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

In Vivo Bone Marrow Cytogenetics Study of Glyphosate in Sprague-Dawley Rats

Study Number: 830083
DMEH Project Number: ML-83-236

Submitted to: Monsanto Agricultural Products Company
Through: , Senior Product Toxicologist

Study Director: , Senior Research Toxicologist

10/20/83
, Study Director Date

10/24/83
Date

10/21/83
, Director EHL Date

Date Report Issued: October 20, 1983

Number of Pages: 20

SUMMARY

The potential for glyphosate to induce chromosomal aberrations in the bone marrow cells of Sprague-Dawley rats was tested. Glyphosate was administered via intraperitoneal injection to male and female rats at a dosage of 1.0 g/kg body weight. Treatment periods were 6, 12 or 24 hrs. No significant increases in chromosomal aberrations were observed in the bone marrow cells at any of the time points tested. The results of this test suggest that glyphosate does not have the potential to produce clastogenic effects in mammalian cells.

TABLE OF CONTENTS

SUMMARY	2
TABLE OF CONTENTS	3
INTRODUCTION	4
MATERIALS AND METHODS	4
Test Materials	4
Animals	5
Administration of Test Chemical	5
Preparation of Bone Marrow Cells	6
Slide Preparation	6
Distribution of Slides for Scoring	7
Scoring of Chromosomal Aberrations	7
Statistical Analysis	8
RESULTS	8
DISCUSSION	9
REFERENCE	10
QUALITY ASSURANCE STATEMENT	11
Appendix I	
Appendix II	

INTRODUCTION

The study was designed to evaluate the potential of the test chemical, glyphosate, to induce morphological aberrations in the chromosomes of bone marrow cells. A well-established cytogenetics assay, the in vivo bone marrow chromosomal aberration assay, was used.

The in vivo bone marrow assay involves the administration of the test chemical in whole animals. At appropriate time intervals, the bone marrow cells were extracted and the morphology of the chromosomes in the mitotic cells are examined for aberrations. The advantage of this assay is the use of whole animals, therefore permitting normal metabolism (activation and detoxification) under a relevant in vivo situation.

The in vivo bone marrow cytogenetics assay has been found sensitive to a wide variety of chemicals. The assay has recently been reviewed by the EPA Gene-Tox program (Preston et al., 1981). The albino rat was used because it is a widely accepted animal species for toxicological studies.

The study was conducted at the Monsanto Company Environmental Health Laboratory (645 S. Newstead, St. Louis, MO 63110). Staining and scoring of the slides were performed in Oak Ridge National Laboratory Biology Division ('s laboratory). The protocol was signed by the study director on August 9, 1983. Experimental work was initiated on August 8, 1983, and completed on August 12, 1983. All rat data except cytogenetic scores are stored in the archives of the Environmental Health Laboratory. The raw data for scoring are stored in Dr. 's laboratory, Biology Division, Oak Ridge National Laboratory.

MATERIALS AND METHODS

Test Materials

Glyphosate (Environmental Health Laboratory sample no. T830044), a white powder, was submitted by the sponsor and received at EHL on June 20, 1983, and was indicated to have a purity of 98.7%. The material was stored at room temperature as recommended. Solutions of the material were made by resuspending the glyphosate in Hank's balanced salt solution (HBSS) (100 mg glyphosate/ml) and

neutralized with 1 N sodium hydroxide to a pH of 7.0. The final solution was a clear solution. The positive control, cyclophosphamide, was purchased from Sigma Chemical Company, St. Louis, Missouri. Cyclophosphamide was dissolved also in HBSS for testing. The glyphosate test solutions were made within 24 hours of the day of testing.

Animals

The animals used in this study were male and female Sprague-Dawley rats (Crl: CD®(SD)BR) (Trademark of Charles River Breeding Laboratories, Wilmington, MA) obtained from Charles River Breeding Laboratory, Portage, MI. Following delivery to the EHL by truck, the animals were quarantined for seven days, during which time individual ear tags were applied. The animals were housed in stainless steel mesh cages suspended over absorbant paper bedding. On the day of test material administration, the rats were approximately 9 weeks old. All animals were considered to be in excellent health at the initiation of the study.

