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ABSTRACT: The test material, MON 0818, 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). The maximum dose levels for mutagenicity testing were selected based on the toxic responses observed in the toxicity screen for each test strain/microsome combination. The maximum dose level for TA100 with and without activation, TA1535 with and without activation, and TA1537 without activation was 0.1 mg/plate. For TA98 without activation and TA1537 with activation, 0.3 mg/plate was the maximum dose level chosen. For TA98 with activation, the maximum dose level was 1 mg/plate. No significant mutagenicity was observed in either the initial assays or the subsequent confirmation assays. Our results therefore indicate that MON 0818 is not a mutagen in *Salmonella typhimurium* under our experimental conditions.

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Ames/Salmonella Mutagenicity Assay of MON 0818

Study Number: 89178
Project Number: ML-89-461

Submitted to: Monsanto Agricultural Company
Through: [redacted] Staff Toxicologist

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[redacted] 10/26/90
Date

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SUMMARY

The test material, MON 0818, 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). The maximum dose levels for mutagenicity testing were selected based on the toxic responses observed in the toxicity screen for each test strain/microsome combination. The maximum dose level for TA100 with and without activation, TA1535 with and without activation, and TA1537 without activation was 0.1 mg/plate. For TA98 without activation and TA1537 with activation, 0.3 mg/plate was the maximum dose level chosen. For TA98 with activation, the maximum dose level was 1 mg/plate. No significant mutagenicity was observed in either the initial assays or the subsequent confirmation assays. Our results therefore indicate that MON 0818 is not a mutagen in Salmonella typhimurium under our experimental conditions.

INTRODUCTION

The purpose of this study was to determine if the test sample, MON 0818, had detectable mutagenic activity towards Ames/Salmonella test strains TA98, TA100, TA1535 and 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 15, 1989. Experimental work was initiated on November 28, 1989, and assays were completed on January 29, 1990.

MATERIALS AND METHODS

Test Materials

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

Name: MON 0818

Identification: Lot No.: PIT-8907-757-I
EHL Test Sample T890096

Stated Purity:
4.2 ± 0.1% Ethylene glycol
18.3 ± 0.2% Polyethylene glycol
71.9 ± 0.3% Polyoxyethylene (15) Tallowamine
4.1 ± 0.07% Water

Appearance: Amber liquid

Stated Expiration Date: None

Storage Conditions: Room Temperature

Source: Monsanto Agricultural Company

Solutions of the test material were prepared on the day of use using dimethyl sulfoxide (DMSO) 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°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 plate incorporation assays was MolTox 0237. The stated protein content was 34.0 mg/ml. This 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 umoles $MgCl_2$, 33 umoles KCl, 5 umoles glucose-6-phosphate, 4 umoles NADP, and 100 umoles 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. 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 $p<.01$ was used in determining statistical significance. Results with $p<.05$ 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 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 are given in Appendix I, Table 1. Toxicity was observed at a level of 0.1 mg/plate and higher with activation and 0.03 mg/plate and higher without activation. The maximum dose levels chosen for mutagenicity testing were 0.1 mg/plate in the presence of activation and 0.03 mg/plate in the absence of activation.

In the initial mutagenicity test, statistically significant increases in revertants/plate ($p<.01$) were observed for TA98 in the absence of activation at the 0.03 mg/plate dose level; and for TA1535 in the absence of activation at the lowest level tested, 0.0003 mg/plate level (Appendix I, Table 3). No significant dose response ($p<.01$) was observed in either case. None of the other initial plate incorporation assay strain/microsome combinations had revertants/plate significantly elevated over control values ($p<.01$) or a significant dose response ($p<.01$). In the repeat assay using TA1535 and TA1537, no significant responses were observed (Appendix I, Tables 4-5). Toxicity was observed at the highest dose levels tested in the initial assay of TA100 in the presence and absence of activation and in the initial and repeat assays of TA1535 in the presence of activation. Because no toxicity was observed for test strains other than TA100 with and without activation and for TA1535 with activation, a second toxicity screen was performed using TA98, TA1535 and TA1537. The results of the screens were used to set maximum dose levels for additional plate incorporation assays. A summary of the toxicity screens are presented in Appendix II, Table 1-3 and these additional tests are discussed below.

