 UG/ML	0882	0129	14.63	0
000081254	ACT	L11	0017-2 PCT.	0735	0042	5.71	0
000081254	ACT	L12	0085-3 PCT.	0810	0135	16.67	0
000081254	ACT	L13	0425-4 PCT.	1054	0126	11.95	0
000081254	ACT	LU1	0017-2 PCT.	0973	0057	5.86	0
000081254	ACT	LU2	0085-3 PCT.	1130	0081	7.17	0
000081254	ACT	LU3	0425-4 PCT.	1414	0053	3.75	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 714710 DETECTOR 000004 SPECIES ICRFLO/MOUSE DATE - 07/25/77

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	1418	0149	0111	10.51	7.83	0
	A-C		SOLVENT	1408	0255	0145	18.11	10.30	0
	ALI		TISSUE	0917	0252	0133	27.48	14.50	0
	ALU		TISSUE	0879	0240	0166	27.30	18.89	0
	ACP	LI	DMN 90 UM/ML	0784	0498	0354	63.52	45.15	0
	ACP	LU	DMN 90 UM/ML	0714	0264	0148	36.97	20.73	0
000081254	ACT	LI1	0005-0 PCT.	0827	0162	0161	19.59	19.47	0
000081254	ACT	LI2	0025-1 PCT.	0843	0204	0124	24.20	14.71	0
000081254	ACT	LI3	0125-2 PCT.	1393	0240	0157	17.23	11.27	0
000081254	ACT	LU1	0005-0 PCT.	0660	0177	0161	26.82	24.39	0
000081254	ACT	LU2	0025-1 PCT.	1377	0236	0149	17.14	10.82	0
000081254	ACT	LU3	0125-2 PCT.	1366	0244	0151	17.86	11.05	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713012 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1195	0370	30.96	0
	A-C		SOLVENT	1493	0136	9.11	0
	ALI		TISSUE	0845	0476	56.33	0
	ALU		TISSUE	1174	0359	30.58	0
	ACP	LI	DMN 90 UM/ML	0658	1082	164.44	0
	ACP	LU	DMN 90 UM/ML	1329	0330	24.83	0
000081254	ACT	LI1	0017-2 PCT.	0941	0272	28.91	0
000081254	ACT	LI2	0085-3 PCT.	0584	0218	37.33	0
000081254	ACT	LI3	0425-4 PCT.	0629	0213	33.86	0
000081254	ACT	LU1	0017-2 PCT.	0955	0261	27.33	0
000081254	ACT	LU2	0085-3 PCT.	0817	0258	31.58	0
000081254	ACT	LU3	0425-4 PCT.	0923	0314	34.02	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713009 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	OKG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	1660	0078	4.70	0
	A-C		SOLVENT	1551	0054	3.48	0
	ALI		TISSUE	1353	0063	4.66	0
	ALU		TISSUE	1429	0093	6.51	0
	ACP	LI	DMN 90 UM/ML	0400	0930	232.50	0
	ACP	LU	DMN 90 UM/ML	0326	0013	3.99	0
000081254	ACT	LI1	0017-2 PCT.	0572	0015	2.62	0
000081254	ACT	LI2	0085-3 PCT.	1200	0061	5.08	0
000081254	ACT	LI3	0425-4 PCT.	1149	0075	6.53	0
000081254	ACT	LU1	0017-2 PCT.	1258	0058	4.61	0
000081254	ACT	LU2	0085-3 PCT.	1315	0065	4.94	0
000081254	ACT	LU3	0425-4 PCT.	1430	0018	1.26	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713008 DETECTOR TA1537 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A•C		AMQ 333 UG/ML	2848	0108	3.79	0
	A-C		SOLVENT	2447	0082	3.35	0
	ALI		TISSUE	1471	0108	7.34	0
	ALU		TISSUE	0882	0114	12.93	0
	ACP	LI	AMQ 333 UG/ML	2030	2362	116.35	0
	ACP	LU	AMQ 333 UG/ML	0954	0260	27.25	0
000081254	ACT	LI1	0017-2 PCT.	1617	0105	6.49	0
000081254	ACT	LI2	0085-3 PCT.	1334	0063	4.72	0
000081254	ACT	LI3	0425-4 PCT.	1552	0075	4.83	0
000081254	ACT	LU1	0017-2 PCT.	0590	0093	15.76	0
000081254	ACT	LU2	0085-3 PCT.	0606	0085	14.03	0
000081254	ACT	LU3	0425-4 PCT.	0618	0052	8.41	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713010 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0863	0039	4.52	0
	A-C		SOLVENT	0716	0039	5.45	0
	ALI		TISSUE	0721	0016	2.22	0
	ALU		TISSUE	0489	0039	7.98	0
	ACP	LI	ANTH 67 UG/ML	0802	1478	184.29	0
	ACP	LU	ANTH 67 UG/ML	1476	0376	25.47	0
000081254	ACT	LI1	0017-2 PCT.	0301	0028	9.30	0
000081254	ACT	LI2	0085-3 PCT.	0463	0021	4.54	0
000081254	ACT	LI3	0425-4 PCT.	0273	0011	4.03	0
000081254	ACT	LU1	0017-2 PCT.	0457	0013	2.84	0
000081254	ACT	LU2	0085-3 PCT.	0408	0021	5.15	0
000081254	ACT	LU3	0425-4 PCT.	0816	0043	5.27	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 716704 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPULATION EP+6	MUTATION EP+0	FREQUENCY EP-8	CONTAMINATION
	A+C		ANTH 67 UG/ML	0521	0030	5.76	0
	A-C		SOLVENT	0555	0043	7.75	0
	ALI		TISSUE	0693	0049	7.07	0
	ALU		TISSUE	0684	0051	7.46	0
	ACP	LI	ANTH 67 UG/ML	0684	0868	126.90	0
	ACP	LU	ANTH 67 UG/ML	0578	0048	8.30	0
000081254	ACT	LI1	0017-2 PCT.	0710	0031	4.37	0
000081254	ACT	LI2	0085-3 PCT.	0544	0035	6.43	0
000081254	ACT	LI3	0425-4 PCT.	0562	0044	7.83	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

