GENETICS ASSAY NO.: 6692

LBI SAFETY NO.: 8189A

10-21-83

MUTAGENICITY EVALUATION OF

B83

IN THE AMES SALMONELLA/MICROSOME PLATE TEST

FINAL REPORT

SUBMITTED TO:

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SUBMITTED BY:

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LBI PROJECT NO.: 20988

REPORT DATE: OCTOBER, 1983

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Litton BIONETICS

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: 6692

A. Identification: B83

B. Date Received: June 20, 1983

C. Physical Description: Clear, pale yellow liquid

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

IV. PROTOCOL NUMBER: 401

V. STUDY DATES:

A. Initiation: August 4, 1983

B. Completion: August 30, 1983

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

VII. RESULTS:

The results of this assay are presented in Tables 1, 2 and 3.

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 to revertant colonies at 75.00 µl/plate (Table 1). The mutagenicity assays were conducted at 7 doses of 0.10 µl to 100.00 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, pale yellow solution.

VIII. INTERPRETATION OF RESULTS:

The results of the test conducted on B83 in the absence of a metabolic activation system were negative (Table 2).

The results of the test conducted on B83 in the presence of a rat liver activation system were negative (Table 2).

Due to the poor growth of TA-100 in the first trial, this portion of the assay ws repeated. The results of this repeat were negative (Table 3).

IX. CONCLUSIONS:

The test material, B83, 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 C. DeGraff 10/7/83

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

REVIEWED BY:

Brian C. Myhr 10/7/83

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

00952198

TABLE 1

TOXICITY TEST WITH TA-100

SPONSOR: Lorillard Research Center

COMPOUND CODE: B83

ASSAY NO.: 6692

SOLVENT: DMSO

DATE INITIATED: August 4, 1983

DATE COMPLETED: August 8, 1983

TEST COMPOUND µl/PLATE	APPEARANCE OF BACK-GROUND LAWN	NUMBER OF COLONIES/PLATE
0 (Control)*	Normal	166 and 202
0.02	Normal	180
0.04	Normal	212
0.07	Normal	182
0.15	Normal	185
0.29	Normal	177
0.59	Normal	126
1.17	Normal	104
2.34	Normal	95
4.69	Normal	69
9.38	Normal	40
18.75	Microcolonies	31
37.50	Microcolonies	15
75.00	Microcolonies	0
150.00	Microcolonies	0

*Solvent Control (50 µl/plate).

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RESULTS

TABLE 2

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: B83
B. SOLVENT: DMSO (50 UL)
C. TEST INITIATION DATES: 08/16/83
D. TEST COMPLETION DATE: 08/19/83
E. S-9 LOT#: 02276
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	---	---	14	12	14	11	13	9	17	11	C	28	31	27	41	71	74
POSITIVE CONTROL**	---	---	649	658	563	211	378	279	1029	33	612	726	851	972	114	106	41
TEST COMPOUND																	
0.100000 UL	---	---	9	10	16	8	4	5	17	11	10	30	35	27	10	10	5
0.500000 UL	---	---	8	9	9	5	4	4	14	11	10	38	35	32	16	12	10
1.000000 UL	---	---	15	7	14	4	10	7	10	13	14	28	26	21	42	10	68
5.000000 UL	---	---	12	18	13	7	4	4	9	8	10	29	29	29	40	55	42
10.000000 UL	---	---	12	14	12	5	4	5	18	13	8	17	25	21	12	49	12
25.000000 UL	---	---	6	11	4	1	2	0	5	9	11	29	21	24	6	19	32
100.000000 UL	---	---	2	1	0	0	0	0	0	1	1	5	12	2	0	0	0
ACTIVATION																	
SOLVENT CONTROL	RAT	LIVER	13	8	11	10	12	11	26	19	15	44	35	45	99	79	59
POSITIVE CONTROL***	RAT	LIVER	271	308	272	16	307	309	1410	1371	1556	1343	1297	1258	1059	1072	1054
TEST COMPOUND																	
0.100000 UL	RAT	LIVER	11	10	16	6	12	4	20	18	21	30	32	45	10	0	0
0.500000 UL	RAT	LIVER	9	10	7	4	5	5	23	17	19	40	45	38	15	10	0
1.000000 UL	RAT	LIVER	19	15	10	5	4	10	26	27	27	40	41	39	65	77	70
5.000000 UL	RAT	LIVER	7	8	12	4	6	4	25	24	25	35	41	28	61	57	59
10.000000 UL	RAT	LIVER	13	5	8	11	4	5	35	24	16	34	35	52	15	61	20
25.000000 UL	RAT	LIVER	7	12	8	2	2	2	20	14	14	40	25	33	60	56	36
100.000000 UL	RAT	LIVER	0	3	10	0	0	1	3	8	5	10	21	17	10	8	4