In the second toxicity screen, toxicity was observed at a level of 0.3 mg/plate and higher for TA98 with activation, and at 0.1 mg/plate and higher without activation. For TA1535, toxicity was observed at a level of 0.1 mg/plate and higher with and without activation. For TA1537, toxicity was observed at a level of 0.3 mg/plate and higher with activation, and at 0.1 mg/plate and higher without activation.

After reviewing the results of the toxicity tests, the maximum dose levels chosen for mutagenicity testing were selected for the repeat assays. They were as follows (mg/plate):

Test Strain	Activation +	Activation -
TA98	1.0	0.3
TA100	0.1	0.1
TA1535	- -	0.1
TA1537	0.3	0.1

Summary tables of the additional plate incorporation test results for MON 0818 are presented in Appendix II, Tables 4-9. Individual plate counts are given in Appendix III, Tables 2-11.

Statistical analyses of the additional plate incorporation assay results indicated that the test sample was not mutagenic. None of the plate incorporation assay strain/microsome combinations had any treatment levels with revertants/plate significantly elevated over control values ($p<.01$) or significant dose response ($p<.01$). Although no dose response analyses could be conducted for the TA100 repeat assays because the solvent control values were higher than the treated plates, there were no indications of a dose response among the treated plates. The apparent response observed for TA98 in the absence of activation in the initial assay at the 0.03 mg/plate level was not reproducible in two subsequent assays that were tested up to a toxic level of 0.3 mg/plate. Also, in the initial assay of TA1535 in the absence of activation, the lowest level tested had an elevated response over controls, however, in three subsequent assays no statistically significant responses ($p<.01$) were observed.

Toxicity was observed for all test strains at the maximum dose level and slight toxicity was often observed at the subsequent dose level.

The positive controls yielded positive responses indicating the adequacy of our experimental conditions in the detection of mutagens (Appendix III, Tables 2-11).

DISCUSSION AND CONCLUSIONS

The observed elevations in revertants/plate seen at the highest dose level in the initial assay of TA98, in the absence of activation, and at the lowest dose level in the initial assay of TA1535, in the absence of activation, were not judged to be an indication of mutagenic activity. In both cases, there were only slight increases in revertants/plate values (less than two-fold) over controls, there were no indications of a dose response, and the results were not reproducible in multiple repeat assays that included testing to a toxic level.

The test sample, MON 0818, 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).

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APPENDIX I

TOXICITY TEST RESULTS FOR TA100

STATISTICAL SUMMARY DATA FOR INITIAL PLATE INCORPORATION ASSAYS

Table 1

Toxicity Test Results With Test Strain TA100

Amount of Test Material Per Plate (mg)	S-9 a	Toxic Response b	Solubility c
0.0003	-	N	S
0.0003	+	N	S
0.001	-	N	S
0.001	+	N	S
0.003	-	N	S
0.003	+	N	S
0.01	-	N	S
0.01	+	N	S
0.03	-	T	S
0.03	+	N	S
0.1	-	T	S
0.1	+	T	S
0.3	-	T	S
0.3	+	T	S
1.0	-	T	S
1.0	+	T	S
3.0	-	T	S
3.0	+	T	S
10.0	-	T	S
10.0	+	T	S

a S-9 Mix was prepared using 10% (v/v) rat liver S-9 preparation (MolTox 0237) in the S-9 Mix.

b N = No toxic response. T = Toxicity observed.

c S = Test material soluble. I = Test material insoluble.