EXPERIMENT 713011		CONTRACT 223-76-2102 DETECTOR TA98		PROJECT 2672 SPECIES SPRDAW/RAT		DATE - 07/25/77	
COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1166	0037	3.17	0
	A-C		SOLVENT	1775	0079	4.45	0
	ALI		TISSUE	0530	0076	14.34	0
	ALU		TISSUE	0575	0081	14.09	0
	ACP	LI	ANTH 67 UG/ML	0462	0686	148.48	0
	ACP	LU	ANTH 67 UG/ML	0547	0340	62.16	0
000081254	ACT	LI1	0017-2 PCT.	0523	0037	7.07	0
000081254	ACT	LI2	0085-3 PCT.	0596	0045	7.55	0
000081254	ACT	LI3	0425-4 PCT.	0576	0034	5.90	0
000081254	ACT	LU1	0017-2 PCT.	0433	0036	8.31	0
000081254	ACT	LU2	0085-3 PCT.	0882	0068	7.71	0
000081254	ACT	LU3	0425-4 PCT.	0541	0050	9.24	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 714711 DETECTOR 0000D4 SPECIES SPRDAW/RAT DATE - 07/25/77

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	0093	0056	8.97	5.40	0
	A-C		SOLVENT	1473	0121	0080	8.21	5.43	0
	ALI		TISSUE	0788	0101	0066	12.82	8.38	0
	ALU		TISSUE	0848	0104	0073	12.26	8.61	0
	ACP	LI	DMN 90 UM/ML	0887	0489	0600	55.13	67.64	0
	ACP	LU	DMN 90 UM/ML	0822	0082	0052	9.98	6.33	0
000081254	ACT	LI1	0005-0 PCT.	1008	0111	0072	11.01	7.14	0
000081254	ACT	LI2	0025-1 PCT.	0874	0097	0059	11.10	6.75	0
000081254	ACT	LI3	0125-2 PCT.	0844	0080	0058	9.48	6.87	0
000081254	ACT	LU1	0005-0 PCT.	0997	0092	0060	9.23	6.02	0
000081254	ACT	LU2	0025-1 PCT.	1077	0098	0062	9.10	5.76	0
000081254	ACT	LU3	0125-2 PCT.	0888	0104	0038	11.71	4.28	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 708304 DETECTOR TA100 SPECIES RHEBUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		DMN 90 UM/ML	0711	0559	78.62	0
	A-C		SOLVENT	0398	0289	72.61	0
	ALI		TISSUE	0520	0477	91.73	0
	ALU		TISSUE	0695	0549	78.99	0
	ACP	LI	DMN 90 UM/ML	0635	1293	203.62	0
	ACP	LU	DMN 90 UM/ML	0507	0490	96.65	0
000081254	ACT	LI1	0017-2 PCT.	0486	0385	79.22	0
000081254	ACT	LI2	0085-3 PCT.	0604	0476	78.81	0
000081254	ACT	LI3	0425-4 PCT.	0549	0396	72.13	0
000081254	ACT	LU1	0017-2 PCT.	0521	0437	83.88	0
000081254	ACT	LU2	0085-3 PCT.	0658	0408	62.01	0
000081254	ACT	LU3	0425-4 PCT.	0733	0480	65.48	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 715803 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-B	CONTAM
	A+C		DMN 90 UM/ML	0722	0070	9.70	0
	A-C		SOLVENT	0734	0101	13.76	0
	ALI		TISSUE	0597	0035	5.86	0
	ALU		TISSUE	0400	0051	12.75	0
	ACP	LI	DMN 90 UM/ML	0601	0406	67.55	0
	ACP	LU	DMN 90 UM/ML	0526	0067	12.74	0
000081254	ACT	LI1	0017-2 PCT.	0250	0034	13.60	2
000081254	ACT	LI2	0085-3 PCT.	0507	0031	6.11	2
000081254	ACT	LI3	0425-4 PCT.	0460	0029	6.30	0
000081254	ACT	LU1	0017-2 PCT.	0753	0073	9.69	2
000081254	ACT	LU2	0085-3 PCT.	0701	0062	8.84	2
000081254	ACT	LU3	0425-4 PCT.	0696	0057	8.19	2

