Litton BIONETICS

MUTAGENICITY EVALUATION OF

B10

IN THE AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

5516 Nicholson Lane
Kensington, Maryland
20795

GENETICS ASSAY NO.: 6376

LBI SAFETY NO.: 7805

MUTAGENICITY EVALUATION OF

B10

IN THE AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

SUBMITTED TO:

LORILLARD RESEARCH CENTER
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P.O. BOX 21688
GREENSBORO, NC 27420

SUBMITTED BY:

LITTON BIONETICS, INC.
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KENSINGTON, MARYLAND 20895

LBI PROJECT NO.: 20988

REPORT DATE: APRIL, 1983

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PREFACE

This report contains a summary of the data compiled during the evaluation of the test compound. The report is organized to present the results in a concise and easily interpretable manner. The first part contains Items I-IX. Items I-IV provide sponsor and test article identification information, type of assay, and the protocol reference number. Item V provides the initiation and completion dates of the study. Item VI identifies the supervisory personnel. Item VII indicates the tables and/or figures containing the test results. The interpretation of the results is in Item VIII. Item IX provides the conclusion and evaluation.

The second part of the report describes the study design, which includes the materials and procedures employed in conducting the assay. This part of the report also contains evaluation criteria used by the study director, and any appendices.

All test and control results presented in this report are supported by raw data which are permanently maintained in the files of the Department of Molecular Toxicology or in the archives of Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland 20895.

Copies of the raw data will be supplied to the sponsor upon request.

The described study was performed in accordance with Good Laboratory Practice regulations except if noted to the contrary. To the best of the signer's knowledge there were no significant deviations from the Good Laboratory Practice regulations which affected the quality or integrity of the study.

I. SPONSOR: Lorillard Research Center

II. MATERIAL (TEST COMPOUND): GENETICS ASSAY NUMBER: 6376

A. Identification: B10

B. Date Received: February 18, 1983

C. Physical Description: Clear, colorless liquid

III. TYPE OF ASSAY: Ames Salmonella/microsome Mutagenesis Assay

IV. PROTOCOL NUMBER: 401

V. STUDY DATES:

A. Initiation: March 16, 1983

B. Completion: March 25, 1983

VI. STUDY DIRECTOR: William G. DeGraff, M.S.

VII. RESULTS:

The results of this assay are presented in Tables 1 and 2.

VIII. INTERPRETATION OF RESULTS:

The test compound was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats.

A negative control consisting of the solvent used for preparing the stock solution and subsequent dilutions of the test material and specific positive compounds were assayed concurrently with the test material. The negative control data were used as the basis for evaluating the results obtained with the test material.

Dose Range

In a preliminary toxicity study conducted at 14 doses of 0.02 µl to 150.00 µl per plate using strain TA-100, the test material caused complete toxicity at the 4.69 µl/plate dose (Table 1). The mutagenicity assays were conducted at 7 doses of 0.01 µl to 5.00 µl per plate. Dimethylsulfoxide (DMSO) was used as a solvent for the preparation of the stock solutions and subsequent dilutions of the test material. In the solvent, the test material formed a clear colorless solution.

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VIII. INTERPRETATION OF RESULTS:

The results of the test conducted on B10 in the absence of a metabolic activation system were negative (Table 2).

The results of the test conducted on B10 in the presence of a rat liver activation system were negative (Table 2).

The addition of S9 mix greatly reduced the toxicity of B10 to all the strains.

IX. CONCLUSIONS:

The test material, B10, did not exhibit genetic activity in any of the assays conducted for this evaluation and was considered not mutagenic under these test conditions according to our evaluation criteria.

