Litton BIONETICS

Mutagenic Evaluation of Compound FDA 75-72 Zinc Stearate USP Final Report

MUTAGENICITY EVALUATION

OF

FDA 75-72

ZINC STEARATE USP

000557-05-1

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000557-05-1

FINAL REPORT

SUBMITTED TO

DEPARTMENT OF HEALTH, EDUCATION AND WELFARE
FOOD AND DRUG ADMINISTRATION
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BIONETICS

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EVALUATION SUMMARY

The test compound, FDA 75-72, 000557-05-1, did not exhibit mutagenic activity in any of the assays employed in these studies.

DATE: May 10, 1977

SPONSOR: U.S. Food and Drug Administration

SUBJECT: Evaluation of Test Compound FDA 75-72 Zinc Stearate USP 000557-05-1

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: October 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:

Component	Final Concentration/ml
1. TPN (sodium salt)	4 µmoles
2. Glucose-6-phosphate	5 µmoles
3. Sodium phosphate (dibasic)	100 µmoles
4. $MgCl_2$	8 µmoles
5. KCl	33 µmoles
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

Assay	Chemical[a]	Solvent	Probable Mutagenic Specificity
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: 000557-05-1
Zinc Stearate USP
FDA 75-72

2. Test solvent: DMF

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:

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:

	Percent Concentration	
Test Doses	Bacteria	Yeast
1/4 50% Survival	0.02875	1.250
1/2 50% Survival	0.0575	2.500
50% Survival	0.115	5.00

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: 000567051

B. TEST DATE: DEC. 16, 1976

TEST		SPECIES	TISSUE	REVERTANTS PER PLATE TA-1535 1	TA-1535 2	TA-1537 1	TA-1537 2	TA-1538 1	TA-1538 2	TA-98 1	TA-98 2	TA-100 1	TA-100 2
1. NON-ACTIVATION													
SOLVENT CONTROL*		---	---	19	26	17	15	18	21	53	47	63	141
POSITIVE CONTROL**		---	---	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000
TEST	0.11500 %	---	---	13	15	11	18	14	18	33	29	64	61
	0.05750 %	---	---	21	31	21	19	17	18	49	44	104	115
	0.02875 %	---	---	20	21	17	21	25	24	51	52	142	149
2. ACTIVATION													
SOLVENT CONTROL*		MOUSE	LIVER	32	28	25	30	34	32	47	49	160	163
		RAT	LIVER	30	29	29	34	23	19	38	47	219	195
		MONKEY	LIVER	26	32	18	23	29	40	42	45	176	194
POSITIVE CONTROL***		MOUSE	LIVER	793	851	252	297	>1000	>1000	>1000	>1000	>1000	>1000
		RAT	LIVER	699	598	264	357	719	894	>1000	>1000	>1000	>1000
		MONKEY	LIVER	539	444	155	181	514	525	>1000	940	>1000	>1000
TEST	0.11500 %	MOUSE	LIVER	15	19	11	15	12	11	22	19	44	57
	0.05750 %	MOUSE	LIVER	10	13	10	11	17	16	34	30	90	95
	0.02875 %	MOUSE	LIVER	12	28	15	12	7	10	50	45	106	97
	0.11500 %	RAT	LIVER	21	17	13	10	17	15	25	20	47	53
	0.05750 %	RAT	LIVER	16	12	14	17	11	12	28	35	70	73
	0.02875 %	RAT	LIVER	11	19	19	13	12	17	42	41	120	117
	0.11500 %	MONKEY	LIVER	11	13	18	14	16	15	17	15	47	74
	0.05750 %	MONKEY	LIVER	23	16	10	19	11	12	41	37	106	103
	0.02875 %	MONKEY	LIVER	17	16	15	14	19	13	47	46	114	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	NNNG	2 UG/PLATE		*** TA-1535	ANTH	100 UG/PLATE
TA-1537	9AA	20 UG/PLATE		TA-1537	AHQ	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	NNNG	2 UG/PLATE		TA-100	ANTH	100 UG/PLATE

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

LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EX#34

COMPOUND FREQUENCY SUMMARY REPORT 02/27/77

NONACTIVATION COMPOUND 000557051

TEST	ORG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	TA98 HIS EX-8	000004 ADE EX-5	000004 THY EX-5	
NAN		56.09	5.34	28.06	10.96	3.79	13.05	19.75	5.37	CONTROLS
NAP		164.41	587.36	137.92	187.12	141.25		101.54	67.35	
NA1		55.61	7.53	16.48	8.77	16.67	5.19	28.46	12.62	TEST DATA
NA2		34.37	6.43	17.08	14.11	4.85		15.63	10.76	
NA3		47.94	4.20	22.21	9.92	3.28		6.84	3.66	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EX#34