AMES/SALMONELLA ASSAY OF MON 0818
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APPENIX I TABLE 2

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR TA98, TA100, TA1535 AND TA1537 WITH S-9

STRAIN	TA98	TA100	TA1535	TA1537
ACTIVATION SYSTEM	WITH S-9	WITH S-9	WITH S-9	WITH S-9
TEST DATE	08-DEC-89	08-DEC-89	08-DEC-89	08-DEC-89
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.001	35.7 (12.7)	119.3 (13.3)	11.7 (4.0)	13.0 (1.7)
0.003	42.3 (9.7)	132.3 (6.8)	4.7 (1.5)	9.0 (3.0)
0.01	38.7 (1.6)	117.0 (19.2)	17.7 (4.6)	12.0 (3.6)
0.03	37.7 (2.5)	117.7 (10.7)	12.7 (0.6)	12.3 (7.5)
0.10	33.0 (1.7)	(T) 0.0 (0.0)	(T) 6.7 (0.6)	9.3 (1.2)
SOLVENT CONTROLS	41.1 (4.7)	123.9 (12.2)	14.9 (2.8)	12.8 (3.7)

T = TOXICITY OBSRVED

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 0818
EHL STUDY 89178 - ML-89-461
APPENDIX I TABLE 3

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR TA98, TA100, TA1535 AND TA1537 WITHOUT S-9

STRAIN	TA98	TA100	TA1535	TA1537
ACTIVATION SYSTEM	NONE	NONE	NONE	NONE
TEST DATE	08-DEC-89	08-DEC-89	08-DEC-89	08-DEC-89
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )			
0.0003	29.7 (4.5)*	118.7 (2.1)	21.3 (5.7)**	8.7 (2.1)
0.001	23.3 (9.1)	118.3 (10.7)	18.7 (3.8)*	8.3 (2.1)
0.003	21.7 (5.9)	107.7 (11.5)	12.7 (1.5)	7.0 (1.7)
0.01	28.7 (8.0)	120.3 (12.7)	12.0 (3.6)	7.0 (2.6)
0.03	35.7 (11.5)** (ST)	53.7 (9.6)	11.0 (3.6)	8.0 (1.7)
SOLVENT CONTROLS	21.4 (5.7)	122.7 (17.7)	12.7 (2.4)	9.0 (2.7)

ST = SLIGHT TOXICITY OBSERVED

SUMMARY ANALYSIS

TREATMENT LEVELS WITH REV/PLATE > CONTROL				
P<=0.05	2	0	2	0
P<=0.01	1	0	1	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 REPEAT ASSAY OF MON 0818
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APPENDIX I TABLE 4

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAY OF TA1535 AND TA1537 WITH S-9

STRAIN	TA1535	TA1537
ACTIVATION SYSTEM	WITH S-9	WITH S-9
TEST DATE	15-DEC-89	15-DEC-89
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
0.001	17.7 (3.5)*	18.7 (6.0)
0.003	13.0 (2.0)	17.0 (2.0)
0.01	11.7 (5.5)	13.7 (1.5)
0.03	12.3 (2.3)	15.7 (3.5)
0.10	(T)12.0 (1.0)	14.3 (1.5)
SOLVENT CONTROLS	12.8 (3.6)	17.2 (6.8)

T = TOXICITY OBSERVED

SUMMARY ANALYSIS

TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	1	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0
DOSE RESPONSE	N	N
LACK OF FIT TEST	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 REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX I TABLE 5

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAY OF TA1535 AND TA1537 WITHOUT S-9

STRAIN	TA1535	TA1537
ACTIVATION SYSTEM	NONE	NONE
TEST DATE	15-DEC-89	15-DEC-89
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
0.0003	23.3 (2.5)*	15.3 (4.2)
0.001	19.0 (0.0)	23.0 (5.0)
0.003	18.7 (5.5)	16.7 (4.7)
0.01	17.0 (2.6)	22.0 (10.4)
0.03	20.7 (1.5)	18.0 (1.0)
SOLVENT CONTROLS	17.8 (4.3)	22.8 (7.4)
SUMMARY ANALYSIS		
TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	1	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0
DOSE RESPONSE	N	N
LACK OF FIT TEST	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

TOXICITY TEST RESULTS FOR TA98, TA1535 AND TA1537

STATISTICAL SUMMARY DATA FOR REPEAT PLATE INCORPORATION ASSAYS

Table 1

Toxicity Test Results With Test Strain TA98

Amount of Test Material Per Plate (mg)	S-9 a	Toxic Response b	Solubility c
0.03	-	N	S
0.03	+	N	S
0.1	-	T	S
0.1	+	N	S
0.3	-	T	S
0.3	+	T	S
1.0	-	T	S
1.0	+	T	S
3.0	-	T	S
3.0	+	T	S
10.0	-	T	S
10.0	+	T	S

a S-9 Mix was prepared using 10% (v/v) rat liver S-9 preparation (MolTox 0237) in the S-9 Mix.

b N = No toxic response. T = Toxicity observed.

c S = Test material soluble. I = Test material insoluble.