SUBMITTED BY:

Study Director:

William G. DeGraff

William G. DeGraff, M.S.
Submammalian Genetics
Department of Molecular Toxicology

4/15/83
Date

REVIEWED BY:

Brian C. Myhr

Brian C. Myhr, Ph.D.
Director
Department of Molecular Toxicology

4-15-83
Date

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TABLE 1

TOXICITY TEST WITH TA-100

SPONSOR: Lorillard Research Center

COMPOUND CODE: B10

ASSAY NO.: 6376

SOLVENT: DMSO

DATE INITIATED: March 16, 1983

DATE COMPLETED: March 21, 1983

TEST COMPOUND µg/PLATE	APPEARANCE OF BACK-GROUND LAWN	NUMBER OF COLONIES/PLATE
0 (Control)*	Normal	127,126
0.02	Normal	145
0.04	Normal	138
0.07	Normal	130
0.15	Normal	104
0.29	Normal	92
0.59	Normal	91
1.17	Normal	127
2.34	Microcolonies	64
4.69	Microcolonies	0
9.38	Clear	0
18.75	Clear	0
37.50	Clear	0
75.00	Clear	0
150.00	Clear	0

*Solvent Control (50 µl/plate).

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RESULTS

TABLE 2

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 810
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 03/22/83
D. TEST COMPLETION DATE: 03/25/83
E. S-9 LOT#: 01002
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE													
			TA-1535			TA-1537			TA-1538			TA-98			TA-100		
			1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
NONACTIVATION																	
SOLVENT CONTROL	---	---	23	17	14	12	11	9	17	19	14	27	32	40	132	143	170
POSITIVE CONTROL**	---	---	1362	1303	1282	403	410	439	947	857	865	881	888	946	1409	1394	1434
TEST COMPOUND																	
0.010000 UL	---	---	28	26	25	9	7	7	20	10	15	45	48	34	141	137	160
0.050000 UL	---	---	19	21	13	12	9	10	10	14	14	45	26	29	149	144	147
0.100000 UL	---	---	21	20	15	12	13	10	11	19	18	33	58	37	170	167	148
0.500000 UL	---	---	13	10	17	4	7	5	18	12	19	38	40	32	157	130	141
1.000000 UL	---	---	16	17	12	6	7	7	13	14	7	28	20	30	103	114	99
2.000000 UL	---	---	4	2	3	6	6	5	10	9	6	17	14	17	95	102	105
5.000000 UL	---	---	0	0	0	0	0	0	0	0	0	5	2	7	0	0	0
ACTIVATION																	
SOLVENT CONTROL	RAT	LIVER	21	23	15	11	5	12	23	25	24	54	71	58	146	130	109
POSITIVE CONTROL***	RAT	LIVER	347	359	334	633	610	654	2303	2080	2075	2314	2942	2655	2900	2742	2917
TEST COMPOUND																	
0.010000 UL	RAT	LIVER	12	12	16	12	9	8	30	27	26	26	54	54	118	128	117
0.050000 UL	RAT	LIVER	13	8	11	15	8	9	30	25	24	42	56	56	106	144	117
0.100000 UL	RAT	LIVER	11	7	8	10	9	11	24	21	26	42	54	64	120	141	144
0.500000 UL	RAT	LIVER	10	13	7	10	10	10	21	24	24	55	54	54	150	127	140
1.000000 UL	RAT	LIVER	10	9	7	9	9	15	16	25	24	58	49	55	111	117	120
2.000000 UL	RAT	LIVER	6	11	9	8	8	8	34	35	22	54	37	55	89	119	104
5.000000 UL	RAT	LIVER	4	3	2	6	10	7	27	32	10	45	59	44	85	87	70

**

TA-1535	SODIUM AZIDE	10 UG/PLATE
TA-1537	9-AMINOACRIDINE	50 UG/PLATE
TA-1538	2-NITROFLUORENE	10 UG/PLATE
TA-98	2-NITROFLUORENE	10 UG/PLATE
TA-100	SODIUM AZIDE	10 UG/PLATE

SOLVENT 50 UL/PLATE

***

TA-1535	2-ANTHRAMINE	2.5 UG/PLATE
TA-1537	2-ANTHRAMINE	2.5 UG/PLATE
TA-1538	2-ANTHRAMINE	2.5 UG/PLATE
TA-98	2-ANTHRAMINE	2.5 UG/PLATE
TA-100	2-ANTHRAMINE	2.5 UG/PLATE