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

SPECIES ICRFLO/MOUSE COMPOUND 000557651

TEST	GRP	TA100 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	TA98 HIS EX-8	000004 ABE EX-5	000004 TRY EX-5	
ACT	A+C	104.08	9.01	5.44	7.54	2.55	14.04	11.23	NEGATIVE CONTROLS
ACT	A-C	74.56	8.33	4.77	10.07	2.07	16.96	13.67	
ACT	ALI	109.86	11.11	4.60	9.73	5.00	19.69	12.92	
ACT	ALU	70.31	8.72	5.66	10.04	4.99	20.41	15.61	
ACT	PLI	251.33	179.70	141.85	222.19	50.00	76.42	76.94	POSITIVE CONTROLS
ACT	PLU	94.27	11.70	7.13	47.40	24.95	10.36	11.81	
ACT	LII	16.02	9.33	3.15	17.66	9.22	14.04	4.63	TEST COMPOUND
ACT	LI2	71.14	9.93	2.03	11.77	5.05	10.57	5.51	
ACT	LI3	72.93	9.40	5.41	10.07	5.61	11.11	6.67	
ACT	LU1	15.64	10.44	3.50	12.82	10.02	11.47	12.07	
ACT	LU2	79.24	9.02	3.30	12.37	5.03	11.34	9.64	
ACT	LU3	96.55	9.07	6.62	12.40	7.19	10.33	5.80	

LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EX834

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

SPECIES SPRGAW/RAT COMPOUND 000557051

TEST	ORG	TA100 HIS EX-R	TA1535 HIS EX-R	TA1537 HIS EX-R	TA1538 HIS EX-R	TA98 HIS EX-R	000004 ADE EX-S	000004 TRY EX-S	
ACT	A+C	161.00	7.00	3.77	46.02	2.66	13.64	14.19	NEGATIVE CONTROLS
ACT	A-C	128.57	7.78	1.64	9.88	6.37	16.00	11.09	
ACT	ALI	128.30	2.66	2.05	13.54	22.50	10.91	9.31	
ACT	ALU	111.50	3.05	2.05	12.30	9.64	17.03	8.67	
ACT	PLI	171.97	138.63	89.01	128.54	299.56	97.06	72.48	POSITIVE CONTROLS
ACT	PLU	112.05	4.45	8.17	32.50	49.31	10.15	3.16	
ACT	LII	8.97	7.15	4.00	4.46	15.17	20.70	6.84	TEST COMPOUND
ACT	LI2	124.23	3.07	2.03	4.68	5.35	10.06	9.48	
ACT	LI3	128.36	4.70	3.10	8.01	10.14	14.90	11.04	
ACT	LU1	9.81	0.07	3.06	4.45	8.35	22.09	13.95	
ACT	LU2	93.54	5.74	2.35	7.27	9.19	11.42	8.90	
ACT	LU3	114.64	4.38	1.21	11.92	8.56	14.91	8.81	

LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
REPORT EXP34

COMPOUND FREQUENCY SUMMARY REPORT 02/22/77

SPECIES RHESUS/MONKEY COMPOUND 000557051

TEST	DHG	TA100 HIS EX-8	TA1535 HIS EX-8	TA1535 HIS EX-8	TA1537 HIS EX-8	TA1538 HIS EX-8	1A98 HIS EX-8	000004 ADE EX-5	000004 TRY EX-5	
ACT	A+C	53.27	2.58		2.45	9.78	5.79	19.41	10.65	NEGATIVE CONTROLS
ACT	A-C	57.26	2.21		2.44	13.84	5.50	10.48	9.49	
ACT	ALI	82.47	3.39	8.45	2.47	12.29	9.56	16.49	10.91	
ACT	ALU	72.94	2.78		1.10	11.95	10.63	15.33	10.17	
ACT	PLI	195.14	36.71		81.54	137.85	123.31	93.48	73.43	POSITIVE CONTROLS
ACT	PLU	141.96	2.37		4.66	15.42	17.43	25.84	17.05	
ACT	LI1	84.04	8.88	6.69	2.71	12.59	7.89	19.00	9.85	TEST COMPOUND
ACT	LI2	126.09	2.04		1.02	14.28	11.89	14.76	7.79	
ACT	LI3	91.18	2.36		1.52	13.90	13.24	13.11	8.93	
ACT	LU1	114.99	8.00	9.62	5.54	13.84	2.36	9.92	3.97	
ACT	LU2	93.95	3.80		1.02	11.63	11.51	21.15	12.24	
ACT	LU3	124.68	2.09		1.75	11.34	9.68	14.29	9.78	

DATA TABLE TERMS AND ABBREVIATIONS

ABBREVIATION OR TERM	DEFINITION OR EXPLANATION
COMPOUND	Client designated compound number appears in this column.
TEST CODES	NAN = Nonactivation: Solvent Control NAP = Nonactivation: Positive Control NA1 = Nonactivation: Test Compound Dose 1 NA2, etc. = Reflects the other dose level(s) 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 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
CONCENTRATION	All test compound dose levels are expressed as a whole number followed by an exponent (negative) identified by the appropriate units. Example: 0025-2PCT = 0.25 percent concentration
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 = 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.

