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Document Title MUTAGENICITY EVALUATION OF m-DIAMINOBENZENE (FINAL REPORT) & ACUTE ORAL TOXICITY STUDIES ON p-PHENYLENEDIAMINES WITH COVER LETTER DATED 061783		
Chemical Category m-DIAMINOBENZENE		

AR027-070

Ba (60)

# Monsanto

Monsanto Company  
800 N. Lindbergh Boulevard  
St. Louis, Missouri 63107  
Phone 314 884 1000  
June 17, 1983

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JUN 17 1983

REGISTERED MAIL

RETURN RECEIPT REQUESTED

U.S. Environmental Protection Agency  
Washington, D.C. 20460  
Washington, Maryland 20852

Dear Sirs:

The enclosed health and safety studies are submitted on behalf of Monsanto Company in accordance with the requirements of Section 8(d) of the Toxic Substances Control Act, and regulations promulgated thereunder. These studies were inadvertently omitted from an earlier submission due to a clerical error. This error was discovered during file reviews in connection with the recent 8(d) final rule (48FR1117A 1/30/83). As indicated in the cover memo with our January 17, 1983 submission, the Monsanto file reviews and data collection involved a number of Monsanto personnel and over 100 different file systems. Unfortunately, the possibility for human error always exists. We have just instituted a new internal practice designed to avoid such errors in the future.

This submission includes health and safety studies for two specific chemical substances. Table 1 enclosed lists the substances and the CAS numbers for the studies included herein. Copies of the studies are indexed per the listing on Table 1. Attached to Table 1 and also included with the copies of the studies is a list, on Form F/1, of the specific studies submitted for each substance.

This submission encompasses a total of four studies.

Sincerely,

J. R. Condray  
Director, Regulatory Management  
Toxic Substances  
Environmental Policy Staff  
(Telephone 314-694-8881)

Enclosure

29-0114

HEALTH AND SAFETY STUDIES - TSCA 8(d)

108-45-2  
CAS Number

m-Diaminobenzene  
Chemical Name

Monsanto Study Number  
BIO-75-137

Study Title  
Mutagenicity Evaluation of CP 25313  
Final Report

\* Indicates a study of a mixture

PROJECT NO. BIO-75-137  
RT FILE

878218595 C

CAS # 108-45-2

C-25AM

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JUN 1983

JUN 25 1983

MUTAGENICITY EVALUATION

OF

BIO-75-137 - CP 25313

FINAL REPORT

*m-diaminobenzene*

SUBMITTED TO

MONSANTO COMPANY  
800 N. LINDBERGH BOULEVARD  
ST. LOUIS, MISSOURI 63166

SUBMITTED BY

LITTON BIONETICS, INC.  
5516 NICHOLSON LANE  
KENSINGTON, MARYLAND 20795

LBI PROJECT NO. 2547

JANUARY 16, 1976



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—Monsanto Company

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MATERIAL: B10-75-137 - CP 25313

SUBJECT: FINAL REPORT MUTAGENICITY PLATE ASSAY

1. OBJECTIVE

The objective of this study was to evaluate the test compound for genetic activity in microbial assays with and without the addition of mammalian metabolic activation preparations.

2. MATERIALS

A. Test Compound

- 1. Date Received: December 22, 1975
- 2. Description: White solid, crystal

B. Indicator Microorganisms

The following strains of indicator microorganisms were used in the evaluation:

- 1. Yeast Strain: Saccharomyces cerevisiae, strain D4
- 2. Bacteria Strains: Salmonella typhimurium, strains
 

TA-1535	TA-98
TA-1537	TA-100
TA-1538	

C. Reaction Mixture

The following reaction mixture was employed in the activation tests:

<u>Component</u>	<u>Final Concentration/ml</u>
1. TPN (sodium salt)	6 μmoles
2. Isocitric acid	35 μmoles
3. Tris buffer, pH 7.4	28 μmoles
4. MgCl <sub>2</sub>	2 μmoles
5. Homogenate fraction equivalent to 25 mg of wet tissue	



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2. MATERIALS (Continued)

D. Tissue Homogenates and Supernatants

The tissue homogenates and 9,000 x g supernatants were prepared from the livers of Sprague-Dawley adult male rats. The animals were pretreated with Aroclor 1254 (500 mg/kg) 5 days before kill.