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RESULTS

TABLE 2 (continued)

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: 810
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 03/22/83
D. TEST COMPLETION DATE: 03/25/83
E. S-9 LOT#: 01002
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE TA-1535 MEAN	TA-1535 S.D.	TA-1537 MEAN	TA-1537 S.D.	TA-1538 MEAN	TA-1538 S.D.	TA-98 MEAN	TA-98 S.D.	TA-100 MEAN	TA-100 S.D.
NONACTIVATION												
SOLVENT CONTROL	---	---	18.0	4.6	10.7	1.5	16.7	2.5	33.0	6.6	148.3	19.6
POSITIVE CONTROL**	---	---	1315.7	41.5	417.3	19.1	889.7	49.8	905.0	35.7	1412.3	20.2
TEST COMPOUND												
0.010000 UL	---	---	26.3	1.5	7.7	1.2	15.0	5.0	42.3	7.4	146.0	12.3
0.050000 UL	---	---	17.7	4.2	10.3	1.5	12.7	2.3	33.3	10.2	146.7	2.5
0.100000 UL	---	---	18.7	3.2	11.7	1.5	16.0	4.4	42.7	13.4	161.7	11.9
0.500000 UL	---	---	13.3	3.5	5.3	1.5	16.3	3.8	36.7	4.2	142.7	13.6
1.000000 UL	---	---	15.0	2.6	6.7	0.6	11.3	3.8	26.0	5.3	105.3	7.8
2.000000 UL	---	---	3.0	1.0	5.7	0.6	8.3	2.1	16.0	1.7	100.7	5.1
5.000000 UL	---	---	0.0	0.0	0.0	0.0	0.0	0.0	4.7	2.5	0.0	0.0
ACTIVATION												
SOLVENT CONTROL	RAT	LIVER	19.7	4.2	9.3	3.8	24.0	1.0	61.0	8.9	148.3	18.6
POSITIVE CONTROL***	RAT	LIVER	346.7	12.5	632.3	22.0	2152.7	130.2	2637.0	314.4	2853.0	96.5
TEST COMPOUND												
0.010000 UL	RAT	LIVER	13.3	2.3	9.7	2.1	27.7	2.1	44.7	16.2	121.0	6.1
0.050000 UL	RAT	LIVER	10.7	2.5	10.7	3.8	26.3	3.2	51.3	8.1	122.3	19.6
0.100000 UL	RAT	LIVER	8.7	2.1	10.3	1.0	23.7	2.5	53.3	11.0	135.0	13.1
0.500000 UL	RAT	LIVER	10.0	3.0	10.0	0.0	23.0	1.7	54.3	0.6	139.0	11.5
1.000000 UL	RAT	LIVER	8.7	1.5	11.0	3.5	21.7	4.9	54.0	4.6	116.0	4.6
2.000000 UL	RAT	LIVER	8.7	2.5	8.0	0.0	30.3	7.2	48.7	10.1	104.0	15.0
5.000000 UL	RAT	LIVER	3.0	1.0	7.7	2.1	23.0	11.5	49.3	8.4	80.7	9.3

**

TA-1535	SODIUM AZIDE	10 UG/PLATE
TA-1537	9-AMINOACRIDINE	50 UG/PLATE
TA-1538	2-NITROFLUORENE	10 UG/PLATE
TA-98	2-NITROFLUORENE	10 UG/PLATE
TA-100	SODIUM AZIDE	10 UG/PLATE

***

TA-1535	2-ANTHRAMINE	2.5 UG/PLATE
TA-1537	2-ANTHRAMINE	2.5 UG/PLATE
TA-1538	2-ANTHRAMINE	2.5 UG/PLATE
TA-98	2-ANTHRAMINE	2.5 UG/PLATE
TA-100	2-ANTHRAMINE	2.5 UG/PLATE

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AMES SALMONELLA/MICROSOME PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate a test material for mutagenic activity in a bacterial assay with and without a mammalian S9 activation system.