Animals were selected for the different test (or control) groups by a computer-generated random number scheme. Each animal was identified by its ear tag and a corresponding bar-coded cage card. Water (supplied by the public water system of St. Louis, MO) was provided ad libitum via an automatic watering system. Purina Laboratory Rodent Chow® No. 5002 (Trademark of the Ralston-Purina Company, St. Louis, MO) was used as the diet and was provided ad libitum, except for a 14-24 hour fasting prior to dosing. This diet has been determined to be adequate for the maintenance of laboratory rodents used in acute studies. No contaminants were reported by the vendor that were likely to interfere with study conduct. The animals were housed in quarters designed to routinely maintain a 12-hour light cycle, a temperature between 70 and 74 degrees F, and relative humidity between 35 and 60%.

Administration of Test Chemical

A volume of 10 ml per kg of HBSS (solvent control) or the 100 mg/ml glyphosate solution was injected intraperitoneally into each of the experimental animals. The final treatment doses were 0 and 1000 mg glyphosate per kg body weight. A volume of 1 ml/kg of 25 mg/ml of cyclophosphamide was similarly injected as the positive control, yielding a final dose of 25 mg/kg.

The different treatment groups are tabulated below:

	No. of Animals					
	Sacrifice Times					
	6 hr		12 hr		24 hr	
Treatment	Male	Female	Male	Female	Male	Female
Solvent (HBSS)	6	6	6	6	6	6
1 g/kg glyphosate	6	6	6	6	6	6
25 mg/kg cyclophosphamide	6	6	6	6	6	6

The rats were fasted for 14-24 hours before the administration of test substances.

Preparation of Bone Marrow Cells

The animals were injected with 2 mg/kg of colchicine 4, 10 and 22 hours after the administration of glyphosate, solvent or positive control. Two hours later (6, 12 or 24 hours after test chemical or solvent control administration), the animals were sacrificed by CO_2 asphyxiation and severance of the spinal cord. Marrow were aspirated from each femur into a 5 ml syringe containing 2 ml of HBSS. The contents of each syringe were added to 5 ml of HBSS in a plastic centrifuge tube. Syringes and centrifuge tubes containing HBSS were kept in an incubator at 37°C until shortly before use for slide preparation for the scoring of chromosomal aberrations.

Slide Preparation

The bone marrow cells were fixed for slide preparation. For fixation, cell suspensions were pelleted by centrifugation (700 x g, 10 min) and the supernatant solution discarded. One ml of 0.075 M KCl (warmed to 37°C) was added to each tube, the pellet was gently disrupted, another 3 ml of KCl was added, and gross debris removed. The tubes were incubated in a 37°C water bath for 30 min before 1 ml of chilled

Cornoy's fixative (methanol/glacial acetic acid, 3/1, v/v) was added and mixed with the hypotonic solution. After pelleting the cells (700 x g, 10 min) and discarding the supernatant solution, 1 ml of chilled fixative was added and mixed with the pellet followed by addition of 4 ml of fixative solution. The fixed cells were stored refrigerated. One to two drops of the cell suspension in fixative were dropped on a clean wet slide and flamed to facilitate spreading the chromosomes. The air-dried slides were transported to Dr. [redacted]'s laboratory where they were stained for 15-20 minutes in a 2% Giemsa solution. The slides were rinsed in water, air dried, and covered with cover-slips.

Distribution of Slide for Scoring

Scoring was performed by Dr. 's group of the Biology Division, Oak Ridge National Laboratory. The three scorers were Dr.), and his two associates Mr. .

The analysis of each treatment group was equally divided between each of the three scorers. The slides from each animal were examined by two different scorers. This scheme was transmitted to Monsanto where the specific samples for each scorer were listed in terms of the previously assigned animal numbers. The numbers were re-listed in a random manner, to avoid identification by any scorer, and transmitted to Oak Ridge.