**

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

C INDICATES CONTAMINATED

***

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: 883
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 08/16/83
D. TEST COMPLETION DATE: 08/19/83
E. S-9 LOT#: 02276
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	---	---	13.3	1.2	11.0	2.0	14.0	4.2	28.7	2.1	62.0	18.2
POSITIVE CONTROL**	---	---	623.3	52.4	289.3	84.0	558.0	500.2	849.7	123.0	87.0	40.0
TEST COMPOUND												
0.100000 UL	---	---	11.7	3.8	5.7	2.1	12.7	3.8	30.7	4.0	8.3	2.9
0.500000 UL	---	---	8.7	0.6	4.3	0.6	11.7	2.1	35.0	3.0	12.7	3.1
1.000000 UL	---	---	12.0	4.4	7.0	3.0	12.3	2.1	25.0	3.6	40.0	29.1
5.000000 UL	---	---	14.3	3.2	5.0	1.7	9.0	1.0	29.0	0.0	45.7	8.1
10.000000 UL	---	---	12.7	1.2	4.7	0.6	13.0	5.0	21.0	4.0	24.3	21.4
25.000000 UL	---	---	7.0	3.6	1.0	1.0	8.3	3.1	24.7	4.0	19.0	13.0
100.000000 UL	---	---	1.0	1.0	0.0	0.0	0.7	0.6	6.3	5.1	0.0	0.0
ACTIVATION												
SOLVENT CONTROL	RAT	LIVER	10.7	2.5	11.0	1.0	20.0	5.6	41.3	5.5	77.3	17.6
POSITIVE CONTROL***	RAT	LIVER	283.7	21.1	210.7	168.6	1445.7	97.5	1299.3	42.5	1061.7	9.3
TEST COMPOUND												
0.100000 UL	RAT	LIVER	12.3	3.2	7.3	4.2	19.7	1.5	35.7	8.1	3.3	5.8
0.500000 UL	RAT	LIVER	8.7	1.5	4.7	0.6	19.7	3.1	41.0	3.6	8.3	7.6
1.000000 UL	RAT	LIVER	14.7	4.5	6.3	3.2	26.7	0.6	40.0	1.0	70.7	6.0
5.000000 UL	RAT	LIVER	9.0	2.6	4.7	1.2	24.7	0.6	34.7	6.5	59.0	2.0
10.000000 UL	RAT	LIVER	8.7	4.0	6.7	3.8	25.0	9.5	40.3	10.1	32.0	25.2
25.000000 UL	RAT	LIVER	9.0	2.6	2.0	0.0	16.0	3.5	32.7	7.5	50.7	12.9
100.000000 UL	RAT	LIVER	4.3	5.1	0.3	0.6	5.3	2.5	16.0	5.6	7.3	3.1

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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

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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

RESULTS TABLE 3

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: B83
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 08/24/83
D. TEST COMPLETION DATE: 08/30/83
E. S-9 LOT#: 02276
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE TA-100		
			1	2	3
NONACTIVATION					
SOLVENT CONTROL	---	---	155	128	130
POSITIVE CONTROL**	---	---	1003	1052	1045
TEST COMPOUND					
0.100000 UL	---	---	156	186	168
0.500000 UL	---	---	171	145	146
1.000000 UL	---	---	118	121	117
5.000000 UL	---	---	95	72	78
10.000000 UL	---	---	74	57	86
25.000000 UL	---	---	64	99	79
100.000000 UL	---	---	32	22	37
ACTIVATION					
SOLVENT CONTROL	RAT	LIVER	174	182	159
POSITIVE CONTROL***	RAT	LIVER	2176	2217	2113
TEST COMPOUND					
0.100000 UL	RAT	LIVER	188	202	165
0.500000 UL	RAT	LIVER	179	157	159
1.000000 UL	RAT	LIVER	130	139	133
5.000000 UL	RAT	LIVER	111	106	109
10.000000 UL	RAT	LIVER	131	125	112
25.000000 UL	RAT	LIVER	109	109	113
100.000000 UL	RAT	LIVER	54	58	65

