HAZLETON
BIOTECHNOLOGIES
5516 NICHOLSON LANE, SUITE 400, KENSINGTON, MARYLAND 20895

GENETICS ASSAY NO.: 8767

HBC SAFETY NO.: 11394-001

MUTAGENICITY EVALUATION OF

CN-322 Ref. No. 004066

IN THE
AMES SALMONELLA/MICROSOME
REVERSE MUTATION ASSAY

FINAL REPORT

SUBMITTED TO:

GREAT LAKES CHEMICAL CORPORATION
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SUBMITTED BY:

HAZLETON BIOTECHNOLOGIES COMPANY
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HBC PROJECT NO.: 20988

REPORT DATE: APRIL 1986

CLIENT CONFIDENTIAL

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PREFACE

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 Genetic Toxicology or in the archives of Hazleton Biotechnologies Company, 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.

SUMMARY

At the request of Great Lakes Chemical Corporation, Hazleton Biotechnologies Company examined CN-322 Ref. No. 004066 for mutagenic activity in the Ames Salmonella/Microsome assays using Salmonella typhimurium strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100. The assays were conducted using two plates per dose level in the presence and absence of a metabolic activation system. The entire assay was performed once as requested by the sponsor.

The test material, CN-322 Ref. No. 004066, did not exhibit genetic activity in these assays and was not mutagenic under these test conditions according to our assay criteria.

SUBMITTED BY:

Study Director:

D.R. Jagannath

4.4.86

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Date

REVIEWED BY:

Brian C. Myhr

4/4/86

Brian C. Myhr, Ph.D.
Director
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Date

EVALUATION OF CN-322 REF. NO. 004066
IN AN AMES SALMONELLA/MICROSOME
REVERSE MUTATION ASSAY

I. 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.

II. RATIONALE:

The Salmonella typhimurium strains used at HBC 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.

III. SPONSOR: Great Lakes Chemical Corporation

IV. MATERIAL TESTED:

A. Genetics Assay No.: 8767

B. Identification: CN-322 Ref. No. 004066

C. Date Received: December 10, 1985

D. Physical Description: Clear amber liquid

V. TYPE OF ASSAY: Ames Salmonella/Microsome Reverse Mutation Assay

VI. PROTOCOL NUMBER:

A. HBC Protocol No.: 401, Edition 11

B. Sponsor's Protocol No.: None

VII. STUDY DATES:

A. Initiation Date: December 31, 1985

B. Completion Date: January 24, 1986

VIII. SUPERVISORY PERSONNEL:

A. Study Driector: D. R. Jagannath, Ph.D.

B. Research Assistant: Doug Everett

IX. MATERIALS:

A. Indicator Microorganisms

The Salmonella typhimurium strains used in this assay were obtained from Dr. Bruce Ames, University of California at Berkeley (Ames et al., 1975). 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

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

IX. MATERIALS: (Continued)

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 (McCann, et al., 1975). 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 the plasmid.

B Media

For daily use, an inoculum from stock culture plates is grown overnight at 37°C in Oxoid Media #2 (nutrient broth) 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., 1975) was purchased commercially and used in this assay.

IX. MATERIALS: (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

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.

   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 and Discussion 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:

IX. MATERIALS: (Continued)

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
	Quinacrine mustard (QM)	Dimethyl-sulfoxide	5.0	TA-1537
Activation	2-anthramine	Dimethyl-sulfoxide	2.5	For all strains

X. EXPERIMENTAL DESIGN:

A. Dosage Selection

Doses were selected for the actual assay based on a preliminary dose rangefinding test with the strain TA-100. Fourteen doses of the test material, 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.

B. Dose Rangefinding Test

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 - 0.15 ml of a solution of the test material to give the appropriate dose.
- 0.1 to 0.2 ml of indicator organism.
- 0.5 ml of 0.2M phosphate buffer, pH 7.4.

X. EXPERIMENTAL DESIGN: (Continued)

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

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.

If the doses are specified by the sponsor, the toxicity studies as outlined in X:B, will not be performed on the test material and the assays are run using the specified doses. These doses will be reported in the results and discussion section and in the results tables.