Scoring of Chromosomal Aberrations

Approximately 50 mitotic cells (300 cells per treatment) were scored for chromosomal aberrations. The following information was recorded:

1. Number of cells scored
2. Number of cells with normal numbers of chromosomes
3. Chromosome-type aberrations:
   a) Dicentric
   b) Ring
   c) Chromosome deletions

4. Chromatid-type aberrations:
   a) Chromatid deletions
   b) Isochromatid deletions
   c) Chromatid interchanges
   d) Chromatid intrachanges
5. Achromatic lesions (gaps)
6. Number of aneuploid cells
7. Location of cells with aberrations

Statistical Analysis

The student's t test was used for data analysis. Treatment samples were considered statistically different from controls if the probability value (p) to be the same as control was ≤ 0.05.

RESULTS

Chromatid-type aberrations were observed both in the solvent control and glyphosate groups at low frequencies. Chromatid deletions, the most frequent category, was observed at a frequency of approximately 1%. At the 6 hr sampling time, glyphosate treatment did not induce higher frequency of chromatid deletions above the solvent control. There were 7 chromatid deletions in 600 cells (male and female) in the solvent control group and 6 in 600 cells in the test compound group (Appendix I, Table 1). For the 12 hr sampling times 2 chromatid deletions per 575 cells and 5 per 577 cells were observed for solvent control and glyphosate treatment, respectively (Appendix I, Table 1). At the 24 hr sampling time, 4 chromatid deletions per 565 cells and 7 per 492 cells were observed for the solvent control and glyphosate treatment, respectively (Appendix I, Table 1). The slightly higher frequencies for the glyphosate treatment group at the 12 hr and 24 hr sampling times were not statistically significant ($p > 0.05$) from the control frequencies (Appendix I, Table 2).

Achromatic lesions are not considered to be chromosome aberrations, but were recorded to show that they were distinguished from chromatid aberrations (Appendix I, Tables 1-3; Appendix II, Tables 1-3). However, the

frequencies were still compared in test compound and solvent control groups. There were no significant differences in achromatic lesion frequency between the glyphosate and solvent treated groups for any of the sampling times (Appendix I, Table 2).

The response to the positive control was scored at the 24 hr sampling time. Because of the apparently high cytotoxicity of cyclophosphamide towards bone marrow cells, only 256 cells were available for scoring in the male animals and only 21 cells were scored in the female animals. Nevertheless, high frequencies of chromosomal aberrations induced by cyclophosphamide were observed (Appendix I, Table 1).

No chromosomal-type aberrations were observed in the glyphosate-treated groups (Appendix II, Tables 1-3).

DISCUSSION

A high concentration of glyphosate (1 g per kg body weight) was administered via an effective route for absorption, intraperitoneal injection, into male and female Sprague-Dawley rats. No significant induction of chromosomal aberrations was observed.

The present study therefore suggests that glyphosate does not have significant clastogenic effects in mammalian cells.

The dose used in this study (1.0 g/kg) was the maximum one could effectively administer into the test animals based on the solubility of glyphosate and the amount of fluid to be injected into a rat.

lc/6216A-4

REFERENCE

Preston, R. J., W. Au, M. A. Bender, J. G. Brewen, A. V. Carrano, J. A. Heddle, A. F. McFee, S. Wolff and J. S. Wassom (1981). Mammalian *in vivo* and *in vitro* cytogenetics assay: A report of the U.S. Gene-Tox program. Mutation Res 87: 143-188.

DMEH QUALITY ASSURANCE AUDIT STATEMENT

Study Number:	830083 ML-83-236
Protocol Amendments:	None
Study Title:	In Vivo Bone Marrow Cytogenetics Study in Sprague-Dawley Rats with Glyphosate
Communication of Findings:	August 11, 24, 1983 October 18, 19, 20, 1983
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 were no significant deviations from Good Laboratory Practice regulations which affected study quality or integrity. The study appears to have been conducted in general compliance with 21 CFR Part 58, Monsanto Standard Operating Procedures and study protocol.