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 (*Macaca mulatta*)
MIXEDB	Dog, Mixed Breed
NEWZEA	New Zealand White Rabbit
UG	Microgram
UM	Micromole
ADE	Adenine
TRY	Tryptophan
DMF	N, N-Dimethyl Formamide

V. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, FDA 75-72, 000557-05-1, 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. The NA1 dose with TA-98 was repeated because of the low plate counts obtained in the initial test which were probably due to a dilution error.

3. Activation suspension tests

The results of these tests were negative. The L11 and LU1 doses using monkey tissue were repeated with TA-1535 because of the low population and revertant counts obtained in the initial test which were probably due to a dilution error.

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 75-72, 000557-05-1, did not exhibit mutagenic activity in any of the assays employed in these studies.

Submitted by:

David J. Brusick, Ph.D. Date 5/11/77
Director
Department of Genetics

Reviewed by:

Robert J. Weir, Ph.D. Date 5/11/77
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 value.

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 convertant 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.

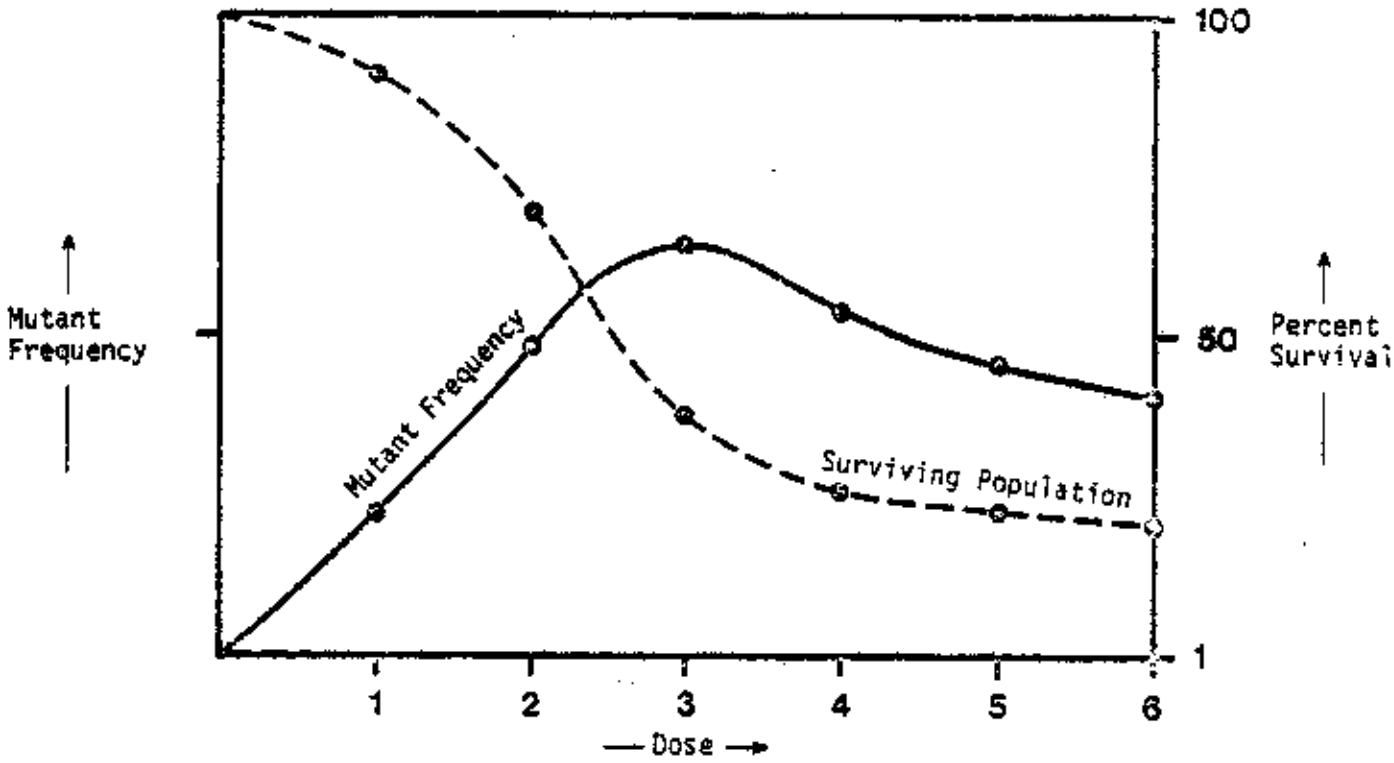
D. Control Tests

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 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	OBSERVED DOSE RESPONSE
(1) Dose levels 1,2 & 3 were used	A typical positive dose response set of data would be obtained.
(2) Dose levels 2, 3 & 4 were used	The intermediate dose level shows a higher mutation frequency than both the low dose and the high dose.
(3) Dose levels 3, 4 & 5 were used	Here an inverted dose response would be observed with the highest dose level showing the lowest response.