C. Mutagenicity Testing

The procedure used is based on the paper published by Ames et. al., 1975) 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 IX:B, Media).

(b) 0.05 ml - 0.15 ml of a solution of the test chemical to yield the appropriate dose.

(b) 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 IX.B: Media). After the top agar had set, the plates were incubated at about 37°C for approximately two 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

X. EXPERIMENTAL DESIGN: (Continued)

(see IX:C.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. Explanation of Evaluation Procedures for Ames Plate Test Data

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 two 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:

- 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 two days.

1. Dose-Response Phenomena

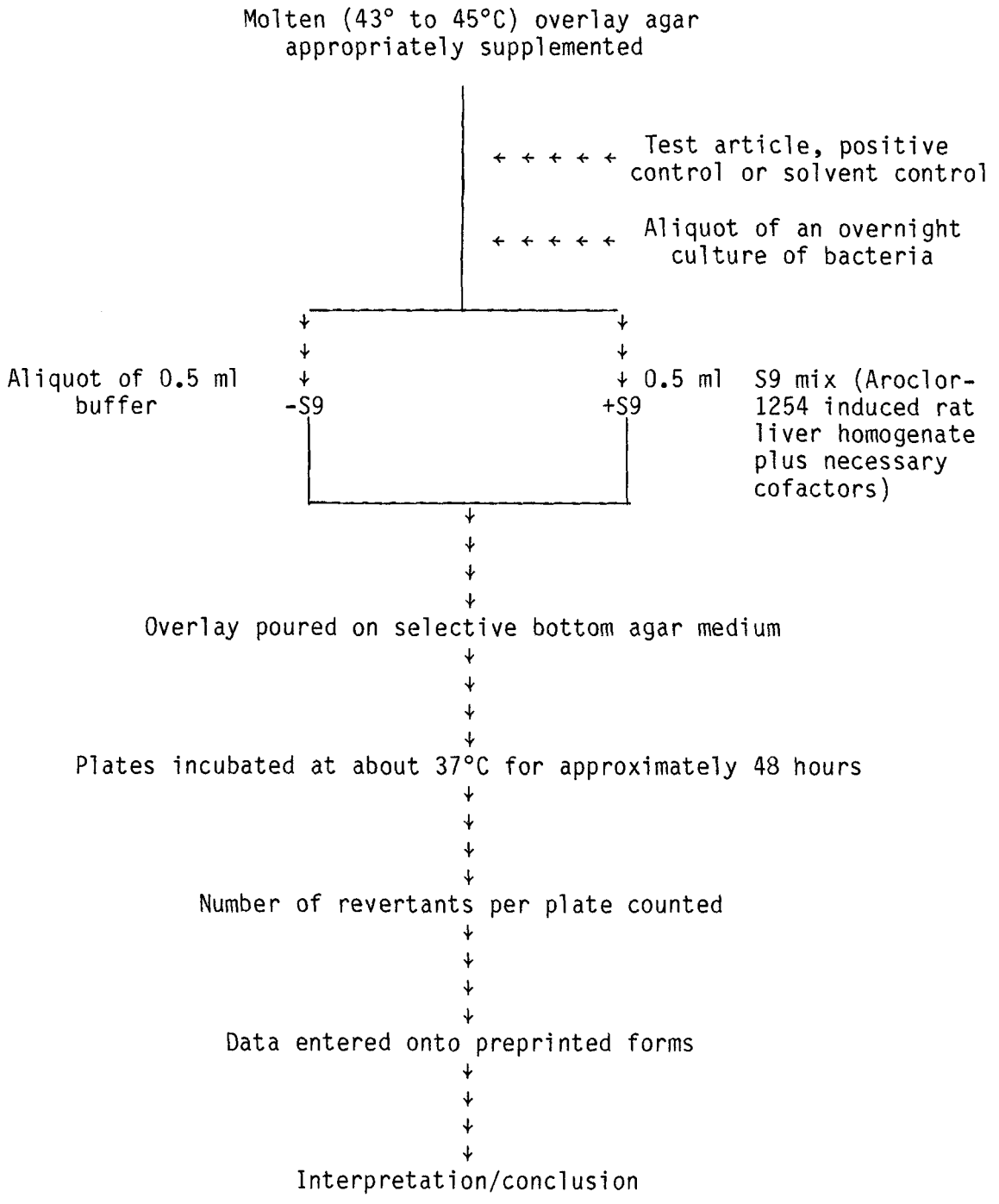
The demonstration of dose-related increases in revertant counts is an important criterion in establishing mutagenicity. Since we employ several doses in the actual assay, a dose response would normally be seen with a mutagenic test material. Additional tests may be performed over a narrower dose range 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.