Table 2

Toxicity Test Results With Test Strain TA1535

Amount of Test Material Per Plate (mg)	S-9 a	Toxic Response b	Solubility c
0.03	-	N	S
0.03	+	N	S
0.1	-	T	S
0.1	+	T	S
0.3	-	T	S
0.3	+	T	S
1.0	-	T	S
1.0	+	T	S
3.0	-	T	S
3.0	+	T	S
10.0	-	T	S
10.0	+	T	S

a S-9 Mix was prepared using 10% (v/v) rat liver S-9 preparation (MolTox 0237) in the S-9 Mix.

b N = No toxic response. T = Toxicity observed.

c S = Test material soluble. I = Test material insoluble.

Table 3

Toxicity Test Results With Test Strain TA1537

Amount of Test Material Per Plate (mg)	S-9 a	Toxic Response b	Solubility c
0.03	-	N	S
0.03	+	N	S
0.1	-	T	S
0.1	+	N	S
0.3	-	T	S
0.3	+	T	S
1.0	-	T	S
1.0	+	T	S
3.0	-	T	S
3.0	+	T	S
10.0	-	T	S
10.0	+	T	S

a S-9 Mix was prepared using 10% (v/v) rat liver S-9 preparation (MolTox 0237) in the S-9 Mix.

b N = No toxic response. T = Toxicity observed.

c S = Test material soluble. I = Test material insoluble.

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX II TABLE 4

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAYS OF TA98 WITH S-9

STRAIN ACTIVATION SYSTEM TEST DATE	TA98 WITH S-9 23-JAN-90	TA98 WITH S-9 26-JAN-90
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
0.01	53.3 (5.1)*	29.3 (4.6)
0.03	47.3 (4.9)	30.3 (5.9)
0.10	53.0 (2.6)*	34.0 (2.6)
(ST) 0.30	36.7 (7.5)	22.3 (5.1)
(T) 1.00	0.0 (0.0)	0.0 (0.0)
SOLVENT CONTROLS	44.3 (5.7)	39.6 (8.4)

ST = SLIGHT TOXICITY OBSERVED
T = TOXICITY OBSERVED

SUMMARY ANALYSIS		
TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	2	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0
DOSE RESPONSE	N	N
LACK OF FIT TEST	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 REPEAT ASSAY OF MON 0818
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APPENDIX II TABLE 5

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAYS OF TA98 WITHOUT S-9

STRAIN		TA98	TA98
ACTIVATION SYSTEM		NONE	NONE
TEST DATE		23-JAN-90	26-JAN-90
AMOUNT/PLATE IN MG		REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
	0.003	31.0 (3.0)	25.3 (1.5)
	0.01	32.7 (11.5)	27.7 (5.8)
	0.03	34.0 (4.6)	34.0 (4.4)
(ST)	0.10	30.0 (4.4)	28.0 (5.2)
(T)	0.30	0.0 (0.0)	0.0 (0.0)
SOLVENT CONTROLS		34.9 (6.1)	37.1 (7.2)

ST = SLIGHT TOXICITY OBSERVED
T = TOXICITY OBSERVED

SUMMARY ANALYSIS

TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	0	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0
DOSE RESPONSE	N	N
LACK OF FIT TEST	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 REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX II TABLE 6

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAYS OF TA100 WITH AND WITHOUT S-9