APPENDIX

Tabulation of Data

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633704 DETECTOR 16100 SPECIES / DATE - 02/24/77

COMPOUND	TEST	DRG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	0797	0447	56.09	0
	NAP		EMS 0.066%	0005	1455	164.41	0
000557051	NA1		0115-3 PCT.	0223	0124	55.61	0
000557051	NA2		0575-4 PCT.	0099	0309	34.37	0
000557051	NA3		2075-5 PCT.	0751	0360	47.94	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102
EXPERIMENT 633402 DETECTOR TA1535 SPECIES / PROJECT 02672 DATE - 02/24/77

COMPOUND	TEST	DRG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	MAN		SOLVENT	0543	0029	5.34	0
	MAP		FMS 0.2%	0433	3710	587.36	0
000557051	MA1		0115-3 PCT.	0146	0011	7.53	0
000557051	MA2		0575-4 PCT.	0746	0040	6.43	0
000557051	MA3		2875-5 PCT.	1004	0042	4.20	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22374-2102 PROJECT 02672
EXPERIMENT 633444 DETECTOR TA1537 SPECIES / DATE - 02/24/77

COMPOUND	TEST	OPG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	NAN		SOLVENT	0643	0129	20.06	0
	NAP		ON 13 UG/ML	0240	0131	137.92	0
000557051	NA1		0115-3 PCT.	1207	0198	16.40	0
000557051	NA2		0575-4 PCT.	1441	0245	17.00	0
000557051	NA3		2875-5 PCT.	0700	0175	22.21	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633403 DETECTOR TA153R SPECIES / DATE - 02/24/77

COMPOUND	TEST	DOS ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	NAN		SOLVENT	0456	0050	10.96	0
	NAP		NF 667 UG/ML	0450	0057	107.32	0
000557051	NA1		0115-3 PCT.	0300	0027	8.77	0
000557051	NA2		0575-4 PCT.	0435	0044	10.11	0
000557051	NA3		2875-5 PCT.	0504	0050	9.92	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634120 DETECTOR TA98 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-8	CONTAM
	NAN		SOLVENT	1397	0053	3.79	0
	NAP		NF 667 UG/ML	1275	1001	141.25	0
000557051	NA1		0115-3 PCT.	0006	0001	16.67	0
000557051	NA2		0575-4 PCT.	1110	0045	4.05	0
000557051	NA3		2875-5 PCT.	1401	0046	3.28	0

REPORT FXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT T01303 DETECTOR TA98 SPECIES / DATE - 02/24/77

COMPOUND	TEST	DMG ID	CONCENTRATION	POPU EP+6	MUTI EP+8	FREQI EP-8	CONTAM
	NAM		SOLVENT	0498	0065	13.05	0
000557051	NAI		0115-3 PCT.	0231	0012	5.19	4

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2182 PROJECT 02672
EXPERIMENT A3490A DETECTOR 000004 SPECIES / DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0633	0125	0034	19.75	5.37	0
	NAP		EMS 1.0 %	0585	0594	0394	101.54	67.35	0
000557051	NA1		0005-0 PCT.	0523	0107	0066	20.46	12.62	0
000557051	NA2		0025-1 PCT.	0576	0090	0062	15.63	10.76	0
000557051	NA3		0125-2 PCT.	1340	0081	0049	6.04	3.66	0