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 713101 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		AMQ 333 UG/ML	0723	0010	1.38	0
	A-C		SOLVENT	0760	0036	4.74	0
	ALI		TISSUE	0519	0051	9.83	0
	ALU		TISSUE	0333	0092	27.63	0
	ACP	LI	AMQ 333 UG/ML	0391	0679	173.66	0
	ACP	LU	AMQ 333 UG/ML	0550	0033	6.00	0
000081254	ACT	LI1	0017-2 PCT.	0819	0074	9.04	0
000081254	ACT	LI2	0085-3 PCT.	0809	0094	11.62	0
000081254	ACT	LI3	0425-4 PCT.	1183	0046	3.89	0
000081254	ACT	LU1	0017-2 PCT.	0448	0046	10.27	0
000081254	ACT	LU2	0085-3 PCT.	0656	0063	9.60	0
000081254	ACT	LU3	0425-4 PCT.	0432	0060	13.89	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 712505 DETECTOR TA1538 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	1093	0085	7.78	0
	A-C		SOLVENT	1176	0031	2.64	0
	ALI		TISSUE	0535	0112	20.93	0
	ALU		TISSUE	0890	0062	6.97	0
	ACP	LI	ANTH 67 UG/ML	1031	1134	109.99	0
	ACP	LU	ANTH 67 UG/ML	0808	0096	11.88	0
000081254	ACT	LI1	0017-2 PCT.	0797	0084	10.54	0
000081254	ACT	LI2	0085-3 PCT.	1212	0102	8.42	0
000081254	ACT	LI3	0425-4 PCT.	0965	0107	11.09	0
000081254	ACT	LU1	0017-2 PCT.	0434	0073	16.82	0
000081254	ACT	LU2	0085-3 PCT.	0816	0095	11.64	0
000081254	ACT	LU3	0425-4 PCT.	0588	0072	12.24	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 710895 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 07/25/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-8	CONTAM
	A+C		ANTH 67 UG/ML	0632	0144	22.78	0
	A-C		SOLVENT	0587	0129	21.98	0
	ALI		TISSUE	0452	0143	31.64	0
	ALU		TISSUE	0735	0148	20.14	0
	ACP	LI	ANTH 67 UG/ML	0624	1190	190.71	0
	ACP	LU	ANTH 67 U6/ML	0584	0158	27.05	0
000081254	ACT	LI1	0017-2 PCT.	0793	0185	23.33	0
000081254	ACT	LI2	0085-3 PCT.	0611	0184	30.11	0
000081254	ACT	LI3	0425-4 PCT.	0576	0169	29.34	0
000081254	ACT	LU1	0017-2 PCT.	0779	0187	24.01	0
000081254	ACT	LU2	0085-3 PCT.	0633	0192	30.33	0
000081254	ACT	LU3	0425-4 PCT.	0726	0192	26.45	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
 COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 223-76-2102 PROJECT 2672
 EXPERIMENT 714739 DETECTOR 0000D4 SPECIES RHESUS/MONKEY DATE - 07/25/77

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	0710	0124	0063	17.46	8.87	0
	A-C		SOLVENT	0798	0114	0058	14.29	7.27	0
	ALI		TISSUE	0795	0153	0087	19.25	10.94	0
	ALU		TISSUE	0803	0158	0088	19.68	10.96	0
	ACP	LI	DMN 90 UM/ML	0766	0687	0523	89.69	68.28	0
	ACP	LU	DMN 90 UM/ML	0683	0128	0053	18.74	7.76	0
000081254	ACT	LI1	0005-0 PCT.	0730	0096	0052	13.15	7.12	0
000081254	ACT	LI2	0025-1 PCT.	0755	0109	0068	14.44	9.01	0
000081254	ACT	LI3	0125-2 PCT.	0765	0152	0069	19.87	9.02	0
000081254	ACT	LU1	0005-0 PCT.	0684	0089	0067	13.01	9.80	0
000081254	ACT	LU2	0025-1 PCT.	0752	0090	0067	11.97	8.91	0
000081254	ACT	LU3	0125-2 PCT.	0772	0138	0059	17.88	7.64	0