E. Positive Control Chemicals

Table 1 below lists the chemicals used for positive controls in the nonactivation and activation assays.

TABLE 1

<u>ASSAY</u>	<u>CHEMICAL*</u>	<u>SOLVENT</u>	<u>PROBABLE MUTAGENIC SPECIFICITY</u>
Nonactivation	Methylnitrosoguanidine (MNNG)	Water or Saline	BPS**
	2-Nitrofluorene (NF)	Dimethylsulfoxide***	FS**
	Quinacrine mustard (QM)	Water or saline	FS**
Activation	2-Anthramine (ANTH)	Dimethylsulfoxide***	BPS**
	2-Acetylaminofluorene (AAF)	Dimethylsulfoxide***	FS**
	8-Aminoquinoline (AMQ)	Dimethylsulfoxide***	FS**
	Dimethylnitrosamine (DMNA)	Saline	BPS**

\* Concentrations given in Results Section

\*\* BPS = Base-pair substitution

FS = Frameshift

\*\*\* Previously shown to be nonmutagenic

### 3. EXPERIMENTAL DESIGN

#### A. Preparation of Tissue Homogenates and 9,000 x g Cell Fractions

Male animals (sufficient to provide the necessary quantities of tissues) were killed by cranial blow, decapitated, and bled. Organs were immediately dissected from the animal using aseptic techniques and were placed in ice-cold 0.25 M sucrose buffered with Tris at a pH of 7.4. Upon collection of the desired quantity of organs, they were washed twice with fresh buffered sucrose and completely homogenized with a motor-driven homogenizing unit at 4C. The organ homogenate obtained from this step was centrifuged for 20 minutes at 9,000 x g in a refrigerated centrifuge. The supernatant from the centrifuged sample was retained and frozen at -80C. Samples from this preparation were used for the activation studies.

#### B. Plate Test (Overlay Method)

Approximately  $10^9$  cells from a log phase culture of each indicator strain were added to test tubes containing 2.0 ml of molten agar supplemented with biotin and a trace of histidine. For non-activation tests, the four dose levels of the test compound were added to the contents of the appropriate tubes and poured over the surfaces of selective agar plates. In activation tests the 9,000 x g tissue supernatant and required cofactors (core reaction mixture) were added to the overlay tubes. Four dose levels of the test chemical were added to the appropriate tubes, which were then mixed and the contents poured over the surface of a minimal agar (selective medium) plate and allowed to solidify. The plates were incubated for 48 to 72 hours at 37C, and scored for the number of colonies growing on each plate. The concentrations of all chemicals are given in the Results Section. Positive and solvent controls using both directly active positive chemicals and those that require metabolic activation were run with each assay.

#### C. Recording and Presenting Data

The numbers of colonies on each plate were counted and recorded on printed forms. These raw data were transferred directly to the report form sheets and presented as revertants per plate for each indicator strain employed in the assay. The positive and the solvent controls are provided as reference points.



