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MAC/Env. & Public Affairs/EHL
(CO./DIV./DEPT./LOCATION)

Final REPORT
(TYPE OF REPORT)

MAC TOXICOLOGY

FEB 18 1992

REPORT NO.: MSL - 11730

JOB/PROJECT NO.: EHL 91184/ML-91-441

DATE: February 7, 1992

TITLE: Ames/Salmonella Mutagenicity Assay of RODEO®

AUTHORS: [redacted]

ABSTRACT: The test material, RODEO, was tested in Ames/Salmonella plate incorporation assays using test strains TA98, TA100, TA1535 and TA1537 in the presence and absence of an Aroclor 1254-induced rat liver homogenate (S-9) activation system. The test chemical was not observed to be toxic or insoluble up to the maximum dose level for mutagenicity testing, 5 mg/plate in the absence and presence of S9 activation. No significant mutagenicity was observed in either the initial assays or the subsequent confirmation assays. These results indicate that RODEO is not a mutagen in the Ames/Salmonella plate incorporation assay under the experimental conditions.

AA016422

REPT. NO.: MSL- 11730 AUTHORS: [redacted]

COPY NO.: 1 TITLE: Ames/Salmonella Mutagenicity Assay of RODEO

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MONSANTO COMPANY
ENVIRONMENTAL HEALTH LABORATORY
645 S. NEWSTEAD
ST. LOUIS, MO 63110

FINAL REPORT

Ames/*Salmonella* Mutagenicity Assay of RODEO®

EHL Study Number: 91184
Sponsor Project Number: ML-91-441

Authors: [redacted]

[redacted] 2/7/92
Date

Study Director
Science Fellow

[redacted] 2/4/92
Date

Manager, Analytical, Biochemical and Genetic Toxicology

SUMMARY

The test material, RODEO, was tested in Ames/*Salmonella* plate incorporation assays using test strains TA98, TA100, TA1535 and TA1537 in the presence and absence of an Aroclor 1254-induced rat liver homogenate (S-9) activation system. The test chemical was not observed to be toxic or insoluble up to the maximum dose level for mutagenicity testing, 5 mg/plate in the absence and presence of S9 activation. No significant mutagenicity was observed in either the initial assays or the subsequent confirmation assays. These results indicate that RODEO is not a mutagen in the Ames/*Salmonella* plate incorporation assay under the experimental conditions.

INTRODUCTION

The purpose of this study was to determine if the test sample, RODEO, had detectable mutagenic activity towards Ames/*Salmonella* test strains TA98, TA100, TA1535 or TA1537 in the presence or absence of an Aroclor 1254-induced rat liver metabolic activation system (S-9 Mix).

This study was conducted at the Monsanto Agricultural Company, Environmental Health Laboratory (645 S. Newstead, St. Louis, MO 63110). The protocol was signed by the Study Director on November 11, 1991. Experimental work was initiated on November 26, 1991, and assays were completed on December 30, 1991.

MATERIALS AND METHODS

Test Materials

Identification and purity of the test material sample is given below:

Name: RODEO®
(Registered Trademark of Monsanto Company, St. Louis, MO)

Identification: Lot No: BS-008-002
EHL Test Sample T910116

Percent Active Ingredient: 40% Glyphosate (acid equivalent)

Appearance: Yellow liquid

Storage Conditions: Room Temperature

Source: Monsanto Agricultural Company

Solutions of the test material were prepared on the day of use using distilled water as solvent. The identity and sources of positive standard materials used in this study are given in Appendix II, Table 1.

Test Strains

The *Salmonella typhimurium* test strains (TA98, TA100, TA1535 and TA1537) were obtained from the laboratory of Dr. [redacted] (Berkeley, CA). The cultures used were inoculated from frozen permanent stocks and grown in nutrient broth at 37° ± 1°C in a shaking incubator. The proper phenotype of each culture was verified by tests for crystal violet sensitivity, ampicillin resistance, requirement for histidine and biotin and spontaneous reversion frequency.