STRAIN	TA100	TA100	TA100
ACTIVATION SYSTEM	WITH S-9	NONE	NONE
TEST DATE	23-JAN-90	23-JAN-90	26-JAN-90
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )		
0.001	103.7 (14.8)	73.0 (14.0)	96.7 (7.1)
0.003	103.0 (12.3)	69.7 (5.8)	98.3 (9.1)
0.01	105.7 (15.3)	68.3 (4.6)	107.7 (9.3)
(ST) 0.03	88.3 (3.8)	44.7 (6.1)	82.7 (3.5)
(T) 0.10	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
SOLVENT CONTROLS	140.1 (9.6)	109.9 (11.0)	132.2 (10.7)

ST = SLIGHT TOXICITY OBSERVED
T = TOXICITY OBSERVED

SUMMARY ANALYSIS

TREATMENT LEVELS WITH REV/PLATE > CONTROL			
P<=0.05	0	0	0
P<=0.01	0	0	0
BARTLETT'S TEST	N	N	N
NO. OUTLIERS (GRUBB'S TEST)	A	A	A
DOSE RESPONSE	A	A	A
LACK OF FIT TEST	A	A	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 REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-481
APPENDIX II TABLE 7

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAYS FOR TA1535 WITHOUT S-9

STRAIN	TA1535	TA1535
ACTIVATION SYSTEM	NONE	NONE
TEST DATE	23-JAN-90	26-JAN-90
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
0.001	10.3 (2.5)	12.0 (5.6)
0.003	11.0 (1.0)	19.7 (7.4)
0.01	9.7 (2.5)	21.3 (3.8)
0.03	13.7 (3.8)	(ST)16.3 (3.5)
(T) 0.10	0.0 (0.0)	0.0 (0.0)
SOLVENT CONTROLS	17.9 (2.9)	16.1 (4.3)

ST = SLIGHT TOXICITY OBSERVED
T = TOXICITY OBSERVED

SUMMARY ANALYSIS

TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	0	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	A	0
DOSE RESPONSE	A	N
LACK OF FIT TEST	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 REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX II TABLE 8

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAYS FOR TA1537 WITH S-9

STRAIN		TA1537	TA1537
ACTIVATION SYSTEM		WITH S-9	WITH S-9
TEST DATE		23-JAN-90	26-JAN-90
AMOUNT/PLATE IN MG		REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
	0.003	13.3 (2.1)	8.7 (7.2)
	0.01	11.0 (1.7)	8.0 (3.5)
	0.03	11.7 (4.9)	12.3 (1.5)
(ST)	0.10	18.7 (3.1)*	12.3 (3.1)
(T)	0.30	0.0 (0.0)	0.0 (0.0)
SOLVENT CONTROLS		12.4 (2.8)	8.9 (2.4)

ST = SLIGHT TOXICITY OBSERVED
T = TOXICITY OBSERVED

SUMMARY ANALYSIS

TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	1	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0
DOSE RESPONSE	N	N
LACK OF FIT TEST	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 REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX II TABLE 9

STATISTICAL SUMMARY OF PLATE INCORPORATION TEST RESULTS FOR REPEAT ASSAYS FOR TA1537 WITHOUT S-9

STRAIN	TA1537	TA1537
ACTIVATION SYSTEM	NONE	NONE
TEST DATE	23-JAN-90	26-JAN-90
AMOUNT/PLATE IN MG	REVERTANTS/PLATE MEAN AND STD. DEV. IN ( )	
0.001	9.3 (3.1)	6.7 (1.2)
0.003	10.0 (3.0)	7.0 (2.6)
0.01	9.7 (3.1)	10.0 (3.6)
0.03	11.7 (2.9)	(ST) 10.0 (2.6)
(T) 0.10	0.0 (0.0)	0.0 (0.0)
SOLVENT CONTROLS	10.3 (3.4)	9.3 (2.1)

ST = SLIGHT TOXICITY OBSERVED
T = TOXICITY OBSERVED

SUMMARY ANALYSIS		
TREATMENT LEVELS WITH REV/PLATE > CONTROL		
P<=0.05	0	0
P<=0.01	0	0
BARTLETT'S TEST	N	N
NO. OUTLIERS (GRUBB'S TEST)	0	0
DOSE RESPONSE	N	*
LACK OF FIT TEST	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 III