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4. SUMMARY OF PLATE TEST RESULTS

A. Name or code designation of the test compound: B10-75-137 - CP 25313

B. Test date: December 23, 1975 through January 6, 1976

C. Concentrations of the test compound: (1) 5 µg (2) 50 µg (3) 250 µg (4) 500 µg/plate

TEST	SPECIES	TISSUE	REVERTANTS PER PLATE					
			TA-1535	TA-1537	TA-1538	TA-98	TA-100	D4*
<u>NONACTIVATION</u>								
Solvent control	---	---	30	28	20	22	61	90
Positive control**	---	---	>10 <sup>3</sup>	>10 <sup>3</sup>	>10 <sup>3</sup>	>10 <sup>3</sup>	>10 <sup>3</sup>	>10 <sup>3</sup>
Test compound	(1)	---	32	10	18	23	45	67
	(2)	---	41	17	25	16	59	83
	(3)	---	45	12	20	32	44	69
	(4)	---	36	19	22	24	58	70
<u>ACTIVATION</u>								
Solvent Control	Rat	Liver	24	38	20	64	78	53
Positive control***	Rat	Liver	551	232	>10 <sup>3</sup>	>10 <sup>3</sup>	>10 <sup>3</sup>	--
Test compound	(1)	Liver	32	53	62	58	121	53
	(2)	Liver	25	53	188	275	220	51
	(3)	Liver	26	106	924	1268	1460	46
	(4)	Liver	19	173	5748	3272	2960	42

\* Try<sup>+</sup> revertants per plate

** TA-1535	MNNG	10 µl/plate	ANTH	100 µg/plate
TA-1537	QM	10 µl/plate	AMQ	100 µg/plate
TA-1538	NF	100 µg/plate	AAF	100 µg/plate
TA-98	NF	100 µg/plate	AAF	100 µg/plate
TA-100	MNNG	10 µl/plate	ANTH	100 µg/plate
D4	MNNG	10 µl/plate	DMNA	.100 µmoles/plate

5. INTERPRETATION OF RESULTS AND CONCLUSIONS

The test compound, BIO-75-137 - CP 25313, was examined for mutagenic activity in a series of in vitro microbial assays employing Salmonella and Saccharomyces indicator organisms. The compound was tested directly and in the presence of liver microsomal enzyme preparations from Aroclor-induced rats. The following results were obtained:

A. Toxicity

The compound was tested at a series of concentrations such that some physiological effect was produced at the high dose. The lowest dose was below the level of detectable toxicity. The dose range was 5  $\mu$ g to 500  $\mu$ g per plate.

B. Nonactivation Test Results

The results of these tests were negative.

C. Activation Test Results

The compound was mutagenic for Salmonella typhimurium strains TA-1537, TA-1538, TA-98, and TA-100.

D. Conclusions

The test compound, BIO-75-137 - CP 25313, was mutagenic for several of the Salmonella indicator organisms employed in the evaluation. All sensitive strains are capable of detecting frameshift mutagens. The compound was active under conditions of metabolic activation indicating that a mutagenic metabolite was responsible for the observed activity.

Submitted by:

  
David Brusick, Ph.D.  
Director  
Department of Genetics

6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS

Plate test data consist of direct revertant colony counts obtained from a set of selective agar plates seeded with populations of mutant cells suspended in a semisolid overlay. Because the test chemical and the cells are incubated in the overlay for 2 to 3 days, and a few cell divisions occur during the incubation period, the test is semi-quantitative in nature. Although these features of the assay reduce the quantitation of results, they provide certain advantages not contained in a quantitative suspension test:

- The small number of cell divisions permits potential mutagens to act on replicating DNA, which is often more sensitive than nonreplicating DNA.
- The combined incubation of the compound and the cells in the overlay permits constant exposure of the indicator cells for 2 to 3 days.

A. Surviving Populations

Plate test procedures do not permit exact quantitation of the number of cells surviving chemical treatment. At low concentrations of the test chemical, the surviving population on the treatment plates is essentially the same as that on the negative control plate. At high concentrations, the surviving population is usually reduced by some fraction. Our protocol normally employs several doses ranging over two or three log concentrations, the highest of these doses being selected to show slight toxicity as determined by subjective criteria.