S-9 Preparation and Mix

The S-9 preparation was purchased from Molecular Toxicology, Inc. (College Park, MD 20742). This preparation was from livers of Aroclor 1254-induced male Sprague-Dawley rats (HillTop Laboratories, Scottsdale, PA,). The procedures used in preparation of the S-9 supernatant solutions were those described by Ames et al. (Ref. 1). The lot number of S-9 used in the toxicity test and plate incorporation assays was MolTox 0339 and the stated protein content was 39.2 mg/ml. The lot of S-9 was tested for metabolic activation capability in a matrix experiment (not shown) in which both percent S-9 in S-9 Mix and amount of positive standard per plate were varied. The S-9 concentration used in these experiments, 10% (v/v), gave acceptable results for positive standards requiring metabolic activation. In addition to S-9, the S-9 Mix contained the following per ml: 8 µmoles $MgCl_2$, 33 µmoles KCl, 5 µmoles glucose-6-phosphate, 4 µmoles NADP, and 100 µmoles sodium phosphate, pH 7.4.

Plate Incorporation Tests

The general procedures used were basically those described by Ames et al. (Ref. 1). Plate incorporation tests were performed by mixing 0.1 ml of bacterial culture, and, if appropriate, 0.5 ml of S-9 mix (as described in Ref. 1) with 2 ml of histidine-biotin top agar (0.5% (w/v) NaCl, 0.6% (w/v) Difco agar, 0.05 mM L-histidine-HCl, 0.05 mM biotin) maintained at 44-48°C. The mixture was poured onto minimal glucose agar plates (Vogel-Bonner medium E of Ref. 2 with 2% glucose and 1.5% Difco agar). Toxicity tests employed the same procedures as those used in the plate incorporation test. Single plates were prepared for each strain/S-9/dose level combination for the toxicity test. Toxicity was judged qualitatively by visual examination of the background lawn and consideration of reduction in revertant colonies. Three replicate plates were prepared for each strain/S-9/combination for the plate incorporation tests. Concurrent positive and negative controls were conducted for plate incorporation tests to demonstrate strain sensitivity and metabolic activation system capability. Plates were examined after at least 48 hrs. at 37° ± 1°C.

Revertant colonies for plates with more than 500 revertant colonies/plate were estimated by counting revertant colonies in several fields under a stereomicroscope and multiplying the counted colonies by a factor relating the total plate area to the area of the counted fields. Revertant colonies measured in this manner are calculated to not more than three significant figures. Revertant colonies on other plates, except as noted, were counted with an Artek Model 880 automatic colony counter or counted by visual examination (<10 revertants/plate).

Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as log10 (revertants/plate). Analysis included Bartlett's test for homogeneity of variance (Ref. 3) and comparison of treatments with controls using within-levels pooled variance and a one-sided t-test (Ref. 4-6).

Grubbs' test was performed to determine if outliers were present (Ref. 7). Statistical significance of dose response was evaluated by regression analysis for log10 transformed doses and revertants/plate (Ref. 8).

A critical level of $p<0.01$ was used in determining statistical significance. Results with $p<0.05$ are also indicated to assist in interpretation of results. Results were considered clearly positive for a strain/microsome combination if revertants/plate values were significantly elevated over control values ($p<.01$) at three or more treatment levels, and there was a statistically significant dose response ($p<.01$).

RESULTS

A toxicity screen was conducted using test strain TA100 with and without S-9 Mix. Results of the toxicity screen is given in Appendix I, Table 1. No toxicity was observed for the test sample up to 5 mg/plate in the absence and presence of activation. The maximum treatment level used in the plate incorporation test was 5 mg/plate in the absence and presence of activation. This level is a generally accepted maximum dose level for this assay for nontoxic soluble materials.

Summary tables of the initial and repeat plate incorporation test results for RODEO are presented in Appendix I, Tables 2-5. Individual plate counts are given in Appendix II, Tables 2-5.