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	T890014
TA98	+	2-acetylaminofluorene	Sigma	T890012
TA100	-	4-nitroquinoline-N-oxide	Sigma	T890014
TA100	+	benzo(a)pyrene	Sigma	T890013
TA1535	-	Sodium Nitrite	Mallinckrodt	T890017
TA1535	+	2-aminoanthracene	Sigma	T890016
TA1537	-	9-aminoacridine	Sigma	T890015
TA1537	+	2-aminoanthracene	Sigma	T890016

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 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 2

INDIVIDUAL PLATE COUNTS FOR TA98, TA100, TA1535 AND TA1537 WITH S-9

STRAIN	TA98			TA100			TA1535			TA1537		
ACTIVATION SYSTEM	WITH S-9			WITH S-9			WITH S-9			WITH S-9		
TEST DATE	08-DEC-89			08-DEC-89			08-DEC-89			08-DEC-89		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.001	31	26	50	108	134	116	8	16	11	11	14	14
0.003	53	34	40	127	130	140	3	5	8	8	12	9
0.01	39	40	37	95	130	126	18	13	22	10	16	10
0.03	38	35	40	127	120	108	13	13	12	8	21	8
0.10	31	34	34	T	T	T	T(6	7	7)	10	8	10
SOLVENT CONTROLS	40	40	36	123	136	121	15	19	C	14	16	9
	48	44	35	135	99	113	15	13	16	18	12	12
	38	41	48	137	125	128	18	12	11	9	17	8
NON-SOLVENT CONTROLS		59			164			16			10	
POSITIVE CONTROLS												
LEVEL 1		140			168			35			14	
LEVEL 2		PE			640			135			29	
LEVEL 3		1700			1700			217			52	

C = PLATE CONTAMINATED
T = TOXICITY OBSERVED
PE = PLATING ERROR

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 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 3

INDIVIDUAL PLATE COUNTS FOR TA98, TA100, TA1535 AND TA1537 WITHOUT S-9

STRAIN	TA98			TA100			TA1535			TA1537		
ACTIVATION SYSTEM	NONE			NONE			NONE			NONE		
TEST DATE	08-DEC-89			08-DEC-89			08-DEC-89			08-DEC-89		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE											
0.0003	25	30	34	117	118	121	23	15	26	11	8	7
0.001	22	15	33	108	125	124	23	17	16	10	6	9
0.003	15	24	26	108	119	98	11	14	13	9	6	6
0.01	23	35	28	114	135	112	13	15	8	6	5	10
0.03	36	24	47	ST(64	45	62)	7	14	12	7	7	10
SOLVENT CONTROLS	18	14	27	115	112	115	16	8	16	5	15	8
	16	22	30	150	117	131	13	13	11	9	8	9
	16	24	26	149	119	98	13	12	12	10	10	7
NON-SOLVENT CONTROLS		29			107			16			15	
POSITIVE CONTROLS												
LEVEL 1		27			129			183			10	
LEVEL 2		59			226			483			18	
LEVEL 3		62			342			2100			32	

ST = SLIGHT 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 : 3 UG; LEVEL 2 : 15 UG; LEVEL 3 : 30 UG;

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 4

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA1535 AND TA1537 WITH S-9

STRAIN		TA1535			TA1537		
ACTIVATION SYSTEM		WITH S-9			WITH S-9		
TEST DATE		15-DEC-89			15-DEC-89		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
0.001		18	14	21	13	18	25
0.003		11	16	13	17	15	19
0.01		8	9	18	15	12	14
0.03		11	15	11	12	19	16
0.10		T(12	13	11)	14	13	16
SOLVENT CONTROLS		7	19	10	10	22	15
		12	11	13	13	14	28
		17	13	13	12	35	8
NON-SOLVENT CONTROLS			12			33	
POSITIVE CONTROLS							
LEVEL 1			35			34	
LEVEL 2			129			52	
LEVEL 3			273			89	

T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA1535 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;
TA1537 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 6 UG; LEVEL 3 : 10 UG;

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 5

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA1535 AND TA1537 WITHOUT S-9

