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DATE: 3 March 1981

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TITLE: AMES/SALMONELLA MUTAGENICITY ASSAY OF MON 8080

AUTHORS: [redacted]

ABSTRACT: The test material, MON 8080, was not mutagenic toward Salmonella typhimurium test strains TA98, TA100, TA1535 or TA1537 in plate incorporation assays conducted with and without a rat liver microsomal activation system. A maximum of 3 ul per plate was used in plate incorporation tests. No mutagenic activity was observed in spot tests conducted with TA98, TA100, TA1535 or TA1537 in the absence of microsomal activation or in the presence of rat liver or mouse liver microsomal activation systems. An amount of 20 ul per spot was used in spot tests. Levels of 3 ul per plate were toxic to all four test strains in the presence and absence of a rat microsomal activation system. Levels of 0.9 ul per plate were toxic to test strains TA98, TA100, TA1535, and TA1537 in the absence and presence of microsomal activation.

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MONSANTO COMPANY
ENVIRONMENTAL HEALTH LABORATORY
645 S. NEWSTEAD
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Ames/Salmonella Mutagenicity Assay of MON 8080

Study Number: 800281
DMEH Project Number: ML-80-294

Submitted to: MAPC
Through: [redacted], Staff Toxicologist

Author and Study Director: [redacted]

[redacted]
Study Director — March 3, 1981 — Date

[redacted]
Supervisor — February 27, 1981 — Date

[redacted]
Laboratory Director — 2/27/81 — Date

Report Issued: March 3, 1981

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SUMMARY

The test material, MON 8080, was not mutagenic toward Salmonella typhimurium test strains TA98, TA100, TA1535 or TA1537 in plate incorporation assays conducted with and without a rat liver microsomal activation system. A maximum of 3 ul per plate was used in plate incorporation tests. No mutagenic activity was observed in spot tests conducted with TA98, TA100, TA1535 or TA1537 in the absence of microsomal activation or in the presence of rat liver or mouse liver microsomal activation systems. An amount of 20 ul per spot was used in spot tests. Levels of 3 ul per plate were toxic to all four test strains in the presence and absence of a rat microsomal activation system. Levels of 0.9 ul per plate were toxic to test strains TA98, TA100, TA1535, and TA1537 in the absence and presence of microsomal activation.

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INTRODUCTION

The purpose of this study was to determine whether significant mutagenic activity could be detected towards any of four Salmonella typhimurium test strains (TA98, TA100, TA1535 or TA1537) in the presence or absence of a mammalian metabolic activation system. This system is used for screening purposes as an indicator of potential mutagenic and oncogenic activity in mammals.

This study was conducted at the Monsanto Company Environmental Health Laboratory (645 S. Newstead, St. Louis, MO 63110). The protocol was signed by the Study Director on October 24, 1980. Experimental work was initiated on October 31, 1980 and completed on November 28, 1980.

MATERIALS AND METHODS

The test material, MON 8080, lot number A-780-00, (Environmental Health Laboratory Sample No. T800067), was received on August 8, 1980. A purity of 87.6% was indicated by the sample submitter. The test material was indicated to be stable for one year at 40-85°F by the sample submitter. Other information is documented in the files of the Environmental Health Laboratory Test and Control Substances Officer. Solutions of the test material were prepared with sterile distilled water. Test material and solutions of the test material were stored in the dark at ambient temperature. The identity and sources of positive standard materials used in this study are presented in Appendix I, Tables 1 and 2.

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°C for less than 16 hr. in a shaking incubator. The proper phenotype of each culture was verified by tests for crystal violet sensitivity, ampicillin resistance and requirement for histidine and biotin.

The S-9 preparations were purchased from Litton Bionetics, Inc. (Kensington, MD 20795). These preparations were from livers of Aroclor 1254-induced male Sprague-Dawley rats (Charles River Laboratories, Crl:CD®(SD)BR) and

Aroclor 1254-induced male CD-1 mice (Charles River Laboratories, Crl:CD-1® (ICR)BR). The procedures used in preparation of the S-9 supernatant solutions were those described by Ames et al. (Ref. 1).

The lot numbers of S-9 used in this study are Litton CE098 and DA006 (rat) and Litton IML-15 (mouse). Each 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

The test procedures used were basically those described by Ames et al. (Ref. 1). Spot 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% Difco agar). A sterile paper disc was placed on the solidified top agar and test material was added to the paper disc. Plate incorporation assays were performed in the same manner except that the test material was added to the top agar instead of being placed on a paper disc. Toxicity tests employed the same procedures as those used in the plate incorporation test. Single plates were prepared for each strain/microsome/dose level combination for the spot and toxicity tests. Three replicate plates were prepared for each strain/microsome/dose level combination for the plate incorporation tests. Concurrent positive and negative controls were conducted for spot and plate incorporation tests to demonstrate strain sensitivity and metabolic activation system capability. Plates were examined after at least 48 hrs. at 37 ± 1°C.