** TA-100 SODIUM AZIDE 10 UG/PLATE

*** TA-100 2-ANTHRAMINE 2.5 UG/PLATE

00522202

RESULTS

TABLE 3 (CONTINUED)

A. NAME OR CODE DESIGNATION OF THE TEST COMPOUND: B83
B. SOLVENT: DMSO
C. TEST INITIATION DATES: 08/24/83
D. TEST COMPLETION DATE: 08/30/83
E. S-9 LOT#: 02276
NOTE: CONCENTRATIONS ARE GIVEN IN MICROLITERS PER PLATE

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE TA-100 MEAN	TA-100 S.D.
NONACTIVATION				
SOLVENT CONTROL	---	---	137.7	15.0
POSITIVE CONTROL**	---	---	1033.3	26.5
TEST COMPOUND				
0.100000 UL	---	---	170.0	15.1
0.500000 UL	---	---	154.0	14.7
1.000000 UL	---	---	118.7	2.1
5.000000 UL	---	---	81.7	11.9
10.000000 UL	---	---	72.3	14.6
25.000000 UL	---	---	80.7	17.6
100.000000 UL	---	---	30.3	7.6
ACTIVATION				
SOLVENT CONTROL	RAT	LIVER	171.7	11.7
POSITIVE CONTROL***	RAT	LIVER	2168.7	52.4
TEST COMPOUND				
0.100000 UL	RAT	LIVER	185.0	18.7
0.500000 UL	RAT	LIVER	165.0	12.2
1.000000 UL	RAT	LIVER	134.0	4.6
5.000000 UL	RAT	LIVER	108.7	2.5
10.000000 UL	RAT	LIVER	122.7	9.7
25.000000 UL	RAT	LIVER	110.3	2.3
100.000000 UL	RAT	LIVER	59.0	5.6

** TA-100 SODIUM AZIDE 10 UG/PLATE

*** TA-100 2-ANTHRAMINE 2.5 UG/PLATE

00952203

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 strains are 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

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3. MATERIALS (continued)

The aforementioned strains have, in addition to the mutation in the histidine operon, a mutation (rfa-) that leads to defective lipopolysaccharide coat, 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 plasmid in the cells.

The indicator strains were kept at 4°C on minimal medium plates supplemented with a trace of biotin, an excess of histidine, and ampicillin (25 μg/ml) for TA-98 and TA-100, to ensure stable maintenance of plasmid pKM101. New stock culture plates are 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.

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3. MATERIALS (continued)

(2) S9 Mix

Components	Concentration 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 were used in this dose selection assay. For the actual assay, at least six doses were selected with the highest dose exhibiting 100% toxicity. Nontoxic chemicals were tested up to 10 mg per plate for solids and 150 μl per plate for liquids.

If the Sponsor specified doses other than those determined above, the tests were run using the specified doses and these doses will be reported in the interpretation of results and in the results tables. If doses were 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 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 on to minimal agar plates (see 3B, Media). After the overlay agar had set, the plates were incubated at 37°C for approximately 2 days. The number of colonies growing on the plates were counted and recorded.

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4. EXPERIMENTAL DESIGN (continued)

B. Toxicity Studies (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 indications 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 a 43°C-45°C water bath the following were added in order:

(a) 2.00 ml of overlay agar (see 3.B. Media).

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

(c) 0.1 ml - 0.2 ml of indicator organism.

(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 ± 2° for approximately 2 days. The number of his+ revertant colonies growing on the plates were counted and recorded.