No toxicity was observed at any of the dose levels for any strain/microsome combinations tested. Results of the statistical analyses of the plate incorporation assays indicated that the test sample was not mutagenic towards any of the *Salmonella typhimurium* test strains used (TA98, TA100, TA1535 and TA1537). None of the strain/microsome combinations had any treatment levels with revertants/plate significantly elevated over control values ($p<0.01$) or a significant dose response ($p<0.01$).

The positive controls yielded the expected positive response indicating the adequacy of the experimental conditions for the detection of mutagens (Appendix II, Tables 2-5).

DISCUSSION AND CONCLUSIONS

The test sample, RODEO, was concluded not to be mutagenic towards any of the *Salmonella typhimurium* test strains used (TA98, TA100, TA1535, and TA1537) in the presence or absence of an Aroclor 1254-induced rat liver homogenate metabolic activation system (S-9 Mix).

REFERENCES

1. Ames, B.N., McCann, J., and Yamasaki, E. (1975). Methods for Detecting Carcinogens and Mutagens with the Salmonella Mammalian-Microsome Test. Mutation Res. 31, 347-364.

2. Vogel, H.J. and Bonner, D.M. (1956). Acetylornithase of E. coli: partial purification and some properties. J. Biol. Chem. 218, 97-106.

3. Bartlett, M.S. (1947). Multivariate analysis. J. R. Statist. Soc. Series B 9, 176-197.

4. Dixon, W.J. and Massey, F.J. (1969). Introduction to Statistical Analysis, 3rd Ed., McGraw-Hill, New York.

5. Ling, R.F. (1974). Comparison of several algorithms for computing sample means and variances. J. Am. Statist. Assoc. 69, 859-866.

6. Munroe, M.E. (1951). Theory of Probability, McGraw-Hill, New York.

7. Grubbs, F.E. and Beck, G. (1972). Extension of sample sizes and percentage points for significance tests of outlying observations. Technometrics. 14, 847-854.

8. Draper, N.R. and Smith, H. (1966). Applied Regression Analysis, Wiley, New York.

APPENDIX I

TOXICITY TEST RESULTS

STATISTICAL SUMMARY DATA FOR PLATE INCORPORATION ASSAYS

Table 1

Toxicity Test Results for RODEO with Test Strain TA100

Amount of Test Material Per Plate (mg)	S-9 Mix Present	Response[a]	Solubility [b]
0.05	-	N	S
0.05	+	N	S
0.15	-	N	S
0.15	+	N	S
0.5	-	N	S
0.5	+	N	S
1.5	-	N	S
1.5	+	N	S
5.0	-	N	S
5.0	+	N	S

[a] N = No toxic response. T = Toxicity observed with no revertant colonies. TR = Toxicity with revertant colonies observed.

[b] S = Test material soluble. I = Test material insoluble.

AMES/SALMONELLA ASSAY OF RODEO
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APPENDIX I - TABLE 2

STATISTICAL SUMMARY OF INITIAL PLATE INCORPORATION TEST RESULTS FOR TA98 AND TA100 WITH AND WITHOUT S9

STRAIN	TA98	TA98	TA100	TA100
ACTIVATION SYSTEM	WITH S-9	NONE	WITH S-9	NONE
TEST DATE	10-DEC-91	10-DEC-91	10-DEC-91	10-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.05	34.3 (5.8)	21.0 (6.2)	150.3 (20.8)	111.0 (17.1)
0.15	40.0 (5.6)	25.7 (1.5)	149.0 (3.6)	116.3 (12.3)
0.50	37.3 (9.9)	29.0 (8.5)	144.7 (17.8)	124.0 (24.0)
1.50	27.0 (7.8)	24.3 (6.0)	151.3 (16.2)	99.7 (18.9)
5.00	21.0 (7.9)	21.7 (3.2)	136.7 (14.3)	73.3 (22.3)
SOLVENT CONTROLS	36.9 (4.3)	26.9 (9.6)	155.8 (9.9)	138.7 (12.2)
SUMMARY ANALYSIS				
TREATMENT LEVELS WITH REV/PLATE > CONTROL				
P<=0.05	0	0	0	0
P<=0.01	0	0	0	0
BARTLETT'S TEST	N	N	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0	0	0
DOSE RESPONSE	N	N	N	N
LACK OF FIT TEST	N	N	N	A