2. RATIONALE

The Salmonella typhimurium strains used at LBI are all histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown in a minimal media petri plate containing a trace of histidine, only those cells that revert to histidine independence (his+) are able to form colonies. The trace amount of histidine allows all the plated bacteria to undergo a few divisions; this growth is essential for mutagenesis to occur. The his+ revertants are easily scored as colonies against the slight background growth. The spontaneous mutation frequency of each strain is relatively constant, but when a mutagen is added to the agar the mutation frequency is increased 2 to 100-fold. Cells which grow to form colonies on the minimal media petri plates are therefore assumed to have reverted, either spontaneously or by the action of a test substance to his+ genotype.

3. MATERIALS

A. Indicator Microorganisms

The Salmonella typhimurium strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley[1-5]. The following five strains are routinely used:

Strain Designation	Gene Affected	Additional Mutations			Mutation Type Detected
		Repair	LPS	R Factor	
TA-1535	his G	Δ uvr B	rfa	-	Base-pair substitution
TA-1537	his C	Δ uvr B	rfa	-	Frameshift
TA-1538	his D	Δ uvr B	rfa	-	Frameshift
TA-98	his D	Δ uvr B	rfa	pKM101	Frameshift
TA-100	his G	Δ uvr B	rfa	pKM101	Base-pair substitution

3. MATERIALS (continued)

A. Indicator Microorganisms

The aforementioned strains have, in addition to the mutation in the histidine operon, a mutation (rfa-) that leads to defective lipopolysaccharide coat and a deletion that covers genes involved in the synthesis of vitamin biotin (bio-) and in the repair of ultraviolet (uv) - induced DNA damage (uvrB-). The rfa- mutation makes the strains more permeable to many large molecules. The uvrB- mutation decreases repair of some types of chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents. The resistant transfer factor plasmid (R factor) pKM101 in TA-98 and TA-100 is believed to cause an increase in error-prone DNA repair that leads to many more mutations for a given dose of most mutagens[5]. In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker to detect the presence of plasmic in the cells.

All indicator strains were kept at 4°C on minimal medium plates supplemented with a trace of biotin and an excess of histidine. The plates with plasmid-carrying strains contain in addition ampicillin (25 µg/ml) to ensure stable maintenance of plasmid pKM101. New stock culture plates were made as often as necessary from frozen master cultures or from single colony reisolates that were checked for their genotypic characteristics (his, rfa, uvrB, bio) and for the presence of plasmid.

B. Media

For daily use, each strain was cultured in Oxoid Media #2 (nutrient broth) for approximately 16 hours at 37°C (titer of 10^8 to 10^9 cells per milliliter) and used in the mutagenicity test.

The minimal media plates for the selection of histidine revertants consisted of the Vogel Bonner medium E (Vogel and Bonner, 1956) with 2% glucose and 1.5% bactoagar. The overlay agar contained the following per 100 ml volume; 0.6 gms of purified agar, 10 ml of 0.5mM L-Histidine-0.5mM Biotin and 0.5 g NaCl according to the method of Ames et al. (1975).

C. Activation System

(1) S9 Homogenate

A 9,000 x g supernatant prepared from Sprague-Dawley adult male rat liver induced by Aroclor 1254 (described by Ames et al.[6]) was purchased commercially and used in this assay.

3. MATERIALS (continued)

C. Activation System (continued)

(2) S9 Mix

Components	Concentration per Milliliter S9 Mix
NADP (sodium salt)	4 µmoles
D-glucose-6-phosphate	5 µmoles
$MgCl_2$	8 µmoles
KCl	33 µmoles
Sodium phosphate buffer pH 7.4	100 µmoles
Organ homogenate from rat liver (S9 fraction)	100 µliters

4. EXPERIMENTAL DESIGN

A. Dosage Selection

Doses were selected for the actual assay based on a preliminary toxicity test with the strain TA-100. Fourteen doses using two-fold dilutions from 10,000 µg per plate for solids and 150 µl per plate for liquids are used in this dose selection assay. For the actual assay, at least six doses are selected with the highest dose exhibiting 100% toxicity. Nontoxic chemicals are tested up to 10 mg per plate for solids and 150 µl per plate for liquids.