B. Dose Response Phenomena

The demonstration of dose-related increases in mutant counts is an important criterion in establishing mutagenicity. A factor that might modify dose-response results for a mutagen would be the selection of doses that are too low (usually mutagenicity and toxicity are related). If the highest dose is far lower than a toxic concentration, no increases may be observed over the dose range selected. Conversely, if the lowest dose employed is highly cytotoxic, the test chemical may kill any mutants that are induced, and the compound will not appear to be mutagenic.



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6. EXPLANATION OF EVALUATION PROCEDURES FOR PLATE ASSAYS (Continued)

C. Control Tests

Positive and negative control assays are conducted with each experiment and consist of direct-acting mutagens for nonactivation assays and mutagens that require metabolic biotransformation in activation assays. Negative controls consist of the test compound solvent in the overlay agar together with the other essential components. The negative control plate for each strain gives a reference point to which the test data are compared. The positive control assay is conducted to demonstrate that the test systems are functional with known mutagens.

D. Interpretation of Results

The demonstration of dose-related increases in mutant counts is the most reliable method to demonstrate mutagenicity. Mutant increases at only one or two doses may be significant if they occur at the higher doses. Increases at low or intermediate concentrations followed by reduced mutant counts at higher doses may indicate that the test chemical has a narrow activity range or that the high dose levels were toxic and the induced revertant cells were killed. We are able to detect the latter possibility by inspecting the background growth, and the former possibility can be investigated by looking at a narrow series of dose levels bracketing the presumptive active range.

It is difficult to detect mutagens with little or no toxicity in this assay since such agents are generally weak mutagens and produce only two to threefold increases in mutant counts. Variations of two to threefold are often within normal fluctuations of the spontaneous counts, and the use of even higher concentrations is often difficult because of the likelihood of overloading the system with large quantities of the chemical. To resolve the mutagenicity of such a chemical, other assays to which statistical evaluations can be applied may be necessary.



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HEALTH AND SAFETY STUDIES - TSCA 8(d)

29-0/15

106-50-3

CAS Number

p-Phenylenediamine

Chemical Name

Monsanto Study Number

Y-78-192

Study Title

Toxicity Studies on: para-phenylenediamine

\* Indicates a study of a mixture

1-78-192

878213600

# YOUNGER LABORATORIES

INCORPORATED

123 CLIFF CAVE ROAD

SAINT LOUIS, MO., 63129



→ Toxicity Studies on: **PARA-PHENYLENEDIAMINE**

CAS# :  
106-50-3

To: **MONSANTO COMPANY**  
St. Louis, Missouri

Lot Number -----

Project No. **Y-78-192**

Date: **October 25th, 1978**

## ACUTE ORAL TOXICITY

Species **Sprague-Dawley Albino Rats**

LD<sub>50</sub> **180** Mg/Kg 95% Confidence Limits **150 - 220** Mg/Kg Slope **5.6**  
 CONDITIONS **Single Oral Dose - 5% Aqueous Solution (warmed to 40° C.)**

Dose Mg/Kg	Avg. Initial Weight		Male	Mortalities/Dosed			Time of Mortality
	Male	Female		Female	Combined		
1 126	235	245	0/3	1/2	1/5	One hour to one day	
2 158	245	240	0/2	2/3	2/5		
3 200	240	245	2/3	1/2	3/5		
4 251	245	240	2/2	3/3	5/5		
5 316	230	240	3/3	2/2	5/5		
6							

Signs of Intoxication **Weight loss (one to six days in survivors), increasing weakness, tremors, collapse, and death.**

Gross Autopsy  
 Decedents : **Hemorrhagic lungs, liver hyperemia, and gastrointestinal inflammation.**

Survivors ( 14 Days) **Viscera appeared normal.**

## ACUTE DERMAL TOXICITY

Species **New Zealand Albino Rabbits**

LD<sub>50</sub> **> 7,940** Mg/Kg 95% Confidence Limits ----- Mg/Kg Slope -----  
 CONDITIONS **Applied as a 40% Aqueous Solution-Suspension -- 24-Hours Exposure**