ALL ANALYSES PERFORMED WITH LOG(10) TRANSFORMED DATA
CODES USED ARE:
* SIGNIFICANT AT P<=0.05 LEVEL
** SIGNIFICANT AT P<=0.01 LEVEL
N NOT SIGNIFICANT AT P<=0.05 LEVEL
A DATA DO NOT ALLOW ANALYSIS TO BE PERFORMED

AMES/SALMONELLA ASSAY OF RODEO
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APPENDIX I - TABLE 3

STATISTICAL SUMMARY OF INITIAL PLATE INCORPORATION TEST RESULTS FOR TA1535 AND TA1537 WITH AND WITHOUT S9

STRAIN	TA1535	TA1535	TA1537	TA1537
ACTIVATION SYSTEM	WITH S-9	NONE	WITH S-9	NONE
TEST DATE	13-DEC-91	13-DEC-91	13-DEC-91	13-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.05	10.3 (4.2)	11.7 (3.2)	8.7 (0.6)	5.7 (2.5)
0.15	11.7 (2.5)	11.0 (3.0)	7.3 (4.0)	4.3 (0.6)
0.50	12.7 (2.9)	11.3 (0.6)	7.0 (0.0)	3.7 (0.6)
1.50	9.3 (1.2)	12.3 (2.1)	9.0 (1.0)	5.7 (2.5)
5.00	7.7 (0.6)	8.0 (2.6)	7.0 (1.0)	3.3 (1.2)
SOLVENT CONTROLS	13.7 (4.4)	16.8 (5.6)	9.4 (3.6)	8.8 (3.8)
SUMMARY ANALYSIS				
TREATMENT LEVELS WITH REV/PLATE > CONTROL				
P<=0.05	0	0	0	0
P<=0.01	0	0	0	0
BARTLETT'S TEST	N	N	*	N
NO. OUTLIERS (GRUBB'S TEST)	0	0	0	0
DOSE RESPONSE	N	N	N	N
LACK OF FIT TEST	N	N	N	N

ALL ANALYSES PERFORMED WITH LOG(10) TRANSFORMED DATA
CODES USED ARE:
* SIGNIFICANT AT P<=0.05 LEVEL
** SIGNIFICANT AT P<=0.01 LEVEL
N NOT SIGNIFICANT AT P<=0.05 LEVEL

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APPENDIX I - TABLE 4

STATISTICAL SUMMARY OF REPEAT PLATE INCORPORATION TEST RESULTS FOR TA98 AND TA100 WITH AND WITHOUT S9

STRAIN	TA98	TA98	TA100	TA100
ACTIVATION SYSTEM	WITH S-9	NONE	WITH S-9	NONE
TEST DATE	17-DEC-91	17-DEC-91	17-DEC-91	17-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.05	28.3 (3.1)	26.7 (2.5)*	147.0 (18.2)	123.0 (9.5)
0.15	32.7 (5.8)	20.7 (8.6)	147.7 (21.5)	110.7 (12.1)
0.50	30.3 (7.5)	22.0 (5.3)	137.0 (20.5)	121.3 (26.8)
1.50	29.7 (3.5)	21.0 (2.6)	135.3 (11.0)	112.7 (15.6)
5.00	31.0 (2.0)	14.3 (3.8)	129.0 (11.4)	93.0 (10.5)
SOLVENT CONTROLS	27.4 (4.9)	18.8 (6.8)	134.6 (16.6)	110.9 (21.8)
SUMMARY ANALYSIS				
TREATMENT LEVELS WITH REV/PLATE > CONTROL				
P<=0.05	0	1	0	0
P<=0.01	0	0	0	0
BARTLETT'S TEST	N	N	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0	0	0
DOSE RESPONSE	N	N	N	N
LACK OF FIT TEST	N	N	N	N