If the Sponsor specifies doses other than those determined above, the tests are run using the specified doses and these doses will be reported in the interpretation of results and in the results tables. If doses are specified, no toxicity testing will be performed to determine doses.

B. Toxicity Studies

To a sterile test tube containing 2.0 ml of overlay agar (placed in a 43°-45°C water bath) the following were added:

- 0.05 - 0.15 ml of a solution of the test material to give the appropriate dose.
- 0.2 ml of an overnight culture.
- 0.5 ml of 0.2M phosphate buffer, pH 7.4.

This mixture was swirled gently and then poured onto minimal agar plates (see 3B, Media). After the overlay had set, the plates were incubated at 37°C for approximately two days. The number of colonies growing on the plates were counted and recorded.

4. EXPERIMENTAL DESIGN (continued)

A reduction in the number of revertants, appearance of microcolonies, or clearing of the background lawn on the test material treated plates as compared to the solvent control plates were considered as an indication of toxicity by the test material.

C. Mutagenicity Testing

The procedure used is based on the paper published by Ames et al.[6] and was performed as follows:

(1) Nonactivation Assay

To a sterile test tube placed in 43°C-45°C water bath the following were added in order:

(a) 2.0 ml of overlay agar (see 3.B. Media).

(b) 0.025 - 0.15 ml of a solution of the test chemical to give the appropriate dose.

(c) 0.1 ml of an undiluted or 0.2 ml of diluted indicator organism(s).

(d) 0.50 ml of 0.2M phosphate buffer, pH 7.4.

This mixture was swirled gently and then poured onto minimal agar plates (see 3B, Media). After the top agar had set, the plates were incubated at 37°C for approximately 2 days. The number of his+ revertant colonies growing on the plates were counted and recorded.

(2) Activation Assay

The activation assays were run concurrently with the nonactivation assay. The only difference was the addition of 0.5 ml of S9 mix (see 3C:2, Activation System) to the tubes in place of 0.5 ml of phosphate buffer which was added in nonactivation assays. All other details were similar to the procedure for nonactivation assays.

A detailed flow diagram for the plate incorporation assay is provided in Figure 1.