REPORT EXN33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633802 DETECTOR TA100 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	A+C		DMM 90 UM/ML	0281	0370	104.00	0
	A-C		SOLVENT	0591	0417	70.56	0
	ALI		TISSUE	0507	0557	109.06	0
	ALU		TISSUE	0747	0585	78.31	0
	ACP	LI	DMM 90 UM/ML	0450	1131	251.33	0
	ACP	LU	DMM 90 UM/ML	0524	0494	94.27	0
000557051	ACT	L11	0115-3 PCT.	0231	0037	16.02	0
000557051	ACT	L12	0575-4 PCT.	0693	0493	71.14	0
000557051	ACT	L13	2875-5 PCT.	0857	0625	72.93	0
000557051	ACT	LU1	0115-3 PCT.	0275	0043	15.64	0
000557051	ACT	LU2	0575-4 PCT.	0602	0477	79.24	0
000557051	ACT	LU3	2875-5 PCT.	0579	0559	96.55	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 633501 DETECTOR TA1535 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUT1 EP+0	FREQ1 EP-0	CONTAM
	A+C		DMN 90 UM/ML	0433	0039	9.01	0
	A-C		SOLVENT	0444	0037	8.33	0
	ALI		TISSUE	0459	0051	11.11	0
	ALU		TISSUE	0447	0039	8.72	0
	ACP	LI	DMN 90 UM/ML	0445	0080	179.78	0
	ACP	LU	DMN 90 UM/ML	0416	0049	11.78	0
000557051	ACT	LI1	0115-3 PCT.	0461	0043	9.33	0
000557051	ACT	LI2	0575-4 PCT.	0433	0043	9.93	0
000557051	ACT	LI3	2875-5 PCT.	0468	0044	9.40	0
000557051	ACT	LU1	0115-3 PCT.	0458	0047	10.44	0
000557051	ACT	LU2	0575-4 PCT.	0438	0043	9.82	0
000557051	ACT	LU3	2875-5 PCT.	0430	0039	9.07	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2142 PROJECT 02672
EXPERIMENT T01005 DETECTOR T41537 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	DRG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	NAN		SOLVENT	0388	0004	1.30	0
	A+C		AMO 333 UG/ML	0533	0029	5.44	0
	A-C		SOLVENT	0688	0029	4.77	0
	AL1		TISSUE	0449	0021	4.68	0
	ALU		TISSUE	0583	0033	5.66	0
	ACP	LI	AMO 333 UG/ML	0466	0661	141.85	0
	ACP	LU	AMO 333 UG/ML	0561	0040	7.13	0
00055705J	ACT	L11	0115-3 PCT.	0444	0014	3.15	0
00055705J	ACT	L12	0575-4 PCT.	0707	0020	2.83	0
00055705J	ACT	L13	2875-5 PCT.	0702	0038	5.41	0
00055705L	ACT	LU1	0115-3 PCT.	0314	0011	3.50	0
00055705L	ACT	LU2	0575-4 PCT.	0364	0012	3.30	0
00055705L	ACT	LU3	2875-5 PCT.	0634	0042	6.62	0