[redacted]
Manager, Quality Assurance

October 21, 1983
Date

APPENDIX I

Summary of Results

Table 1 Chromosomal Aberration Frequencies Observed in Rat Bone Marrow Cells Treated with Solvent Control, Glyphosate (1 g/kg) or Positive Control Cyclophosphamide (25 mg/kg)

Table 2 Statistical Analysis of Data

CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION OF MONSANTO COMPANY

Table 1

Chromosomal Aberration Frequencies Observed in Rat Bone Marrow Cells Treated with Solvent Control, Glyphosate (1 g/kg) or Positive Control Cyclophosphamide (25 mg/kg)

	Number of Cells	Normal[1]	Chromatid Deletions[2]	Chromatid Interchanges	Chromatid Intrachanges	Achromatic Lesions[3]	Aneuploid Cells[4]
A. 6 hr Sampling Time							
Vehicle Control							
Male	300	296	3	1	0	0	13
Female	300	292	4	0	0	5	22
Total	600	588	7	1	0	5	35
Test Compound							
Male	300	293	3	0	0	6	21
Female	300	291	3	0	0	6	22
Total	600	584	6	0	0	12	43
B. 12 hr Sampling Time							
Vehicle Control							
Male	300	297	1	0	0	2	17
Female	275	271	1	0	0	1	31
Total	575	568	2	0	0	3	48
Test Compound							
Male	300	294	3	0	0	3	21
Female	277	270	2	0	0	6	21
Total	577	564	5	0	0	9	42

Table 1 (Cont.)

Chromosomal Aberration Frequencies Observed in Rat Bone Marrow Cells Treated with Solvent Control, Glyphosate (1 g/kg) or Positive Control Cyclophosphamide (25 mg/kg)

	Number of Cells	Normal[1]	Chromatid Deletions[2]	Chromatid Interchanges	Chromatid Intrachanges	Achromatic Lesions[3]	Aneuploid Cells[4]
C. 24 hr Sampling Time							
Vehicle Control							
Male	300	296	1	0	0	3	19
Female	265	259	3	0	0	5	27
Total	565	555	4	0	0	8	46
Test Compound							
Male	192	190	2	0	0	0	13
Female	300	289	5	0	0	6	22
Total	492	479	7	0	0	6	35
Positive Control							
Male	256	148	217	76	6	34	22
Female	21	16	14	1	0	3	1
Total	277	164	231	77	6	37	23

[1]Number of normal cells includes aneuploid cells.
[2]This category includes chromatid deletions, isochromatid deletions, and chromosome-type terminal deletions (indistinguishable from isochromatid deletions).
[3]Achromatic lesions are not considered to be aberrations. They are recorded to indicate that a distinction was made between deletions and achromatic lesions.
[4]Almost all aneuploids were minus one chromosome, and since slides had been flamed these cells were considered to be technical artifacts.

Note: Chromosome-type interchanges and intrachanges not observed.

EHL 830083
PAGE AI 3

Table 2

Statistical Analysis of Data[1]

A. Chromatid Deletions

	Observed Frequencies[2]		
Sampling Time	Control	Glyphosate	p[3]
12 hr	0.0035	0.0087	0.26
24 hr	0.0071	0.0142	0.26

B. Achromatic Lesions

	Observed Frequencies[2]		
Sampling Time	Control	Glyphosate	p[3]
6 hr	0.0083	0.020	0.08
12 hr	0.0052	0.016	0.08

[1]Performed only on data where the observed frequency for glyphosate treatment was higher than that of the solvent control.

[2]No. of aberrations - no of cells scored.

[3]Probability to be the same as solvent control as determined by the student's t test.

CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION OF MONSANTO COMPANY

APPENDIX II

Individual Animal Data

Table 1 Chromosomal Aberration Frequencies Observed at the 6 hr Sampling Time

Table 2 Chromosomal Aberration Frequencies Observed at the 12 hr Sampling Time

Table 3 Chromosomal Aberration Frequencies Observed at the 24 hr Sampling Time

CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION OF MONSANTO COMPANY

Table 1

Chromosomal Aberration Frequencies Observed at the 6 hr Sampling Time

	A	B	C	D	E	F	G	H	I	J	K	L
A. Solvent Control												
Male:	M0001	50	49(50)	0	0	0	0	0	0	0	0	1
	M0003	50	47(50)	0	0	0	0	0	0	0	0	3
	M0004	50	48(50)	0	0	0	0	0	0	0	0	2
	M0019	50	43(48)	0	0	0	1	0	1	0	0	5
	M0024	50	47(49)	0	0	0	1	0	0	0	0	2
	M0025	50	49(49)	0	0	0	1	0	0	0	0	0
Female:	F0002	50	45(47)	0	0	0	1	1	0	0	1	2
	F0003	50	45(48)	0	0	1	0	0	0	0	1	3
	F0004	50	44(49)	0	0	0	0	0	0	0	1	5
	F0011	50	46(50)	0	0	0	0	0	0	0	0	4
	F0013	50	44(48)	0	0	0	0	1	0	0	2	4
	F0018	50	46(50)	0	0	0	0	0	0	0	0	4
B. 1 g/kg glyphosate treatment												
Male:	M0002	50	48(50)	0	0	0	0	0	0	0	0	2
	M0005	50	47(49)	0	0	0	0	0	0	0	1	2
	M0007	50	45(49)	0	0	0	0	0	0	0	1	4
	M0008	50	41(47)	0	0	0	1	0	0	0	3	6
	M0009	50	45(49)	0	0	0	1	1	0	0	0	4
	M0011	50	46(49)	0	0	0	0	0	0	0	1	3
Female:	F0001	50	47(48)	0	0	0	0	0	0	0	2	1
	F0005	50	43(49)	0	0	0	0	0	0	0	1	6
	F0008	50	45(49)	0	0	0	0	0	0	0	1	4
	F0009	50	44(47)	0	0	0	1	0	0	0	2	3
	F0012	50	46(49)	0	0	0	0	1	0	0	0	3
	F0014	50	44(49)	0	0	0	1	0	0	0	0	5

[A]Animal Number
[B]No. of Cells Scored
[C]Normal (Normal + Aneuploid)
[D]Dicentric
[E]Ring
[F]Chromosome Deletions
[G]Chromatid Deletions
[H]Isochromatid Deletions
[I]Chromatid Interchanges
[J]Chromatid Intrachanges
[K]Achromatic Lesions
[L]Aneuploid

Table 2

Chromosomal Aberration Frequencies Observed at the 12 hr Sampling Time

	A	B	C	D	E	F	G	H	I	J	K	L
A. Solvent Control												
Male:	M0032	50	47(50)	0	0	0	0	0	0	0	0	3
	M0033	50	46(49)	0	0	0	0	0	0	0	1	3
	M0038	50	43(48)	0	0	1	0	0	0	0	1	5
	M0041	50	47(50)	0	0	0	0	0	0	0	0	3
	M0043	50	49(50)	0	0	0	0	0	0	0	0	1
	M0045	50	48(50)	0	0	0	0	0	0	0	0	2
Female:	F0026	50	46(49)	0	0	0	0	0	0	0	1	3
	F0028	50	44(47)	0	0	0	1	0	0	0	0	3
	F0029	50	43(50)	0	0	0	0	0	0	0	0	7
	F0039	50	47(50)	0	0	0	0	0	0	0	0	3
	F0040	25	20(25)	0	0	0	0	0	0	0	0	5
	F0045	50	40(50)	0	0	0	0	0	0	0	0	10
B. 1 g/kg glyphosate treatment												
Male:	M0012	50	47(49)	0	0	0	0	0	0	0	1	2
	M0013	50	42(48)	0	0	0	0	1	0	0	1	6
	M0015	50	42(49)	0	0	0	0	0	0	0	1	7
	M0016	50	49(49)	0	0	0	0	1	0	0	0	0
	M0018	50	49(50)	0	0	0	0	0	0	0	0	1
	M0022	50	44(49)	0	0	0	1	0	0	0	0	5
Female:	F0019	50	45(48)	0	0	0	0	1	0	0	1	4
	F0025	50	48(50)	0	0	0	0	0	0	0	0	2
	F0027	27	22(25)	0	0	0	0	0	0	0	3	4
	F0030	50	46(49)	0	0	0	0	0	0	0	1	3
	F0032	50	45(48)	0	0	0	1	0	0	0	1	3
	F0033	50	45(50)	0	0	0	0	0	0	0	0	5