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Statistical analysis was performed on plate incorporation assay results after transforming revertant/plate values as $\log_{10}$ (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). Grubb's test was performed to determined if outliers were present (Ref. 7). Dose response was evaluated with regression analysis for $\log_{10}$ transformed doses and revertants/plate (Ref. 8). Significance of dose-response was evaluated by a t-test.

There were no known deviations from the standard operating procedure or circumstances which might have affected the quality and integrity of the results. A signed quality assurance audit statement and supplemental study information are given in Appendix II.

RESULTS

A toxicity screen was conducted using test strain TA100 with and without a rat microsomal activation system. Results of the toxicity screen are given in Appendix I, Table 3. In the toxicity screen, the test material was toxic at a level of 1 ul/plate in the absence of an exogenous metabolic activation system and at a level of 1 ul/plate in the presence of a rat liver metabolic activation system.

Results of a spot test conducted with the test material are presented in Appendix I, Table 4. The test material was toxic to the bacteria as indicated by the zones of growth inhibition. In the initial spot test conducted with test strain TA1537 (data not shown) acceptable positive control values were not observed. The results of a repeat spot test with TA1537 are included in Appendix I, Table 4. No mutagenic response was observed in this assay towards any of the four test strains in the absence of an exogenous metabolic activation system or in the presence of mouse or rat liver metabolic activation systems.

Plate incorporation tests were conducted with maximum levels of 3 ul/plate in the absence of an exogenous metabolic activation system and 3 ul/plate in the presence of a rat liver metabolic activation system. These maximum levels tested were toxic in the plate incorporation test. Results from plate incorporation tests are presented in Appendix I, Tables 6, 7, and 8.

Statistical analysis was performed on plate incorporation test data as described in Materials and Methods. Results of this analysis are summarized in Appendix I, Table 5. None of the test strain results had 3 treatments with revertants/plate significantly greater than controls ($p < 0.01$) a significant dose response ($p < 0.01$.).

DISCUSSION AND CONCLUSIONS

No mutagenic activity was detected in spot tests conducted with the test material at a maximum level of 20 ul per spot. Although the spot test is not as sensitive as the plate incorporation assay for most materials, it does detect activity for certain volatile compounds.

The test material was tested at a maximum level of 3 ul per plate in the absence of a metabolic activation system and 3 ul per plate in the presence of a metabolic activation system in plate incorporation tests. These levels were observed to be toxic to TA100 in the toxicity screen and were toxic to all four test strains in the plate incorporation tests. The maximum concentration tested represents a reasonable maximum concentration for this assay system.

The plate incorporation test results indicated no significant mutagenic activity for this test material.

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APPENDIX I

DATA TABLES

Table 1 - Description of Positive Standard Compounds and Solutions Used in This Study

Material	Source	EHL Sample Number[a]	Solution Concentration	Solution Monsanto Notebook Page
2-acetylaminofluorene	Sigma	T800085	0.30 mg/ml	1633935A
2-aminoanthracene	Sigma	T790105	0.30 mg/ml	1633926B
9-aminoacridine	Sigma	T790104	0.30 mg/ml	1633929
benzo(a)pyrene	Aldrich	T790096	0.02 mg/ml	1633932A
4-nitroquinoline-N-oxide	K&K Labs	T790090	1 ug/ml	1633905
4-nitroquinoline-N-oxide	K&K Labs	T790090	0.5 ug/ml	1633931A
$NaNO_2$	Mallinckrodt	T790092	100 mg/ml	1633928
tris (2,3-dibromopropyl) phosphate	Aldrich	T790095	0.30 mg/ml	1633927

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

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Table 2 - Positive Standards Used for Test Strains in This Study

Strain	S-9 Mix	Compound[a]
TA98	-	4-nitroquinoline-N-oxide
TA98	+	2-acetylaminofluorene
TA100	-	4-nitroquinoline-N-oxide
TA100	+	benzo(a)pyrene
TA1535	-	$NaNO_2$
TA1535	+	tris(2,3-dibromopropyl) phosphate
TA1537	-	9-aminoacridine
TA1537	+	2-aminoanthracene

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

Table 3 - Toxicity Test Results

Amount of Test Substance Per Plate	S-9[a]	Toxic Response[b]
3 ul	+	P
3 ul	-	P
1 ul	+	P (slight)
1 ul	-	P (moderate)
0.2 ul	+	N
0.2 ul	-	N

[a]S-9 Mix was prepared using 10% (v/v) rat liver S-9 preparation (Litton CE098) in the S-9 Mix.