2. 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

FIGURE 1

REVERSE MUTATION ASSAY
(Agar Incorporation Method)



X. EXPERIMENTAL DESIGN: (Continued)

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.

3. 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.

4. 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 are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

E. 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

Data sets will be evaluated as positive if a dose response is observed over a minimum of three test concentrations and the increase in revertants is equal to or greater than three times the solvent control value at the peak of the dose response. The solvent control value should be within the normal range for evaluating the results (see also X:D.1, Dose response phenomena).

2. Strains TA-98 and TA-100

Data sets will be evaluated as positive if a dose response is observed over a minimum of three test concentrations and the

X. EXPERIMENTAL DESIGN: (Continued)

increase in revertants achieves a doubling of the solvent control value at the peak of the dose response. The solvent control value should be within the normal range for evaluating the results (see also X:D.1, Dose response phenomena).

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

XI. RESULTS AND DISCUSSION:

At the request of Great Lakes Chemical Corporation, Hazleton Biotechnologies Company examined CN-322 Ref. No. 004066 for mutagenic activity in the Microbial Reverse Mutation Assays employing five Salmonella typhimurium strains in the presence and absence of liver microsomal enzyme preparations from Aroclor induced rats. Solvent control and specific positive control compounds were assayed concurrently with the test material. The concurrent solvent control data were used as the basis for evaluating the results.

A. Test Material Handling

The test material, CN-322 Ref. No. 004066, was stored at room temperature in the dark. Dimethylsulfoxide (DMSO) was used in the preliminary solubility test and the test material was found to be soluble in this solvent. The test material formed a clear amber solution in DMSO. Various dilutions of the test material were then performed in DMSO.

B. Preliminary Dose Rangefinding Studies

Doses for the mutagenesis assays were selected from a preliminary study conducted on the test material at 14 doses from 0.02 µl to 150.0 µl per plate using the strain TA-100. In this preliminary study, the test material was not toxic to the indicator strain as evidenced by the normal background lawn on the minimal media plates (Table 1).

XI. RESULTS AND DISCUSSION: (Continued)

These results were used to select 8 doses for the mutagenicity assays. The doses selected for the mutation assays ranged from 0.1 µl per plate to 150.0 µl per plate. The mutation assays were conducted at 8 doses using two plates per dose level.

C. Mutation Assays

Table 2 provides the summary of the test results for the test material, CN-322 Ref. No. 004066. The data is given as mean revertants per plate with standard deviation for each treatment and control group. Individual plate counts are given in Table 2A.

The results of the assays conducted on the test material at dose levels ranging from 0.1 µl to 150.0 µl per plate in the absence and presence of metabolic activation did not exhibit increased numbers of his+ revertant colonies (Table 2). The test material was also non-toxic to all the indicator organisms up to the highest assayed concentration of 150.0 µl per plate.

The positive control treatments in both the nonactivation and S-9 activation assays induced large increases in the revertant numbers with all the indicator strains, which demonstrates the effectiveness of the S-9 activation system and ability of the test system to detect known mutagens.

XII. CONCLUSION:

The test material, CN-322 Ref. No. 004066, did not exhibit genetic activity in any of the assays conducted in this evaluation and was not mutagenic to Salmonella typhimurium indicator organisms under these test conditions according to our evaluation criteria.