STRAIN		TA1535			TA1537		
ACTIVATION SYSTEM		NONE			NONE		
TEST DATE		15-DEC-89			15-DEC-89		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
0.0003		23	21	26	14	12	20
0.001		19	19	19	28	23	18
0.003		19	13	24	22	13	15
0.01		15	16	20	34	17	15
0.03		19	22	21	18	17	19
SOLVENT CONTROLS		24	14	17	31	30	19
		20	18	10	28	16	13
		15	20	22	14	24	30
NON-SOLVENT CONTROLS			28			16	
POSITIVE CONTROLS							
LEVEL 1			176			12	
LEVEL 2			580			43	
LEVEL 3			1400			93	

POSITIVE CONTROL AMOUNTS
TA1535 -S-9: LEVEL 1 : 500 UG; LEVEL 2 : 2500 UG; LEVEL 3 : 5000 UG;
TA1537 -S-9: LEVEL 1 : 3 UG; LEVEL 2 : 15 UG; LEVEL 3 : 30 UG;

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 6

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA98 WITH S-9

STRAIN		TA98			TA98		
ACTIVATION SYSTEM		WITH S-9			WITH S-9		
TEST DATE		23-JAN-90			26-JAN-90		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
0.01		59	52	49	32	24	32
0.03		44	53	45	37	28	26
0.10		50	55	54	31	35	36
(ST) 0.30		37	29	44	18	28	21
(T) 1.00		T	T	T	T	T	T
SOLVENT CONTROLS		51	52	40	43	36	26
		44	40	45	40	45	48
		34	48	45	36	52	30
NON-SOLVENT CONTROLS		61			52		
POSITIVE CONTROLS							
LEVEL 1		208			103		
LEVEL 2		541			285		
LEVEL 3		920			1000		

T = TOXICITY OBSERVED
ST = SLIGHT TOXICITY OBSERVED

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

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 7

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA98 WITHOUT S-9

STRAIN		TA98			TA98		
ACTIVATION SYSTEM		NONE			NONE		
TEST DATE		23-JAN-90			26-JAN-90		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
	0.003	28	31	34	24	27	26
	0.01	21	44	33	31	21	31
	0.03	29	36	38	29	36	37
(ST)	0.10	25	32	33	31	31	22
(T)	0.30	T	T	T	T	T	T
SOLVENT CONTROLS		44	39	31	38	46	50
		28	39	35	33	28	39
		34	39	25	33	30	37
NON-SOLVENT CONTROLS		46			32		
POSITIVE CONTROLS							
LEVEL 1		31			36		
LEVEL 2		44			49		
LEVEL 3		74			168		

T = TOXICITY OBSERVED
ST = SLIGHT TOXICITY OBSERVED

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

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
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APPENDIX III TABLE 8

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA100 WITH AND WITHOUT S-9

STRAIN	TA100			TA100			TA100		
ACTIVATION SYSTEM	WITH S-9			NONE			NONE		
TEST DATE	23-JAN-90			23-JAN-90			26-JAN-90		
AMOUNT/PLATE IN MG	REVERTANTS/PLATE								
0.001	100	91	120	73	59	87	98	89	103
0.003	117	98	94	73	63	73	105	88	102
0.01	119	109	89	71	71	63	118	105	100
(ST) 0.03	84	91	90	50	T(38)	46	86	83	79
(T) 0.10	T	T	T	T	T	T	T	T	T
SOLVENT CONTROLS	140	138	142	108	110	113	114	126	124
	131	144	122	111	125	87	143	136	139
	150	140	154	101	114	120	133	149	127
NON-SOLVENT CONTROLS		167			116			128	
POSITIVE CONTROLS									
LEVEL 1		268			127			148	
LEVEL 2		760			220			197	
LEVEL 3		1400			340			313	

T = TOXICITY OBSERVED
ST = SLIGHT TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
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 REPEAT ASSAY OF MON 0818
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APPENDIX III TABLE 9