REPORT EX933 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22336-2182 PROJECT 02672
EXPERIMENT 634106 DETECTOR TA1538 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU E+6	MUTI E+0	FREQ1 E-8	CONTAM
	A+C		ANTH 67 UG/ML	0716	0054	7.54	0
	A-C		SOLVENT	0561	0061	10.87	0
	ALI		TISSUE	0514	0050	9.73	0
	ALU		TISSUE	0488	0049	10.04	0
	ACP	LI	ANTH 67 UG/ML	0531	1183	222.79	0
	ACP	LU	ANTH 67 UG/ML	0543	0366	67.40	0
000557051	ACT	LI1	0125-3 PCT.	0470	0083	17.66	0
000557051	ACT	LI2	0578-4 PCT.	0603	0071	11.77	0
000557051	ACT	LI3	2475-5 PCT.	0576	0058	10.07	0
000557051	ACT	LU1	0125-3 PCT.	0507	0065	12.82	0
000557051	ACT	LU2	0575-4 PCT.	0405	0060	12.37	0
000557051	ACT	LU3	2075-5 PCT.	0516	0065	12.60	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2182 PROJECT 02672
EXPERIMENT 634104 DETECTOR TA98 SPECIES ICRFLO/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	A+C		ANTH 67 UG/ML	1605	0041	2.55	0
	A-C		SOLVENT	1531	0044	2.87	0
	ALI		TISSUE	1051	0061	5.80	0
	ALU		TISSUE	0941	0047	4.99	0
	ACP	LI	ANTH 67 UG/ML	1101	0605	58.00	0
	ACP	LU	ANTH 67 UG/ML	1010	0252	24.95	0
000557051	ACT	LI1	0115-3 PCT.	0553	0051	9.22	0
000557051	ACT	LI2	0575-4 PCT.	1043	0061	5.85	0
000557051	ACT	LI3	2875-5 PCT.	1141	0064	5.61	0
000557051	ACT	LU1	0115-3 PCT.	0385	0033	10.82	0
000557051	ACT	LU2	0575-4 PCT.	0892	0052	5.83	0
000557051	ACT	LU3	2875-5 PCT.	0998	0064	7.19	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634005 DETECTOR 000004 SPECIES (CAFLD/MOUSE DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMM 90 UM/ML	0740	0105	0004	14.04	11.23	0
	A-C		SOLVENT	0578	0090	0079	16.96	13.67	0
	ALI		TISSUE	0650	0120	0004	19.69	12.92	0
	ALU		TISSUE	0583	0119	0091	20.41	15.61	0
	ACP	LI	DMM 90 UM/ML	0386	0295	0297	76.42	76.94	0
	ACP	LU	DMM 90 UM/ML	0601	0125	0075	10.36	11.01	0
000557051	ACT	LI1	0005-0 PCT.	0691	0097	0032	14.04	4.63	0
000557051	ACT	LI2	0025-1 PCT.	0672	0071	0037	10.57	5.51	0
000557051	ACT	LI3	0125-2 PCT.	0765	0085	0051	11.11	6.67	0
000557051	ACT	LU1	0005-0 PCT.	0645	0074	0083	11.47	12.87	0
000557051	ACT	LU2	0025-1 PCT.	0591	0067	0057	11.34	9.64	0
000557051	ACT	LU3	0125-2 PCT.	0707	0073	0041	10.33	5.80	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634208 DETECTOR TA100 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		DMM 90 UM/ML	0441	0718	161.00	0
	A-C		SOLVENT	0644	0828	128.57	0
	ALT		TISSUE	0643	0825	128.30	0
	ALU		TISSUE	0717	0800	111.58	0
	ACP	LT	DMM 90 UM/ML	0365	0635	173.97	0
	ACP	LU	DMM 90 UM/ML	0581	0651	112.05	0
000557051	ACT	LT1	0115-3 PCT.	0591	0053	0.97	0
000557051	ACT	LT2	0575-4 PCT.	0487	0605	124.23	0
000557051	ACT	LT3	2075-5 PCT.	0555	0668	120.36	0
000557051	ACT	LU1	0115-3 PCT.	0591	0058	9.81	0
000557051	ACT	LU2	0575-4 PCT.	0712	0664	93.54	0
000557051	ACT	LU3	2075-5 PCT.	0608	0697	114.64	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02472
EXPERIMENT 203901 DETECTOR TA1535 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	A+C		DMN 98 UN/ML	0343	0024	7.00	0
	A-C		SOLVENT	0160	0014	7.78	0
	ALI		TISSUE	0527	0014	2.66	0
	ALU		TISSUE	0545	0021	3.85	0
	ACP	11	DMN 98 UN/ML	0422	0585	138.63	0
	ACP	10	DMN 98 UN/ML	0472	0021	4.45	0
000557051	ACT	L11	0115-3 PCT.	0160	0012	7.14	0
000557051	ACT	L12	0575-4 PCT.	0594	0023	3.87	0
000557051	ACT	L13	2875-5 PCT.	0774	0037	4.78	0
000557051	ACT	L01	0115-3 PCT.	0161	0013	8.07	0
000557051	ACT	L02	0575-4 PCT.	0575	0033	5.74	0
000557051	ACT	L03	2875-5 PCT.	0754	0033	4.38	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2142 PROJECT 02672
EXPERIMENT 634206 DETECTOR T41537 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	OBG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	A+C		AMO 333 UG/ML	1460	0055	3.77	0
	A-C		SOLVENT	1430	0023	1.60	0
	ALI		TISSUE	1075	0022	2.05	0
	ALU		TISSUE	1157	0033	2.85	0
	ACP	LI	AMO 333 UG/ML	0302	0340	89.01	0
	ACP	LU	AMO 333 UG/ML	1240	0102	8.17	0
000557051	ACT	L11	0115-3 PCT.	0500	0020	4.00	0
000557051	ACT	L12	0575-4 PCT.	1379	0028	2.03	0
000557051	ACT	L13	2075-5 PCT.	0850	0027	3.18	0
000557051	ACT	LU1	0115-3 PCT.	0415	0016	3.84	0
000557051	ACT	LU2	0575-4 PCT.	0935	0022	2.35	0
000557051	ACT	LU3	2075-5 PCT.	1071	0013	1.21	0