[b]N = No toxic response, P = Toxic response. Lower concentrations tested (0.04 ul and 0.01 ul/plate) were not toxic.

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Table 4 - Spot Test Results[a]

Strain	Response[b] No S-9	Rat S-9[c]	Mouse S-9 [d]
TA98	N(3.2)	N(2.4)	N(2.5)
TA100	N(3.0)	N(2.0)	N(2.2)
TA1535	N(3.1)	N(2.5)	N(2.3)
TA1537	N(2.8)	N(2.1)	N(0)

[a]Results are reported for 20 ul of test material per spot in a volume of 200 ul.

[b]N = No mutagenic response (halo of revertants) observed. Numbers in parentheses are diameters of zones of inhibition in cm. and indicate toxicity.

[c]Litton Lot CE098 at a concentration of 10% (v/v) in the S-9 Mix.

[d]Litton Lot IML-15 at a concentration of 10% (v/v) in the S-9 Mix.

Table 5 - Results of Statistical Analysis of Plate Incorporation Data

Strain	Test[a]	S-9[b]	No. of Treatments Greater Than Control[c]	Dose Response[d]
TA98	1778782	+	0	N
TA98	1778781	-	0	N
TA100	1778782	+	0	N
TA100	1778781	-	0	N
TA1535	1778787	+	0	N
TA1535	1778786	-	0	N
TA1537	1778787	+	0	N
TA1537	1778786	-	3	N
TA1537	1778790	-	0	N

[a]Monsanto notebook page.

[b]-, no S-9 Mix; +, rat liver S-9 Mix.

[c]Evaluated by t-test, ($p < .01$ critical value) using $\log_{10}$ (revertants/plate) transformed data and within levels pooled variance.

[d]Dose response evaluated by t-test using $\log_{10}$ (dose) and $\log_{10}$ (revertants/plate) transformed data. N = dose response not significant ($p < 0.01$ critical level) or negative.

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TABLE 6. PLATE INCORPORATION TEST RESULTS FOR SAMPLE T800067 WITHOUT MICROSOMAL ACTIVATION.

STUDY	800281											
TEST SAMPLE NO.	T800067			T800067			T800067			T800067		
STRAIN	TA98			TA100			TA1535			TA1537		
CULTURE NBP	1633884			1633884			1633886			1633886		
ACTIVATION SYSTEM	WITHOUT S-9			WITHOUT S-9			WITHOUT S-9			WITHOUT S-9		
S-9 PREPARATION	NONE			NONE			NONE			NONE		
% S-9 IN MIX	NONE			NONE			NONE			NONE		
SAMPLE SOLUTION NBP	1778780			1778780			1778785			1778785		
SOLVENT	WATER			WATER			WATER			WATER		
TEST NBP	1778781			1778781			1778786			1778786		
TEST DATE	6-NOV-80			6-NOV-80			14-NOV-80			14-NOV-80		
DOSE AMOUNT/PLATE IN UL	REVERTANTS/PLATE											
0.003	23	19	23	116	134	134	10	12	9	5	8	7
0.012	27	26	38	132	154	121	9	12	12	12	5	8
0.06	37	17	33	126	130	131	13	11	13	9	5	7
0.30	31	25	23	141	135	134	18	6	12	6	7	5
0.90	T19	T22	T27	T94	T125	T89	T13	T7	T8	T7	T7	T
3.00	T	T	T	T67	T117	T70	T	T	T	T	T	T
SOLVENT CONTROLS	27	32	30	148	117	138	14	11	8	5	3	4
	29	25	22	124	137	131	19	14	19	6	1	2
	35	37	26	149	107	124	13	17	11	1	2	4
NON-SOLVENT CONTROLS		29			124			6			3	
POSITIVE CONTROLS												
LEVEL 1		55			154			417			4800	
LEVEL 2		84			325			734			8600	
LEVEL 3		136			549			1900			10000	

NBP = MONSANTO NOTEBOOK PAGE
T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS

TA98 - S-9:	LEVEL 1 : .1 UG	LEVEL 2 : .5 UG	LEVEL 3 : 1 UG		
TA100 - S-9:	LEVEL 1 : .05 UG	LEVEL 2 : .25 UG	LEVEL 3 : .5 UG		
TA1535 - S-9:	LEVEL 1 : 1 MG	LEVEL 2 : 5 MG	LEVEL 3 : 10 MG		
TA1537 - S-9:	LEVEL 1 : 30 UG	LEVEL 2 : 60 UG	LEVEL 3 : 90 UG		

TABLE 7. PLATE INCORPORATION TEST RESULTS FOR SAMPLE T800067 WITH MICROSOMAL ACTIVATION.