ALL ANALYSES PERFORMED WITH LOG(10) TRANSFORMED DATA
CODES USED ARE:
- * SIGNIFICANT AT P<=0.05 LEVEL
- ** SIGNIFICANT AT P<=0.01 LEVEL
- N NOT SIGNIFICANT AT P<=0.05 LEVEL

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APPENDIX I - TABLE 5

STATISTICAL SUMMARY OF REPEAT PLATE INCORPORATION TEST RESULTS FOR TA1535 AND TA1537 WITH AND WITHOUT S9

STRAIN	TA1535	TA1535	TA1537	TA1537
ACTIVATION SYSTEM	WITH S-9	NONE	WITH S-9	NONE
TEST DATE	27-DEC-91	27-DEC-91	27-DEC-91	27-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.05	12.7 (1.5)	14.3 (4.0)	7.3 (2.1)	7.3 (0.6)
0.15	11.7 (5.5)	11.3 (2.5)	5.3 (1.5)	7.0 (2.0)
0.50	11.0 (2.6)	13.3 (1.2)	7.3 (2.3)	7.0 (1.0)
1.50	11.7 (4.7)	13.7 (7.4)	7.7 (3.2)	6.0 (3.0)
5.00	11.7 (2.5)	12.7 (1.5)	5.3 (1.5)	5.3 (2.5)
SOLVENT CONTROLS	12.4 (2.8)	12.3 (5.0)	9.2 (2.9)	7.0 (1.2)
SUMMARY ANALYSIS				
TREATMENT LEVELS WITH REV/PLATE > CONTROL				
P<=0.05	0	0	0	0
P<=0.01	0	0	0	0
BARTLETT'S TEST	N	N	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0	0	0
DOSE RESPONSE	N	N	N	N
LACK OF FIT TEST	N	N	N	N

ALL ANALYSES PERFORMED WITH LOG(10) TRANSFORMED DATA
CODES USED ARE:
* SIGNIFICANT AT P<=0.05 LEVEL
** SIGNIFICANT AT P<=0.01 LEVEL
N NOT SIGNIFICANT AT P<=0.05 LEVEL

APPENDIX II

POSITIVE STANDARDS

INDIVIDUAL PLATE INCORPORATION TEST DATA

Table 1

Positive Standards Used for Test Strains in This Study

Strain	S-9 Mix	Compound [a]	Source	EHL Sample Number [b]
TA98	-	4-nitroquinoline-N-oxide	Sigma	T900135
TA98	+	2-acetylaminofluorene	Sigma	T900137
TA100	-	4-nitroquinoline-N-oxide	Sigma	T900135
TA100	+	Benzo(a)pyrene	Sigma	T900138
TA1535	-	Sodium Nitrite	Mallinckrodt	T890017
TA1535	+	2-aminoanthracene	Sigma	T900134
TA1537	-	9-aminoacridine	Sigma	T900136
TA1537	+	2-aminoanthracene	Sigma	T900134

[a] Amounts per plate used are given in plate incorporation test data tables.

[b] Additional information on strength, stability, and purity is contained in the files of the Environmental Health Laboratory Test and Control Substances Officer.

AMES/SALMONELLA ASSAY OF RODEO
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APPENDIX II - TABLE 2

INDIVIDUAL PLATE COUNTS FOR INITIAL TEST USING TA98 AND TA100 WITH AND WITHOUT S9

STRAIN	TA98			TA98			TA100			TA100		
ACTIVATION SYSTEM	WITH S-9			NONE			WITH S-9			NONE		
TEST DATE	10-DEC-91			10-DEC-91			10-DEC-91			10-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.05	41	31	31	26	23	14	127	167	157	130	106	97
0.15	41	34	45	24	27	26	150	152	145	130	113	106
0.50	42	26	44	20	30	37	165	137	132	148	124	100
1.50	36	22	23	18	30	25	141	170	143	108	113	78
5.00	15	18	30	24	23	18	140	149	121	99	59	62
SOLVENT CONTROLS	35	38	32	25	34	20	154	154	176	168	144	135
	34	36	38	42	22	17	149	161	154	126	133	131
	35	47	37	41	19	22	160	139	155	140	132	139
NON-SOLVENT CONTROLS		37			22			159			126	
POSITIVE CONTROLS												
LEVEL 1		102			44			202			276	
LEVEL 2		316			99			1930			1190	
LEVEL 3		726			191			2200			1500	