Dose Mg/Kg	Initial Weight		Male	Mortalities/Dosed			Time of Mortality
	Male	Female		Female	Combined		
1 5,010	---	2.1	---	0/1	0/1		
2 7,940	2.0	2.1	0/1	0/1	0/2		
3							
4							
5							
6							

Signs of Intoxication **Weight loss (two to four days).**

Gross Autopsy  
 Decedents

Survivors ( 14 Days) **Viscera appeared normal.**

1100603

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**ACUTE EYE IRRITATION** (Max. Score = 110. Cornea + Iris + Conjunctivae) Species New Zealand Albino Rabbits  
 Irritation Score (24, 48, 72 Hour Avg.) 15.5 Classification Mild \* Exposure 24 Hours  
 Dose and Conditions 30 Mg. Applied as Finely Ground Sample (Dosage equivalent to 0.1 ml. /  
 \* F.H.S.A.: **CLASSED** as an Eye Irritant // Volume) - F.H.S.A.

Hours	1		24		48		72		120		168	
	Cornea	Conjunctivae Iris										
Animal Number												
1	0	0	16	5	0	14	5	0	12	5	0	8
2	0	0	16	5	0	14	5	0	10	5	0	8
3	0	0	16	5	0	14	5	0	12	5	0	8
4	0	0	16	5	0	14	5	0	10	0	0	8
5	0	0	16	5	0	14	5	0	10	5	0	8
6	0	0	16	5	0	16	5	0	12	0	0	8
Mean Score	16.0		19.3		16.0		11.3		2.3		0.0	

**Comments:** Immediate: Discomfort was moderate with eyes tightly closed  
 10 Minutes: Slight erythema, copious discharge  
 1 Hour: Severe erythema, slight edema, copious discharge  
 24 Hours: Areas of barely perceptible corneal dullness, severe erythema (necrosis), very slight to slight edema, copious discharge containing slight whitish exudate  
 48 - 120 Hours: Gradual improvement  
 168 Hours: All scored zero

**PRIMARY SKIN IRRITATION** (Max. Score = 8. Erythema + Edema) Species New Zealand Albino Rabbits  
 Irritation Score (24, 72 Hour Avg.) 0.0 X Classification Non-Irritating \* X Exposure: 24 Hours  
 Dose and Conditions 0.5 Gm. Applied as Finely Ground Sample Moistened with Water / F.H.S.A.  
 \* F.H.S.A.: **NOT CLASSED** as a Primary Skin Irritant

HOURS	4		24		48		72		168	
	ERYTHEMA I A	EDEMA I A								
ANIMAL NUMBER										
1			X X	0 0	X X	0 0	X X	0 0	X X	0 0
2			X X	0 0	X X	0 0	X X	0 0	X X	0 0
3			X X	0 0	X X	0 0	X X	0 0	X X	0 0
4			X X	0 0	X X	0 0	X X	0 0	X X	0 0
5			X X	0 0	X X	0 0	X X	0 0	X X	0 0
6			X X	0 0	X X	0 0	X X	0 0	X X	0 0
Mean Score			0.0		0.0		0.0		0.0	

**Comments:** X = No evaluation of erythema (sample color stained skin deep purple to black color)  
 Sample classed as non-irritating since other parameters indicated no apparent skin changes

**INHALATION TOXICITY** Species

Mortality .....		Initial sample .....	g	Chamber temperature ..	°C
Concentration .....	Mg/L	Recovered sample ....	g	Chamber humidity ....	%
Exposure .....	Hrs	Condensed sample ....	g	Chamber volume .....	L
Sample temperature...	°C	Vaporized sample ....	g	Air flow rate .....	L/Min

Signs of Intoxication

Gross Autopsy

Decedents

Survivors ( Days) 1110604

## CERTIFICATE OF AUTHENTICITY

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