XIII. EXPERIMENTAL DATA TABLES

TABLE 1

TOXICITY TEST WITH TA-100

SPONSOR: Great Lakes Chemical Corporation

COMPOUND CODE: CN-322 Ref. No. 004066

ASSAY NO.: 8767

SOLVENT: DMSO

DATE INITIATED: December 31, 1985

DATE COMPLETED: January 2, 1986

TEST COMPOUND µl/PLATE	APPEARANCE OF BACK-GROUND LAWN	NUMBER OF COLONIES/PLATE
0 (Control)*	Normal	176 and 149
0.02	Normal	179
0.04	Normal	203
0.07	Normal	186
0.15	Normal	190
0.29	Normal	215
0.59	Normal	182
1.17	Normal	178
2.34	Normal	192
4.69	Normal	203
9.38	Normal	192
18.75	Normal	181
37.50	Normal	178
75.00	Normal	186
150.00	Normal	188

*Solvent Control: DMSO (50 µl/plate).

TABLE 2
Summary Of Test Results

RESULTS

A. Name or code designation of the test compound: CN-322 REF. NO. 004066
B. Solvent: DMSO (50 UL)
C. Test initiation date: 01/21/86
D. Test completion date: 01/24/86
E. S-9 Lot #: R-274
NOTE: Concentrations are given in Microliters per plate.

Test	Species	Tissue	MEAN REVERTANTS PER PLATE WITH STANDARD DEVIATIONS									
			TA-1535		TA-1537		TA-1538		TA-98		TA-100	
			MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.	MEAN	S.D.
NONACTIVATION												
Solvent Control	---	---	29.0	2.8	10.0	2.8	20.5	0.7	21.5	2.1	150.5	12.0
Positive Control**	---	---	1724.0	52.3	911.5	58.7	1426.5	51.6	1080.0	2.8	1765.5	24.7
Test Compound												
0.10 UL	---	---	30.0	2.8	9.0	2.8	22.0	2.8	22.5	2.1	142.5	7.8
1.00 UL	---	---	29.0	7.1	6.0	1.4	23.0	0.0	22.5	0.7	135.0	7.1
5.00 UL	---	---	32.0	1.4	10.0	0.0	20.5	4.9	19.5	0.7	144.0	5.7
10.00 UL	---	---	26.0	1.4	12.0	1.4	18.0	2.8	20.5	0.7	142.0	7.1
25.00 UL	---	---	29.5	0.7	10.0	4.2	24.0	4.2	17.5	4.9	152.0	11.3
50.00 UL	---	---	26.0	4.2	12.0	0.0	22.5	3.5	21.5	2.1	130.5	2.1
100.00 UL	---	---	33.5	2.1	8.0	0.0	17.0	4.2	23.5	0.7	145.5	3.5
150.00 UL	---	---	31.5	4.9	11.5	2.1	22.5	2.1	24.5	6.4	149.5	4.9
ACTIVATION												
Solvent Control	RAT	LIVER	11.5	2.1	10.5	2.1	26.0	4.2	28.5	4.9	126.5	13.4
Positive Control***	RAT	LIVER	217.5	33.2	232.0	9.9	1726.5	78.5	1766.0	24.0	2304.5	34.6
Test Compound												
0.10 UL	RAT	LIVER	14.0	5.7	10.0	1.4	35.0	2.8	31.0	0.0	120.5	6.4
1.00 UL	RAT	LIVER	11.0	1.4	11.0	2.8	31.0	0.0	36.0	0.0	122.0	8.5
5.00 UL	RAT	LIVER	13.5	3.5	14.0	0.0	36.0	2.8	30.0	8.5	138.0	21.2
10.00 UL	RAT	LIVER	10.0	1.4	11.0	1.4	33.0	0.0	30.5	2.1	122.5	16.3
25.00 UL	RAT	LIVER	12.5	3.5	8.0	0.0	35.0	0.0	39.0	14.1	127.0	26.9
50.00 UL	RAT	LIVER	14.5	4.9	9.5	2.1	34.5	3.5	39.5	2.1	139.0	5.7
100.00 UL	RAT	LIVER	11.5	2.1	12.0	2.8	38.5	0.7	28.5	6.4	137.5	2.1
150.00 UL	RAT	LIVER	15.5	3.5	13.0	0.0	36.0	5.7	38.0	0.0	128.5	4.9

