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ABSTRACT: The test material, MON 14445, 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 observed to be toxic at the maximum dose level for mutagenicity testing, 0.5 mg/plate in the absence of S9 activation and 1.5 mg/plate in the presence of S9 activation. No significant mutagenicity was observed in either the initial assays or the subsequent confirmation assays. These results indicate that MON 14445 is not a mutagen in the Ames/Salmonella plate incorporation assay under the experimental conditions.

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

FINAL REPORT

Ames/*Salmonella* Mutagenicity Assay of MON 14445 (DIRECT® Herbicide Formulation)

EHL Study Number: 91185
Sponsor Project Number: ML-91-442

Authors:

2/7/92
Date

Study Director
Science Fellow

2/4/92
Date

Manager, Analytical, Biochemical and Genetic Toxicology

SUMMARY

The test material, MON 14445, 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 observed to be toxic at the maximum dose level for mutagenicity testing, 0.5 mg/plate in the absence of S9 activation and 1.5 mg/plate in the presence of S9 activation. No significant mutagenicity was observed in either the initial assays or the subsequent confirmation assays. These results indicate that MON 14445 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, MON 14445, 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: MON 14445 (DIRECT® Herbicide Formulation)
(Registered Trademark of Monsanto Company, St. Louis, MO)

Identification: Lot No: PSGF-1001 (mgf. lot no.)
EHL Test Sample T910112

Percent Active Ingredient: 72% Glyphosate (acid equivalent)

Appearance: Off-white granules

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 [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. Toxicity of the test sample was observed at 0.5 mg/plate in the absence and presence of activation. The maximum treatment levels used in the plate incorporation test was 0.5 mg/plate in the absence of activation and 1.5 mg/plate in the presence of activation based on clear indications of toxic responses at these levels.

Summary tables of the initial and repeat plate incorporation test results for MON 14445 are presented in Appendix I, Tables 2-5. Individual plate counts are given in Appendix II, Tables 2-5.

Toxicity was observed at the maximum dose levels tested for all strain/microsome combinations, and occasionally at lower dose levels. 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, MON 14445, 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

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APPENDIX I

TOXICITY TEST RESULTS

STATISTICAL SUMMARY DATA FOR PLATE INCORPORATION ASSAYS

Table 1

Toxicity Test Results for MON 14445 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	-	TR	S
0.15	+	N	S
0.5	-	T	S
0.5	+	TR	S
1.5	-	T	S
1.5	+	T	S
5.0	-	T	S
5.0	+	T	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 MON 14445
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APPENDIX I - TABLE 2

STATISTICAL SUMMARY OF INITIAL PLATE INCORPORATION TEST RESULTS FOR TA98, TA100, TA1535 AND TA1537 WITH S9

STRAIN	TA98	TA100	TA1535	TA1537
ACTIVATION SYSTEM	WITH S-9	WITH S-9	WITH S-9	WITH S-9
TEST DATE	10-DEC-91	10-DEC-91	13-DEC-91	13-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.015	28.0 (9.5)	142.7 (15.4)	10.7 (2.1)	6.3 (1.2)
0.05	27.7 (6.7)	153.3 (37.1)	8.3 (2.5)	8.7 (3.5)
0.15	33.7 (11.0)	111.0 (7.0)	9.0 (2.0)	8.3 (2.5)
0.50	24.0 (8.7)	(T) 89.3 (13.7)	(T) 7.0 (1.0)	(T) 7.3 (0.6)
1.50	(T) 28.0 (0.0)	TOXIC	TOXIC	(T) 6.3 (2.5)
SOLVENT CONTROLS	36.9 (4.3)	155.8 (9.9)	13.7 (4.4)	9.4 (3.6)

T = TOXICITY OBSERVED
TOXIC = TOXICITY OBSERVED, NO REVERTANTS COUNTED

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	A	A	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
A DATA DO NOT ALLOW ANALYSIS TO BE PERFORMED

AMES/SALMONELLA ASSAY OF MON 14445
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APPENDIX I - TABLE 3

STATISTICAL SUMMARY OF INITIAL PLATE INCORPORATION TEST RESULTS FOR TA98, TA100, TA1535 AND TA1537 WITHOUT S9

STRAIN	TA98	TA100	TA1535	TA1537
ACTIVATION SYSTEM	NONE	NONE	NONE	NONE
TEST DATE	10-DEC-91	10-DEC-91	13-DEC-91	13-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.005	21.3 (6.0)	127.3 (21.8)	11.7 (2.5)	8.0 (1.0)
0.015	28.3 (3.1)	120.3 (4.9)	10.7 (3.8)	4.7 (1.2)
0.05	26.0 (10.5)	120.3 (15.2)	14.7 (3.8)	5.0 (1.0)
0.15	32.3 (10.3)	90.7 (16.0)	11.7 (3.5)	7.0 (2.6)
0.50	TOXIC	TOXIC	TOXIC	(T) 6.7 (1.5)
SOLVENT CONTROLS	26.9 (9.6)	138.7 (12.2)	16.8 (5.6)	8.8 (3.8)