[A]Animal Number
[B]No. of Cells Scored
[C]Normal (Normal + Aneuploid)
[D]Dicentric
[E]Ring
[F]Chromosome Deletions
[G]Chromatid Deletions
[H]Isochromatid Deletions
[I]Chromatid Interchanges
[J]Chromatid Intrachanges
[K]Achromatic Lesions
[L]Aneuploid

Table 3

Chromosomal Aberration Frequencies Observed at the 24 hr Sampling Time

	A	B	C	D	E	F	G	H	I	J	K	L
A. Solvent Control												
Male:	M0048	50	48(50)	0	0	0	0	0	0	0	0	2
	M0049	50	45(48)	0	0	0	1	0	0	0	1	3
	M0056	50	45(50)	0	0	0	0	0	0	0	0	5
	M0058	50	46(50)	0	0	0	0	0	0	0	0	4
	M0059	50	48(50)	0	0	0	0	0	0	0	0	2
	M0060	50	45(48)	0	0	0	0	0	0	0	2	3
Female:	F0048	33	32(33)	0	0	0	0	0	0	0	0	1
	F0054	50	44(50)	0	0	0	0	0	0	0	0	6
	F0056	50	48(49)	0	0	0	0	1	0	0	0	1
	F0057	50	40(48)	0	0	0	0	1	0	0	1	9
	F0058	32	26(29)	0	0	0	1	0	0	0	4	3
	F0060	50	43(50)	0	0	0	0	0	0	0	0	7
B. 1 g/kg glyphosate												
Males:	M0029	10	9(10)	0	0	0	0	0	0	0	0	1
	M0035	5	3(5)	0	0	0	0	0	0	0	0	2
	M0039	27	26(27)	0	0	0	0	0	0	0	0	1
	M0040	50	49(49)	0	0	0	1	0	0	0	0	0
	M0044	50	43(49)	0	0	0	0	1	0	0	0	6
	M0057	50	47(50)	0	0	0	0	0	0	0	0	3
Female:	F0038	50	45(48)	0	0	0	1	1	0	0	0	3
	F0042	50	46(49)	0	0	0	0	0	0	0	1	3
	F0049	50	40(46)	0	0	0	0	0	0	0	4	6
	F0051	50	46(50)	0	0	0	0	0	0	0	0	4
	F0052	50	43(48)	0	0	0	1	1	0	0	0	5
	F0055	50	47(48)	0	0	0	0	1	0	0	1	1

CONTAINS TRADE SECRET OR OTHERWISE CONFIDENTIAL INFORMATION OF MONSANTO COMPANY

Table 3 (Cont.)

Chromosomal Aberration Frequencies Observed at the 24 hr Sampling Time

	A	B	C	D	E	F	G	H	I	J	K	L
C. Positive Control (25 mg/kg cyclophosphamide)												
Male:	M0046	50	21(25)	0	0	0	25	13	25	1	5	4
	M0047	23	13(14)	0	0	0	8	5	2	1	3	1
	M0050	50	30(35)	0	0	0	22	8	7	0	2	5
	M0051	50	31(35)	0	0	0	19	6	7	0	4	4
	M0053	51	24(28)	0	0	0	40	25	20	3	9	7
	M0054	32	10(11)	0	0	0	24	22	15	1	11	1
Female:	F0041	1	0(1)	0	0	0	0	0	0	0	0	1
	F0043	6	6(6)	0	0	0	0	0	0	0	0	0
	F0047	1	1(1)	0	0	0	0	0	0	0	0	0
	F0050	4	0(0)	0	0	0	10	4	1	0	2	0
	F0053	1	0(0)	0	0	0	0	0	0	0	1	0
	F0059	8	8(8)	0	0	0	0	0	0	0	0	0

[A]Animal Number
[B]No. of Cells Scored
[C]Normal (Normal + Aneuploid)
[D]Dicentric
[E]Ring
[F]Chromosome Deletions
[G]Chromatid Deletions
[H]Isochromatid Deletions
[I]Chromatid Interchanges
[J]Chromatid Intrachanges
[K]Achromatic Lesions
[L]Aneuploid