(2) Activation Assay

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

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

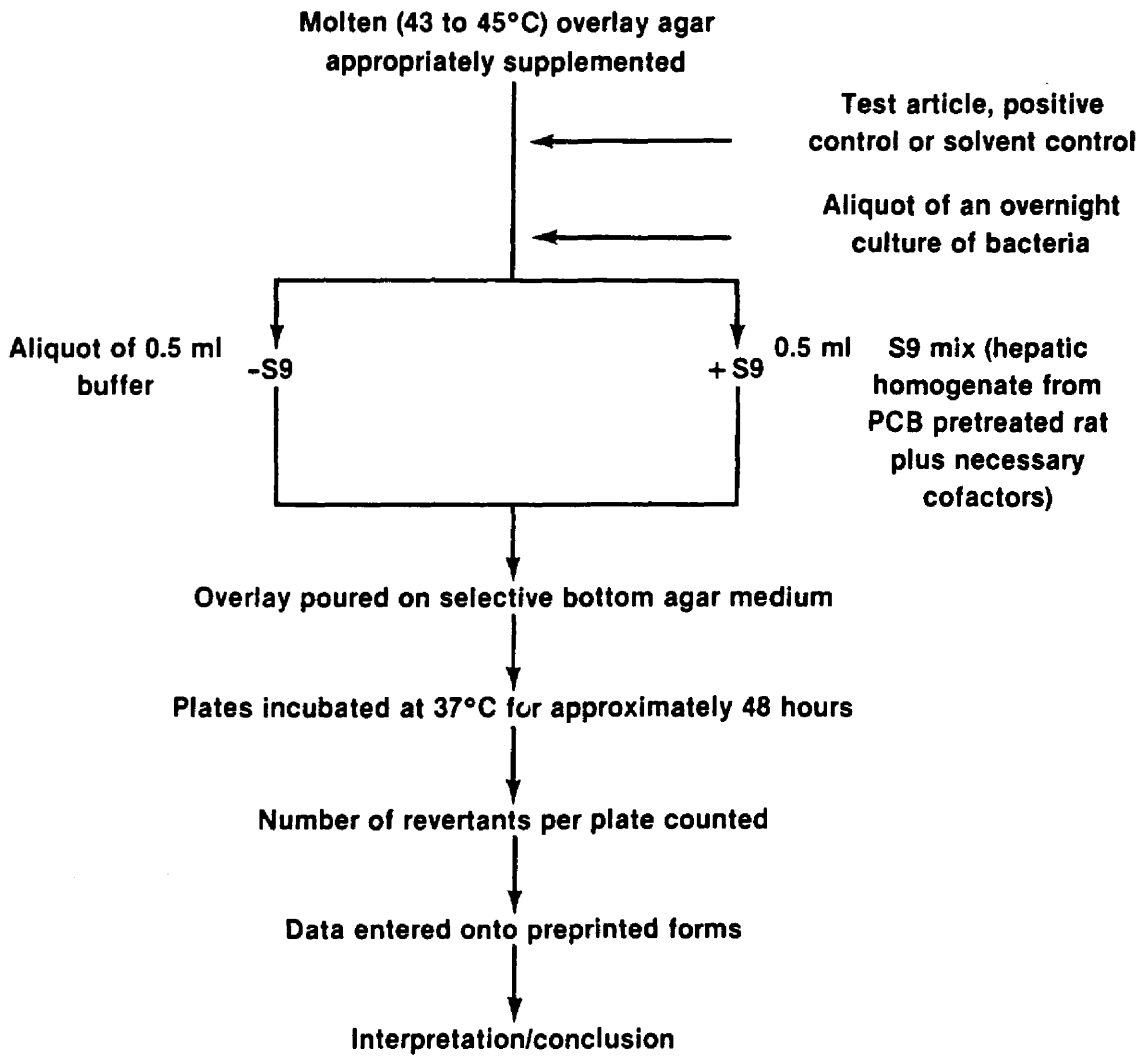
D. Control Compounds

(1) 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.

00952207

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 S9 mixture were assayed concurrently with the test material. The following positive controls were employed in the assays:

Assay	Chemical	Solvent	Concentrations per Plate (μg)	Salmonella Strains
Nonactivation	Sodium azide (SA)	Water	10.0	TA-1535, TA-100
	2-Nitrofluorene (NF)	Dimethyl-sulfoxide	10.0	TA-1538, TA-98
	9-aminoacridine (9-AA)	Ethanol	50.0	TA-1537
Activation	2-amino-anthracene (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 in replicating DNA, which is often more sensitive than nonreplicating DNA.

- The combined incubation of the test material 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 ranges 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. Occasionally, exceptions to this pattern may be seen.

00952210

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 over a narrower dose range if the mutagenic test material fails to exhibit a dose-response in the initial assay. However, occasionally 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 nonactivation assays and mutagens requiring metabolic biotransformation in activation assays. Negative controls consist of the test material solvent in the overlay agar, together with the other essential components. The negative control plate for each strain gives a reference point to which the test data is compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

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

PROJECT 20988 LBI Assay No. 6692

TYPE of STUDY Ames Plate Test

This final study report was reviewed by the LBI Quality Assurance Unit on 9.29.83. A report of findings was submitted to the Study Director and to Management on 9.30.83.

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.

Auditor, Quality Assurance Unit

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Litton BIONETICS