T = TOXICITY OBSERVED
TOXIC = TOXICITY OBSERVED, NO REVERTANTS COUNTED

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

AMES/SALMONELLA ASSAY OF MON 14445
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APPENDIX I - TABLE 4

STATISTICAL SUMMARY OF REPEAT PLATE INCORPORATION TEST RESULTS FOR TA98, TA100, TA1535 AND TA1537 WITH S9

STRAIN	TA98	TA100	TA1535	TA1537
ACTIVATION SYSTEM	WITH S-9	WITH S-9	WITH S-9	WITH S-9
TEST DATE	17-DEC-91	17-DEC-91	27-DEC-91	27-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.015	28.7 (2.3)	144.3 (21.5)	9.3 (2.5)	8.7 (2.1)
0.05	30.0 (3.6)	143.3 (24.0)	9.3 (2.3)	13.0 (5.7)
0.15	32.0 (4.4)	124.0 (5.0)	10.3 (2.1)	8.3 (2.1)
0.50	26.3 (4.0)	(T) 85.0 (41.9)	(T) 10.0 (2.8)	(T) 7.7 (0.6)
1.50	(T) 24.0 (8.5)	TOXIC	TOXIC	TOXIC
SOLVENT CONTROLS	27.4 (4.9)	134.6 (16.6)	12.4 (2.8)	9.2 (2.9)

T = TOXICITY OBSERVED
TOXIC = TOXICITY OBSERVED, NO REVERTANTS COUNTED

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

AMES/SALMONELLA ASSAY OF MON 14445
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APPENDIX I - TABLE 5

STATISTICAL SUMMARY OF REPEAT PLATE INCORPORATION TEST RESULTS FOR TA98, TA100, TA1535 AND TA1537 WITHOUT S9

STRAIN	TA98	TA100	TA1535	TA1537
ACTIVATION SYSTEM	NONE	NONE	NONE	NONE
TEST DATE	17-DEC-91	17-DEC-91	27-DEC-91	27-DEC-91
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.005	26.3 (8.5)	122.7 (10.0)	7.7 (0.6)	8.3 (2.1)
0.015	20.7 (9.5)	125.0 (26.9)	7.0 (1.4)	6.3 (1.5)
0.05	21.0 (2.6)	122.3 (9.3)	10.7 (2.5)	8.0 (2.6)
0.15	21.7 (4.6)	91.0 (4.4)	10.3 (1.5)	6.3 (1.5)
0.50	TOXIC	TOXIC	TOXIC	(T) 6.7 (1.5)
SOLVENT CONTROLS	18.8 (6.8)	110.9 (21.8)	12.3 (5.0)	7.0 (1.2)

T = TOXICITY OBSERVED
TOXIC = TOXICITY OBSERVED, NO REVERTANTS COUNTED

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
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 MON 14445
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APPENDIX II - TABLE 2

INDIVIDUAL PLATE COUNTS FOR INITIAL TEST USING TA98, TA100, TA1535 AND TA1537 WITH S9

STRAIN ACTIVATION SYSTEM TEST DATE	TA98 WITH S-9 10-DEC-91			TA100 WITH S-9 10-DEC-91			TA1535 WITH S-9 13-DEC-91			TA1537 WITH S-9 13-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.015	23	39	22	153	150	125	9	13	10	5	7	7
0.05	22	26	35	196	129	135	6	8	11	12	9	5
0.15	41	39	21	119	108	106	7	11	9	11	8	6
0.50	20	34	18	T(105	83	80)	T(6	7	8)	T(8	7	7)
1.50	T	T(28)	T	T	T	T	T	T	T	T(9	4	6)
SOLVENT CONTROLS	35	38	32	154	154	176	11	23	17	12	15	11
	34	36	38	149	161	154	15	11	9	7	12	3
	35	47	37	160	139	155	15	10	12	8	10	7
NON-SOLVENT CONTROLS		37			159			10			9	
POSITIVE CONTROLS												
LEVEL 1		102			202			55			24	
LEVEL 2		316			1930			302			56	
LEVEL 3		726			2200			1060			201	

T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS

TA98 +S-9:	LEVEL 1 : 3 UG;	LEVEL 2 : 15 UG;	LEVEL 3 : 30 UG;
TA100 +S-9:	LEVEL 1 : .2 UG;	LEVEL 2 : 1 UG;	LEVEL 3 : 2 UG;
TA1535 +S-9:	LEVEL 1 : 1 UG;	LEVEL 2 : 5 UG;	LEVEL 3 : 10 UG;
TA1537 +S-9:	LEVEL 1 : 1 UG;	LEVEL 2 : 5 UG;	LEVEL 3 : 10 UG;