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA1535 WITHOUT S-9

STRAIN		TA1535			TA1535		
ACTIVATION SYSTEM		NONE			NONE		
TEST DATE		23-JAN-90			26-JAN-90		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
	0.001	8	13	10	7	11	18
	0.003	10	12	11	17	14	28
	0.01	7	12	10	23	17	24
	0.03	12	18	11	ST(13	16	20)
(T)	0.10	T	T	T	T	T	T
SOLVENT CONTROLS		15	21	18	14	13	17
		20	18	15	14	11	13
		15	23	16	24	21	18
NON-SOLVENT CONTROLS			24			17	
POSITIVE CONTROLS							
LEVEL 1			190			155	
LEVEL 2			890			870	
LEVEL 3			1200			1600	

T = TOXICITY OBSERVED
ST = SLIGHT TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA1535 -S-9: LEVEL 1 : 500 UG; LEVEL 2 : 2500 UG; LEVEL 3 : 5000 UG;

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 10

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA1537 WITH S-9

STRAIN		TA1537			TA1537		
ACTIVATION SYSTEM		WITH S-9			WITH S-9		
TEST DATE		23-JAN-90			26-JAN-90		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
0.003		14	11	15	4	5	17
0.01		12	12	9	10	4	10
0.03		6	15	14	14	12	11
0.10	(ST)	18	18	22	9	13	15
0.30	(T)	T	T	T	T	T	T
SOLVENT CONTROLS		8	14	11	8	6	11
		12	16	12	11	9	13
		15	9	15	8	6	8
NON-SOLVENT CONTROLS			16			11	
POSITIVE CONTROLS							
LEVEL 1			15			13	
LEVEL 2			20			24	
LEVEL 3			37			39	

T = TOXICITY OBSERVED
ST = SLIGHT TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA1537 +S-9: LEVEL 1 : 1 UG; LEVEL 2 : 5 UG; LEVEL 3 : 10 UG;

AMES/SALMONELLA REPEAT ASSAY OF MON 0818
EHL STUDY 89178 - ML-89-461
APPENDIX III TABLE 11

INDIVIDUAL PLATE COUNTS FOR REPEAT ASSAYS FOR TA1537 WITHOUT S-9

STRAIN		TA1537			TA1537		
ACTIVATION SYSTEM		NONE			NONE		
TEST DATE		23-JAN-90			26-JAN-90		
AMOUNT/PLATE IN MG		REVERTANTS/PLATE					
	0.001	6	10	12	6	8	6
	0.003	7	13	10	8	9	4
	0.01	13	9	7	9	14	7
	0.03	10	10	15	ST(11	7	12)
(T)	0.10	T	T	T	T	T	T
SOLVENT CONTROLS		7	8	13	13	9	10
		7	8	12	8	8	9
		17	9	12	12	6	9
NON-SOLVENT CONTROLS		18			10		
POSITIVE CONTROLS							
LEVEL 1		13			9		
LEVEL 2		15			17		
LEVEL 3		45			38		

T = TOXICITY OBSERVED
ST = SLIGHT TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS
TA1537 -S-9: LEVEL 1 : 3 UG; LEVEL 2 : 15 UG; LEVEL 3 : 30 UG;

APPENDIX IV

QUALITY ASSURANCE STATEMENT,

GLP COMPLIANCE STATEMENT

and

SUPPLEMENTAL STUDY INFORMATION

S&EH QUALITY ASSURANCE AUDIT STATEMENT

Study Number: 89178
ML-89-461

Protocol Amendments: None

Study Title: Ames/Salmonella Assay of MON 0818

Dates of Inspections and Communication of Findings:
November 30, 1989
December 08, 1989
May 18, 1990
September 06, 1990

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

September 14, 1990
Date

GLP STATEMENT OF COMPLIANCE

Monsanto Environmental Health Laboratory Study Number:
89178/ML-89-461

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.

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.

These deviations should not impact the interpretation of the study.

[redacted]

Study — 10/26/90 Date

[redacted]

Laboratory Director — 11/12/90 Date

SUPPLEMENTAL STUDY INFORMATION

Scientists and Professionals Participating in Study

Study Director:

Professionals:

Technical Support:

Location of Study Material

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