REPORT EXA33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02472
EXPERIMENT 635201 DETECTOR TA1538 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU E+6	MUTI E+0	FREQ1 E-0	CONTAM
	A+C		ANTH 67 UG/ML	0528	0243	46.02	0
	A-C		SOLVENT	0507	0050	9.00	0
	ALT		TISSUE	0539	0073	13.54	0
	ALU		TISSUE	0626	0077	12.30	0
	ACP	LT	ANTH 67 UG/ML	0515	0662	128.54	0
	ACP	LU	ANTH 67 UG/ML	0408	0159	32.50	0
000557051	ACT	LT1	0115-3 PCT.	0279	0013	4.66	0
000557051	ACT	LT2	0575-4 PCT.	0363	0017	4.68	0
000557051	ACT	LT3	2075-5 PCT.	0512	0041	8.01	0
000557051	ACT	LU1	0115-3 PCT.	0268	0012	4.48	0
000557051	ACT	LU2	0575-4 PCT.	0305	0020	7.27	0
000557051	ACT	LU3	2075-5 PCT.	0520	0062	11.92	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2162 PROJECT 02672
EXPERIMENT 634207 DETECTION TA98 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	BRG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-8	CONTAM
	A+C		AMTH 67 UG/ML	1729	0046	2.64	0
	A-C		SOLVENT	0973	0062	6.37	0
	ALI		TISSUE	0368	0081	22.58	0
	ALU		TISSUE	0643	0062	9.64	0
	ACP	LI	AMTH 67 UG/ML	0227	0680	299.56	0
	ACP	LU	AMTH 67 UG/ML	0726	0359	49.31	0
000557051	ACT	L11	0115-3 PCT.	0323	0049	15.17	0
000557051	ACT	L12	0575-4 PCT.	1046	0056	5.35	0
000557051	ACT	L13	2075-5 PCT.	0414	0042	10.14	0
000557051	ACT	LH1	0115-3 PCT.	0479	0040	8.35	0
000557051	ACT	LH2	0575-4 PCT.	0653	0060	9.19	0
000557051	ACT	LH3	2075-5 PCT.	0631	0054	8.56	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634285 DETECTOR 000004 SPECIES SPRDAW/RAT DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	A+C		DMM 90 UM/ML	0638	0087	0065	13.64	10.19	0
	A-C		SOLVENT	0550	0080	0061	16.00	11.09	0
	ALI		TISSUE	0677	0120	0063	18.91	9.31	0
	ALW		TISSUE	0646	0110	0056	17.03	8.67	0
	ACP	11	DMM 90 UM/ML	0442	0429	0320	97.06	72.40	0
	ACP	LW	DMM 90 UM/ML	0601	0061	0019	10.15	3.16	0
000557051	ACT	L11	0005-0 PCT.	0541	0132	0037	28.70	6.84	0
000557051	ACT	L12	0025-1 PCT.	0654	0071	0062	10.86	9.48	0
000557051	ACT	L13	0125-2 PCT.	0634	0095	0070	14.98	11.04	0
000557051	ACT	L01	0005-0 PCT.	0360	0087	0053	22.09	13.95	0
000557051	ACT	L02	0025-1 PCT.	0674	0077	0060	11.42	8.90	0
000557051	ACT	L03	0125-2 PCT.	0637	0095	0051	14.91	8.01	0

REPORT EX031 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2142 PROJECT 02672
EXPERIMENT 634209 DETECTOR TA100 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU E+6	MUTI E+0	FREQ1 E-0	CONTAM
	A+C		DMN 90 UM/ML	0336	0179	53.27	0
	A-C		SOLVENT	0468	0268	57.26	0
	ALI		TISSUE	0485	0400	82.47	0
	ALU		TISSUE	0436	0318	72.94	0
	ACP	L1	DMN 90 UM/ML	0350	0683	195.14	0
	ACP	LU	DMN 90 UM/ML	0386	0312	181.96	0
000557051	ACT	L11	0115-3 PCT.	0213	0179	84.04	0
000557051	ACT	L12	0575-4 PCT.	0345	0435	126.09	0
000557051	ACT	L13	2875-5 PCT.	0408	0372	91.18	0
000557051	ACT	LU1	0115-3 PCT.	0367	0422	134.99	0
000557051	ACT	LU2	0575-4 PCT.	0347	0326	93.95	0
000557051	ACT	LU3	2875-5 PCT.	0235	0293	124.68	0

REPORT EXR33 LITTON BIONETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 434802 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	DRG ID	CONCENTRATION	POPU EP+6	MUTI EP+8	FREQI EP-8	CONTAM
	A+C		DMN 98 UM/ML	0854	0022	2.58	0
	A-C		SOLVENT	0905	0020	2.21	0
	ALI		TISSUE	1003	0034	3.39	0
	ALU		TISSUE	0792	0022	2.78	0
	ACP	LI	DMN 98 UM/ML	0768	0279	36.71	0
	ACP	LU	DMN 98 UM/ML	0938	0022	2.37	0
000557051	ACT	L11	0115-3 PCT.	0024	0000	0.00	0
000557051	ACT	L12	0575-4 PCT.	1177	0024	2.04	0
000557051	ACT	L13	2875-5 PCT.	1100	0026	2.36	0
000557051	ACT	LU1	0115-3 PCT.	0043	0000	0.00	0
000557051	ACT	LU2	0575-4 PCT.	0773	0030	3.88	0
000557051	ACT	LU3	2875-5 PCT.	1051	0022	2.09	0

REPORT EXP33 LITTON BIOMEDICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 701207 DETECTOR TA1535 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQI EP-6	CONTAM
	ALI		TISSUE	0500	0049	0.45	0
000557051	ACT	LT1	0115-3 PCT.	0269	0018	6.69	0
000557051	ACT	LU1	0115-3 PCT.	0156	0015	9.62	0