AMES/SALMONELLA ASSAY OF MON 14445
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APPENDIX II - TABLE 3

INDIVIDUAL PLATE COUNTS FOR INITIAL TEST USING TA98, TA100, TA1535 AND TA1537 WITHOUT S9

STRAIN	TA98			TA100			TA1535			TA1537		
ACTIVATION SYSTEM	NONE			NONE			NONE			NONE		
TEST DATE	10-DEC-91			10-DEC-91			13-DEC-91			13-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.005	27	15	22	145	134	103	14	12	9	7	8	9
0.015	31	29	25	126	118	117	15	8	9	4	6	4
0.05	37	25	16	134	104	123	19	12	13	6	5	4
0.15	41	35	21	106	74	92	15	12	8	5	10	6
0.50	T	T	T	T	T	T	T	T	T	T(7	8	5)
SOLVENT CONTROLS	25	34	20	168	144	135	14	25	13	8	16	4
	42	22	17	126	133	131	11	16	12	12	6	11
	41	19	22	140	132	139	13	23	24	7	10	5
NON-SOLVENT CONTROLS		22			126			20			7	
POSITIVE CONTROLS												
LEVEL 1		44			276			114			19	
LEVEL 2		99			1190			428			181	
LEVEL 3		191			1500			1940			1970	

T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS

TA98 -S-9:	LEVEL 1 : .02 UG;	LEVEL 2 : .1 UG;	LEVEL 3 : .2 UG;
TA100 -S-9:	LEVEL 1 : .02 UG;	LEVEL 2 : .1 UG;	LEVEL 3 : .2 UG;
TA1535 -S-9:	LEVEL 1 : 500 UG;	LEVEL 2 : 2500 UG;	LEVEL 3 : 5000 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, TA100, TA1535 AND TA1537 WITH S9

STRAIN	TA98			TA100			TA1535			TA1537		
ACTIVATION SYSTEM	WITH S-9			WITH S-9			WITH S-9			WITH S-9		
TEST DATE	17-DEC-91			17-DEC-91			27-DEC-91			27-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.015	26	30	30	168	139	126	7	12	9	8	11	7
0.05	27	29	34	171	128	131	12	8	8	17	9	C
0.15	27	35	34	124	119	129	8	12	11	9	10	6
0.50	31	24	24	T(95	121	39)	T(8	12)	T	T(8	8	7)
1.50	T(30	18)	T	T	T	T	T	T	T	T	T	T
SOLVENT CONTROLS	34	26	26	126	157	145	11	13	10	7	9	8
	37	22	24	107	147	120	15	16	15	12	6	10
	28	26	24	131	152	126	8	14	10	15	10	6
NON-SOLVENT CONTROLS		24			123			14			8	
POSITIVE CONTROLS												
LEVEL 1		85			176			93			22	
LEVEL 2		234			447			431			80	
LEVEL 3		761			1470			708			T	

C = PLATE CONTAMINATED
T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA98 +S-9: LEVEL 1 : 3 UG; LEVEL 2 : 15 UG; LEVEL 3 : 30 UG;
TA100 +S-9: LEVEL 1 : .2 UG; LEVEL 2 : 1 UG; LEVEL 3 : 2 UG;
TA1535 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;
TA1537 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;

AMES/SALMONELLA ASSAY OF MON 14445
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APPENDIX II - TABLE 5

INDIVIDUAL PLATE COUNTS FOR REPEAT TEST USING TA98, TA100, TA1535 AND TA1537 WITHOUT S9

STRAIN	TA98			TA100			TA1535			TA1537		
ACTIVATION SYSTEM	NONE			NONE			NONE			NONE		
TEST DATE	17-DEC-91			17-DEC-91			27-DEC-91			27-DEC-91		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.005	26	18	35	115	119	134	8	8	7	6	9	10
0.015	28	10	24	141	94	140	8	6	C	5	8	6
0.05	19	24	20	130	125	112	8	11	13	7	11	6
0.15	27	19	19	94	86	93	9	10	12	8	5	6
0.50	T	T	T	T	T	T	T	T	T	T(7	8	5)
SOLVENT CONTROLS	30	15	30	114	104	127	14	12	8	8	7	5
	14	18	20	133	102	81	18	11	22	7	7	9
	15	15	12	94	94	149	11	8	7	6	8	6
NON-SOLVENT CONTROLS		22			101			10			5	
POSITIVE CONTROLS												
LEVEL 1		30			136			149			26	
LEVEL 2		71			1030			436			68	
LEVEL 3		272			1890			1010			321	

C = PLATE CONTAMINATED
T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA98 -S-9: LEVEL 1 : .02 UG; LEVEL 2 : .1 UG; LEVEL 3 : .2 UG;
TA100 -S-9: LEVEL 1 : .02 UG; LEVEL 2 : .1 UG; LEVEL 3 : .2 UG;
TA1535 -S-9: LEVEL 1 : 500 UG; LEVEL 2 : 2500 UG; LEVEL 3 : 5000 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:	91185 ML-91-442
Protocol Amendments:	None
Study Title:	Ames/Salmonella Mutagenicity Assay of MON 14445 (DIRECT® Herbicide Formulation)
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: 91185/ML-91-442

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]
Study Director

2/3/92
Date

[redacted]
Laboratory Director

2/4/92
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