STUDY	800281			
TEST SAMPLE NO.	T800067	T800067	T800067	T800067
STRAIN	TA98	TA100	TA1535	TA1537
CULTURE NBP	1633885	1633885	1633886	1633886
ACTIVATION SYSTEM	WITH S-9	WITH S-9	WITH S-9	WITH S-9
S-9 PREPARATION	LITTON DAO-06	LITTON DAO-06	LITTON DAO-06	LITTON DAO-06
% S-9 IN MIX	10	10	10	10
SAMPLE SOLUTION NBP	1778780	1778780	1778785	1778785
SOLVENT	WATER	WATER	WATER	WATER
TEST NBP	1778782	1778782	1778787	1778787
TEST DATE	7-NOV-80	7-NOV-80	16-NOV-80	16-NOV-80

DOSE AMOUNT/PLATE IN UL	REVERTANTS/PLATE											
0.003	31	20	30	146	126	106	9	13	13	1	7	2
0.012	28	19	30	153	157	88	9	8	9	3	4	2
0.06	30	31	43	114	90	104	11	C	7	1	6	7
0.30	23	36	22	91	109	108	11	9	5	4	6	9
0.90	T22	T20	T18	T102	T106	T99	T11	T4	T3	T12	T4	T3
3.00	T13	T	T	T97	T96	T64	T2	T13	T	T	T	T
SOLVENT CONTROLS	36	28	38	123	126	114	7	7	8	5	5	5
	17	36	28	101	104	105	8	11	12	4	9	2
	28	33	27	112	128	92	4	3	8	5	11	9
NON-SOLVENT CONTROLS		23			C			5			6	
POSITIVE CONTROLS												
LEVEL 1		612			402			20			104	
LEVEL 2		7700			680			57			204	
LEVEL 3		5500			868			97			132	

NBP = MONSANTO NOTEBOOK PAGE
C = PLATE CONTAMINATED
T = TOXICITY OBSERVED

POSITIVE CONTROL AMOUNTS

TA98	+ S-9:	LEVEL 1 :	15 UG	LEVEL 2 :	30 UG	LEVEL 3 :	45 UG	
TA100	+ S-9:	LEVEL 1 :	1 UG	LEVEL 2 :	2 UG	LEVEL 3 :	3 UG	
TA1535	+ S-9:	LEVEL 1 :	3 UG	LEVEL 2 :	15 UG	LEVEL 3 :	30 UG	
TA1537	+ S-9:	LEVEL 1 :	3 UG	LEVEL 2 :	15 UG	LEVEL 3 :	30 UG	

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TABLE 8. PLATE INCORPORATION RETEST OF SAMPLE T800067 WITHOUT MICROSOMAL ACTIVATION.

STUDY	800281
TEST SAMPLE NO.	T800067
STRAIN	TA1537
CULTURE NBP	1633887
ACTIVATION SYSTEM	WITHOUT S-9
S-9 PREPARATION	NONE
% S-9 IN MIX	NONE
SAMPLE SOLUTION NBP	1778789
SOLVENT	H2O
TEST NBP	1778790
TEST DATE	28-NOV-80

DOSE AMOUNT/PLATE IN UL	REVERTANTS/PLATE		
0.006	16	11	16
0.012	14	9	16
0.018	13	14	22
SOLVENT CONTROLS	15	14	16
	14	10	20
NON-SOLVENT CONTROLS		13	
POSITIVE CONTROLS			
LEVEL 1		73	
LEVEL 2		96	
LEVEL 3		94	

NBP = MONSANTO NOTEBOOK PAGE

POSITIVE CONTROL AMOUNTS
TA1537 - S-9: LEVEL 1 : 30 UG #LEVEL 2 : 60 UG #LEVEL 3 : 90 UG :

APPENDIX II

QUALITY ASSURANCE AUDIT STATEMENT
and
SUPPLEMENTAL STUDY INFORMATION

DMEH QUALITY ASSURANCE AUDIT STATEMENT

Study No:	800281 ML-80-294
Amendments:	None
Study Title:	Ames/Salmonella Mutagenicity Assay of MON 8080
Communication of Findings:	November 13, 1980 February 5, 1981
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. The study appears to have been conducted in compliance with 21 CFR Part 58, Monsanto Standard Operating Procedures and study protocol.

[redacted]
Manager, Quality Assurance

February 9, 1981
Date

SUPPLEMENTAL STUDY INFORMATION

Scientists and Professionals Participating in Study

Study Director: [redacted]

Supervisor of Study Director: [redacted]

Location of Study Material

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

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