**	TA-1535	SODIUM AZIDE	10 UG/PLATE	***	TA-1535	2-ANTHRAMINE	2.5 UG/PLATE
	TA-1537	QUINACRINE MUSTARD	5 UG/PLATE		TA-1537	2-ANTHRAMINE	2.5 UG/PLATE
	TA-1538	2-NITROFLUORENE	10 UG/PLATE		TA-1538	2-ANTHRAMINE	2.5 UG/PLATE
	TA-98	2-NITROFLUORENE	10 UG/PLATE		TA-98	2-ANTHRAMINE	2.5 UG/PLATE
	TA-100	SODIUM AZIDE	10 UG/PLATE		TA-100	2-ANTHRAMINE	2.5 UG/PLATE

TABLE 2A
Individual Plate Counts

RESULTS

A. Name or code designation of the test compound: CN-322 REF. NO. 004066
B. Solvent: DMSO (50 UL)
C. Test initiation date: 01/21/86
D. Test completion date: 01/24/86
E. S-9 Lot #: R-274
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	1	2	1	2	1	2	1	2
NONACTIVATION												
Solvent Control	---	---	27	31	8	12	21	20	23	20	159	142
Positive Control**	---	---	1761	1687	953	870	1463	1390	1082	1078	1783	1748
Test Compound												
0.10 UL	---	---	28	32	11	7	20	24	21	24	148	137
1.00 UL	---	---	34	24	5	7	23	23	23	22	130	140
5.00 UL	---	---	31	33	10	10	24	17	20	19	140	148
10.00 UL	---	---	25	27	11	13	20	16	21	20	147	137
25.00 UL	---	---	29	30	7	13	21	27	14	21	144	160
50.00 UL	---	---	29	23	12	12	20	25	20	23	132	129
100.00 UL	---	---	32	35	8	8	14	20	24	23	148	143
150.00 UL	---	---	28	35	13	10	24	21	29	20	153	146
ACTIVATION												
Solvent Control	RAT	LIVER	10	13	9	12	29	23	25	32	117	136
Positive Control***	RAT	LIVER	241	194	225	239	1671	1782	1783	1749	2329	2280
Test Compound												
0.10 UL	RAT	LIVER	18	10	11	9	37	33	31	31	125	116
1.00 UL	RAT	LIVER	12	10	13	9	31	31	36	36	128	116
5.00 UL	RAT	LIVER	16	11	14	14	38	34	24	36	153	123
10.00 UL	RAT	LIVER	11	9	12	10	33	33	29	32	111	134
25.00 UL	RAT	LIVER	10	15	8	8	35	35	49	29	146	108
50.00 UL	RAT	LIVER	18	11	8	11	32	37	38	41	135	143
100.00 UL	RAT	LIVER	13	10	14	10	38	39	33	24	139	136
150.00 UL	RAT	LIVER	13	18	13	13	32	40	38	38	132	125

** TA-1535 SODIUM AZIDE 10 UG/PLATE
TA-1537 QUINACRINE MUSTARD 5 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

XIV. REFERENCES:

1. 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.

2. 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.

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

XV. QUALITY ASSURANCE STATEMENT:

PROJECT: 20988 HBC Assay No.: 8767

TYPE of STUDY: Ames Salmonella/Microsome Plate Test

PROTOCOL NO.: 401, Edition 11

In compliance with the Good Laboratory Practice Regulations this study was inspected by the HBC Quality Assurance Unit. The data presented in the report accurately reflect data collected during the conduct of the study. Dates of inspection, phase inspected and reporting dates are as follows:

PHASE INSPECTED	DATE OF INSPECTION	DATE OF REPORT TO MANAGEMENT AND STUDY DIRECTOR
Final Report	3.31.84	3.31.84

Marshall T. Hyman 3/31/84
Auditor, Quality Assurance Unit