REPORT ERR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 634210 DETECTOR TA1537 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	DRG ID	CONCENTRATION	POPN EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	A+C		AMO 333 UG/ML	2044	0050	2.45	0
	A-C		SOLVENT	1436	0035	2.44	0
	ALI		TISSUE	1784	0037	2.07	0
	ALW		TISSUE	2174	0024	1.10	0
	ACP	L1	AMO 333 UG/ML	0454	0370	81.50	0
	ACP	L0	AMO 333 UG/ML	1003	0004	4.66	0
000557051	ACT	L11	0115-3 PCT.	0737	0020	2.71	0
000557051	ACT	L12	0575-4 PCT.	1567	0016	1.02	0
000557051	ACT	L13	2075-5 PCT.	1041	0028	1.52	0
000557051	ACT	LH1	0115-3 PCT.	0596	0033	5.54	0
000557051	ACT	L02	0575-4 PCT.	1102	0012	1.02	0
000557051	ACT	L03	2075-5 PCT.	1655	0029	1.75	0

REPORT EX033 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 635102 DETECTOR TA153A SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+6	MUTI EP+0	FREQ1 EP-0	CONTAM
	A+C		ANTH 67 UG/ML	0501	0049	9.78	0
	A-C		SOLVENT	0549	0076	13.84	0
	ALI		TISSUE	0602	0074	12.29	0
	ALU		TISSUE	0569	0068	11.95	0
	ACP	LI	ANTH 67 UG/ML	0529	0725	137.05	0
	ACP	LU	ANTH 67 UG/ML	0493	0076	15.42	0
000557051	ACT	L11	0115-3 PCT.	0421	0053	12.59	0
000557051	ACT	L12	0575-4 PCT.	0500	0071	14.20	0
000557051	ACT	L13	2075-5 PCT.	0554	0077	13.90	0
000557051	ACT	L01	0115-3 PCT.	0477	0066	13.84	0
000557051	ACT	L02	0575-4 PCT.	0430	0050	11.63	0
000557051	ACT	L03	2075-5 PCT.	0494	0056	11.34	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 2237A-2102 PROJECT 02672
EXPERIMENT 634302 DETECTOR TA98 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU E+6	MUTI E+0	FREQ1 E-0	CONTAM
	A+C		ANTH 67 UG/ML	2229	0129	5.39	0
	A-C		SOLVENT	1691	0093	5.50	0
	ALI		TISSUE	0973	0093	9.56	0
	ALU		TISSUE	1359	0144	10.43	0
	ACP	L1	ANTH 67 UG/ML	1137	1402	123.31	0
	ACP	LU	ANTH 67 UG/ML	1308	0228	17.43	0
000557051	ACT	LI1	0115-3 PCT.	0659	0052	7.89	0
000557051	ACT	LI2	0575-4 PCT.	1326	0147	11.09	0
000557051	ACT	LI3	2875-5 PCT.	1299	0172	13.24	0
000557051	ACT	LU1	0115-3 PCT.	1017	0024	2.36	0
000557051	ACT	LU2	0575-4 PCT.	1772	0204	11.51	0
000557051	ACT	LU3	2875-5 PCT.	2081	0192	9.60	0

REPORT EXR33 LITTON BIOMETICS MUTAGENIC ACTIVITY SYSTEM
COMPOUND SUMMARY BACKUP DETAIL

CONTRACT 22376-2102 PROJECT 02672
EXPERIMENT 781006 DETECTOR 000004 SPECIES RHESUS/MONKEY DATE - 02/24/77

COMPOUND	TEST	ORG ID	CONCENTRATION	POPU EP+4	MUT1 EP+1	MUT2 EP+1	FREQ1 EP-5	FREQ2 EP-5	CONTAM
	NAN		SOLVENT	0357	0101	0000	28.29	0.00	0
	A-C		DMN 90 UM/ML	0742	0144	0079	19.41	10.65	0
	A-C		SOLVENT	0790	0146	0075	18.48	9.49	0
	ALI		TISSUE	0770	0127	0084	16.49	10.91	0
	ALU		TISSUE	0698	0107	0071	15.33	10.17	0
	ACP	LI	DMN 90 UM/ML	0414	0307	0304	93.48	73.43	0
	ACP	LU	DMN 90 UM/ML	0387	0100	0066	25.84	17.05	0
000557051	ACT	LI1	0005-0 PCT.	0650	0124	0064	19.08	9.85	0
000557051	ACT	LI2	0025-1 PCT.	0603	0089	0047	14.76	7.79	0
000557051	ACT	LI3	0125-2 PCT.	0694	0091	0062	13.11	8.93	0
000557051	ACT	LU1	0005-0 PCT.	0655	0065	0026	9.92	3.97	0
000557051	ACT	LU2	0025-1 PCT.	0539	0114	0066	21.15	12.24	0
000557051	ACT	LU3	0125-2 PCT.	0742	0106	0072	14.29	9.70	0