POSITIVE CONTROL AMOUNTS
TA98 +S-9: LEVEL 1 : 3 UG; LEVEL 2 : 15 UG; LEVEL 3 : 30 UG;
TA98 -S-9: LEVEL 1 : .02 UG; LEVEL 2 : .1 UG; LEVEL 3 : .2 UG;
TA100 +S-9: LEVEL 1 : .2 UG; LEVEL 2 : 1 UG; LEVEL 3 : 2 UG;
TA100 -S-9: LEVEL 1 : .02 UG; LEVEL 2 : .1 UG; LEVEL 3 : .2 UG;

AMES/SALMONELLA ASSAY OF RODEO
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APPENDIX II - TABLE 3

INDIVIDUAL PLATE COUNTS FOR INITIAL TEST USING TA1535 AND TA1537 WITH AND WITHOUT S9

STRAIN	TA1535			TA1535			TA1537			TA1537		
ACTIVATION SYSTEM	WITH S-9			NONE			WITH S-9			NONE		
TEST DATE	13-DEC-91			13-DEC-91			13-DEC-91			13-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.05	9	7	15	14	13	8	9	9	8	6	3	8
0.15	12	14	9	14	11	8	5	12	5	4	4	5
0.50	16	11	11	12	11	11	7	7	7	3	4	4
1.50	10	8	10	14	10	13	10	8	9	6	8	3
5.00	8	8	7	6	11	7	7	8	6	4	4	2
SOLVENT CONTROLS	11	23	17	14	25	13	12	15	11	8	16	4
	15	11	9	11	16	12	7	12	3	12	6	11
	15	10	12	13	23	24	8	10	7	7	10	5
NON-SOLVENT CONTROLS		10			20			9			7	
POSITIVE CONTROLS												
LEVEL 1		55			114			24			19	
LEVEL 2		302			428			56			181	
LEVEL 3		1060			1940			201			1970	

POSITIVE CONTROL AMOUNTS
TA1535 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;
TA1535 -S-9: LEVEL 1 : 500 UG; LEVEL 2 : 2500 UG; LEVEL 3 : 5000 UG;
TA1537 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;
TA1537 -S-9: LEVEL 1 : 10 UG; LEVEL 2 : 50 UG; LEVEL 3 : 100 UG;

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APPENDIX II - TABLE 4

INDIVIDUAL PLATE COUNTS FOR REPEAT TEST USING TA98 AND TA100 WITH AND WITHOUT S9

STRAIN	TA98			TA98			TA100			TA100		
ACTIVATION SYSTEM	WITH S-9			NONE			WITH S-9			NONE		
TEST DATE	17-DEC-91			17-DEC-91			17-DEC-91			17-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.05	25	29	31	24	27	29	159	156	126	113	124	132
0.15	36	36	26	30	13	19	172	140	131	115	120	97
0.50	30	23	38	20	28	18	157	138	116	117	97	150
1.50	30	26	33	23	18	22	146	136	124	127	115	96
5.00	31	29	33	17	10	16	137	134	116	83	104	92
SOLVENT CONTROLS	34	26	26	30	15	30	126	157	145	114	104	127
	37	22	24	14	18	20	107	147	120	133	102	81
	28	26	24	15	15	12	131	152	126	94	94	149
NON-SOLVENT CONTROLS		24			22			123			101	
POSITIVE CONTROLS												
LEVEL 1		85			30			176			136	
LEVEL 2		234			71			447			1030	
LEVEL 3		761			272			1470			1890	