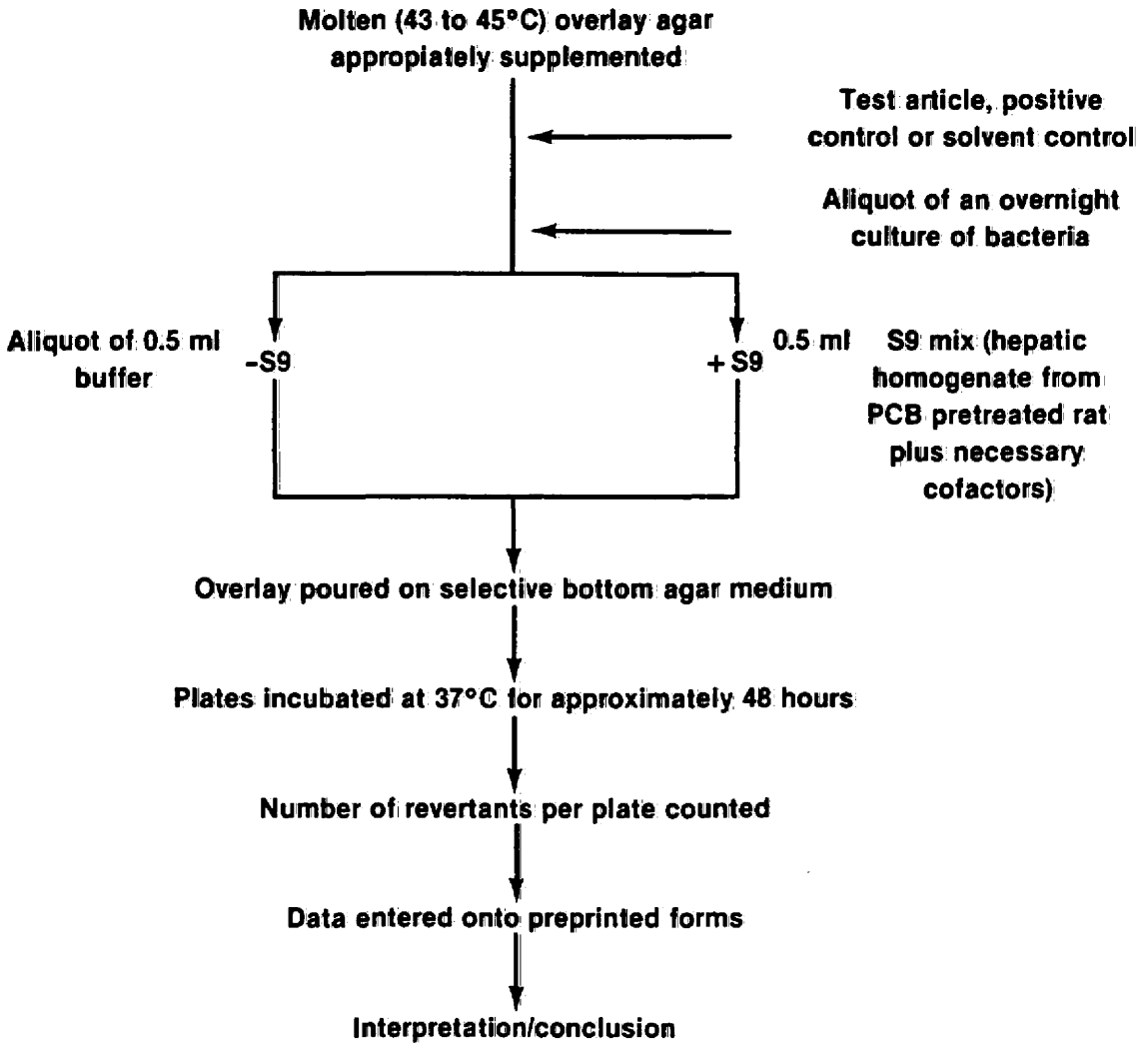
D. Control Compounds

(1) Negative Control Article

A negative control consisting of the solvent used for the test material was assayed concurrently with the test material. The solvent control was employed for each indicator strain and was used in the absence and presence of S9 mix.

Figure 1

REVERSE MUTATION ASSAY
(Agar Incorporation Method)



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4. EXPERIMENTAL DESIGN (continued)

D. Control Compounds (continued)

(1) Negative Control Article

The solvent was tested at a single concentration equal to the maximum volume used to administer the highest dose of the test article or at the concentration indicated in the Results Table(s) of this report. The solvent used to prepare the stock solution of the test article is given in the Results Section of this report. All dilutions of the test article were made using this solvent.

(2) Positive Control Articles

Strain specific positive controls and positive controls to ensure the efficacy of the metabolizing mixture were assayed concurrently with the test material. The following positive controls were employed in the assays:

Assay	Chemical	Solvent	Concentration per plate (µg)	Salmonella Strains
Nonactivation	Sodium azide (SA)	Water	10.0	TA-1535, TA-100
	2-Nitrofluorene (2-NF)	Dimethyl-sulfoxide	10.0	TA-1538, TA-98
	9-aminoacridine (9-AA)	Ethanol	50.0	TA-1537
Activation	2-aminoanthracene (2-AA)	Dimethyl-sulfoxide	2.5	For all strains

5. EVALUATION CRITERIA

Statistical methods are not currently used and evaluation is based on the criteria included in this protocol.

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 material and the cells are incubated in the overlay for approximately 2 days and a few cell divisions occur during the incubation period, the test is semiquantitative in nature. Although these features reduce the quantitation of result, they provide certain advantages not contained in a quantitative suspension test:

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5. EVALUATION CRITERIA (continued)

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.