POSITIVE CONTROL AMOUNTS

TA98 +S-9:	LEVEL 1 :	3 UG;	LEVEL 2 :	15 UG;	LEVEL 3 :	30 UG;
TA98 -S-9:	LEVEL 1 :	.02 UG;	LEVEL 2 :	.1 UG;	LEVEL 3 :	.2 UG;
TA100 +S-9:	LEVEL 1 :	.2 UG;	LEVEL 2 :	1 UG;	LEVEL 3 :	2 UG;
TA100 -S-9:	LEVEL 1 :	.02 UG;	LEVEL 2 :	.1 UG;	LEVEL 3 :	.2 UG;

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APPENDIX II - TABLE 5

INDIVIDUAL PLATE COUNTS FOR REPEAT TEST USING TA1535 AND TA1537 WITH AND WITHOUT S9

STRAIN	TA1535			TA1535			TA1537			TA1537		
ACTIVATION SYSTEM	WITH S-9			NONE			WITH S-9			NONE		
TEST DATE	27-DEC-91			27-DEC-91			27-DEC-91			27-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.05	13	14	11	18	15	10	5	8	9	7	7	8
0.15	9	8	18	11	14	9	4	7	5	5	7	9
0.50	13	8	12	12	14	14	6	10	6	8	6	7
1.50	8	17	10	11	22	8	9	10	4	3	9	6
5.00	12	14	9	13	14	11	7	4	5	5	3	8
SOLVENT CONTROLS	11	13	10	14	12	8	7	9	8	8	7	5
	15	16	15	18	11	22	12	6	10	7	7	9
	8	14	10	11	8	7	15	10	6	6	8	6
NON-SOLVENT CONTROLS		14			10			8			5	
POSITIVE CONTROLS												
LEVEL 1		93			149			22			26	
LEVEL 2		431			436			80			68	
LEVEL 3		708			1010			T			321	

T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA1535 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;
TA1535 -S-9: LEVEL 1 : 500 UG; LEVEL 2 : 2500 UG; LEVEL 3 : 5000 UG;
TA1537 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;
TA1537 -S-9: LEVEL 1 : 10 UG; LEVEL 2 : 50 UG; LEVEL 3 : 100 UG;

APPENDIX III

QUALITY ASSURANCE STATEMENT,

GLP COMPLIANCE STATEMENT

and

SUPPLEMENTAL STUDY INFORMATION

S&EH QUALITY ASSURANCE AUDIT STATEMENT

Study Number:	91184 ML-91-441
Protocol Amendments:	None
Study Title:	Ames/Salmonella Mutagenicity Assay of RODEO®
Dates of Inspections and Communication of Findings:	November 15, 1991 December 10, 1991 January 27, 1992
Quality Assurance Review Conducted by:	[redacted]
Results:	The Quality Assurance review indicates the final report accurately presents the raw data as developed during the study. There appears to be no significant deviation from applicable GLP regulations that adversely affected study quality or integrity.

[redacted]
Quality Assurance Director

FEBRUARY 6, 1992
Date

GLP STATEMENT OF COMPLIANCE

Monsanto Environmental Health Laboratory Study Number: 91184/ML-91-441

To the best of our knowledge this study was conducted in general accordance with U.S. Environmental Protection Agency Good Laboratory Practice (GLP) Standards; the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) GLP Standards; and the OECD GLP Principles, with the following exceptions:

1. Characterization of test and control substances was not conducted according to the standards as part of this study.

2. Test and control substance concentrations and homogeneity in carrier were not confirmed.

3. The stability of test and control substances, neat and after mixing with carrier were not determined. Mixtures of test substance with carrier were prepared on each day of use.

[redacted] 2/3/92

Study Director Date

[redacted] 2/4/92

Laboratory Director Date

SUPPLEMENTAL STUDY INFORMATION

Study Sponsor: Monsanto Agricultural Company
Submitted to: [redacted] Staff Toxicologist

Scientists and Professionals Participating in Study

Study Director: [redacted]

Study Coordinator:

Technical support:

Supervisor of Study Director:

Location of Study Material

Type	Location
Specimens	No specimens saved
Raw data	EHL archives
Final report	EHL archives