- The combined incubation of the test article and the cells in the overlay permits constant exposure of the indicator cells for approximately 2 days.

A. Evaluation Criteria for Ames Assay

Because the procedures used to evaluate the mutagenicity of the test material were semiquantitative, the criteria used to determine positive effects were inherently subjective and were based primarily on a historical data base. Most data sets were 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 test material producing a positive response equal to three times the solvent control value is considered mutagenic.

(2) Strains TA-98 and TA-100

If the solvent control value is within the normal range, a test material producing a positive response equal to twice the solvent control value for TA-98 and TA-100 is considered mutagenic.

The following normal range of revertants for solvent controls are generally considered acceptable:

TA-1535:	8-30
TA-1537:	4-30
TA-1538:	10-35
TA-98 :	20-75
TA-100 :	80-250

(3) Pattern

Because TA-1535 and TA-100 are both derived from the same parental strain (G-46) and because TA-1538 and TA-98 are both derived from the same parental strain (D3052), to some extent 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. Generally, if a strain responds to a mutagen in nonactivation tests, it will do so in activation tests. Occassionally, exceptions to this pattern may be seen.

5. EVALUATION CRITERIA (continued)

B. Dose-Response Phenomena

The demonstration of dose-related increases in revertant counts is an important criterion in establishing mutagenicity. Since we employ several doses in the actual assay, a dose response would normally be seen with a mutagenic test material. Additional tests may be performed at a narrower dose range, if the mutagenic test material fails to exhibit a dose-response in the initial assay. Occassionally, however, it is difficult to generate a dose-response and the test material will be evaluated based on the available data.

C. Reproducibility

If a test material produces a response in a single test which cannot be reproduced in additional runs, the initial positive test data lose significance.

D. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for the non-activation assays and mutagens requiring metabolic biotransformation for the activation assays. Negative controls consist of the test material solvent in the overlay agar together with the other essential components. The negative control data for each strain provide a reference to which the test data is compared. The positive control assays are conducted to demonstrate that the test systems are functional with known mutagens.

REFERENCES

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2. Ames, B.N., Gurney, E.G., Miller, J.A. and Bartsch, H.: Carcinogens as frameshift mutagens: Metabolites and derivatives of 2-acetyl-aminofluorene and other aromatic amine carcinogens. Proc. Nat. Acad. Sci. USA, 69:3128-3132, 1972.

3. Ames, B.N., Lee, F.D. and Durston, W.E.: An improved bacterial test system for the detection and classification of mutagens and carcinogens. Proc. Nat. Acad. Sci. USA, 70:782-786, 1973.

4. Ames, B.N., Durston, W.E., Yamasaki, E. and Lee, F.D.: Carinogens are mutagens: A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. USA, 70:2281-2285, 1973.

5. McCann, J., Springarn, N.E., Kobori, J. and Ames, B.N.: Detection of carcinogens as mutagens: Bacterial tester strains with R factor plasmids. Proc. Nat. Acad. Sci. USA, 72:979-983, 1975.

6. Ames, B.N., McCann, J. and Yamasaki, E.: Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. Mutation Res., 31:347-364, 1975.

7. Vogel, H.J. and Bonner, D.M.: Acetylornithinase of E. coli; Partial purification and some properties. J. Biol. Chem., 218:97-106, 1956.

Q.A. Inspection Statement
(reference 21 CFR 58.35(b)(7))

PROJECT 10988 LBI Assay No. 6376

TYPE of STUDY Ames Plate Test

This final study report was reviewed by the LBI Quality Assurance Unit on 4-12-83. A report of findings was submitted to the Study Director and to Management on 4-12-83.

The short-term nature of this study precluded inspection while it was in process. The Quality Assurance Unit inspects an in-process study of this type approximately once per month to assure that no significant problems exist that are likely to affect the integrity of this type of study.

Marshall L. Hyman
Auditor, Quality Assurance Unit

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