<u>Monsanto</u>

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AUTHORS:

ABSTRACT:

The purpose of this study was to obtain a preliminary indication of the potential for MON 0818 to induce chromosomal effects when tested in a mouse bone marrow micronucleus screening assay. MON 0818 was administered by a single intraperitoneal (i.p.) injection to male and female CD-1 mice at a target dose of approximately 100 mg/kg body weight. Negative control groups were treated with vehicle only (corn oil, 10 ml/kg body weight) and positive control groups were treated, via i.p. injection, with cyclophosphamide (60 mg/kg body weight). Mouse bone marrow was sampled at approximately 24 and 48 hours after dosing for the vehicle and MON 0818 dosed groups. A single sampling time of 24 hours after dosing was used for the cyclophosphamide positive control group. Slides of bone marrow cells were prepared from five animals/time point for each group and scored for the occurrence of micronucleated polychromatic erythrocytes (micronucleated PCE) and PCE/total erythrocyte ratios.

Based on results from the toxicity rangefinding experiments, a target dose of 100 mg/kg body weight was selected as the maximum dose level for male and female mice that would insure a reasonable probability of observing signs of toxicity but allow survival of the treated animals through the 48 hour time point. In the main micronucleus experiment, MON 0818 was not toxic to the male and female mice treated at 100 mg/kg body weight, no clinical signs of toxicity or death were observed. No statistically significant decreases in mean body weight change were observed for any of the MON 0818 treated groups or the positive control group compared to the vehicle control group. No statistically significant decreases in mean PCE/total erythrocyte ratio were observed for any of the MON 0818 treated groups or positive control groups.

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STUDY TITLE

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Monsanto Study Number: ML-89-463
Laboratory Project Number: EHL 89182

REPORT SIGNATURE PAGE

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SUMMARY

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Based on results from the toxicity rangefinding experiments, a target dose of 100 mg/kg body weight was selected as the maximum dose level for male and female mice that would insure a reasonable probability of observing signs of toxicity but allow survival of the treated animals through the 48 hour time point. In the main micronucleus experiment, MON 0818 was not toxic to the male and female mice treated at 100 mg/kg body weight, no clinical signs of toxicity or death were observed. No statistically significant decreases in mean body weight change were observed for any of the MON 0818 treated groups or the positive control group compared to the vehicle control group. No statistically significant decreases in mean PCE/total erythrocyte ratio were observed for any of the MON 0818 treated groups or positive control groups.

No statistically significant increases in micronucleated PCE frequency, compared to control values, were observed for MON 0818 dosed groups at either of the sacrifice times. The positive control (cyclophosphamide) yielded the expected increase in micronucleated PCE frequency indicating the adequacy of the experimental conditions.

The observations and findings of this study indicate that MON 0818 did not induce increases in micronucleated PCE frequencies in mouse bone marrow cells under the experimental conditions utilized in this study.

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INTRODUCTION

The study was designed to obtain a preliminary indication of the potential of the test chemical, MON 0818, to induce chromosome effects in an in vivo mammalian assay, the mouse bone marrow micronucleus screening assay. The study was performed under the provisions of a generic protocol used for screening studies and was not designed to be in full compliance with EPA and/or FDA Good Laboratory Practice regulations. However, the data collection procedures were, in general, conducted in accordance with applicable EPA and/or FDA GLP requirements.

The *in vivo* micronucleus assay has been found to be sensitive to a variety of chemical agents. The assay has been reviewed by the EPA Gene-Tox program (Heddle et al., 1983 and Mayournin et al., 1990). It is generally accepted that induction of micronucleus formation in the assay is indicative of either clastogenic effects or malsegregation of chromosomes. An advantage of this assay is that it evaluates effects on somatic cells of mice that are treated in vivo and thus is relevant to the assessment of potential in vivo mammalian genotoxic effects (MacGregor et al., 1987).

This study was conducted at the Environmental Health Laboratory (645 S. Newstead, St. Louis, MO 63110, USA), Monsanto Company. The study was conducted using an abbreviated mouse bone marrow micronucleus assay protocol (Micronucleus Screen Protocol 003), issued 5-Oct-89 and was not intended to meet regulatory guidelines. Experimental work was initiated on 6-Nov-89 and completed on 5-Feb-90.

MATERIALS AND METHODS

Test Materials

Identification and purity of the test material is given below:

Name:

MON 0818

Identification: Batch/Lot No.: PIT-8907-757-I

EHL Test Sample: T890096

Stated Purity: $4.2 \pm 0.1\%$ Ethylene glycol

 $18.3 \pm 0.2\%$ Polyethylene glycol

 $71.9 \pm 0.3\%$ Polyoxyethylene (15) tallowamine

 $4.1 \pm 0.07\%$ Water

Appearance:

Amber liquid

Storage

Conditions: Room Temperature

Expiration: Not indicated on sample submission form.

Source: Monsanto Company

Animals

The animals used in this study were ten to eleven week old male and female CD-1® mice (Source: Charles River Laboratories, Portage, MI) (Registered trademark of Charles River Laboratories Inc., Wilmington, MA). Upon receipt, the animals were quarantined for a minimum of seven days. Only animals considered to be normal were released from quarantine and used for testing. Prior to testing, the mice were uniquely identified using ear tags and corresponding cage cards. The animals were housed two per cage prior to dosing and one per cage after dosing. The animals were housed in suspended, stainless steel cages with stainless steel mesh bottoms.

Animals were selected for the different test (or control) groups by a computer-generated randomization scheme. Water (supplied by the public water system of St. Louis, MO) was provided ad libitum via an automatic watering system. Certified Rodent Diet #5002 (PMI, St. Louis, MO) was used as the diet and was provided ad libitum. This diet has been determined to be nutritionally acceptable for the maintenance of laboratory rodents and has been certified by the manufacturer not to contain contaminants likely to interfere with the study. The animals were housed in rooms designed to routinely maintain a 12-hour light cycle, a temperature between 64°-79°F, and relative humidity between 40-70%. There were no excursions in animal room environmental conditions which had any obvious impact on the results of the study. Animal housing and husbandry were in accordance with the provisions of the 'Guide to the Care and Use of Laboratory Animals', USPHS-NIH Publication No. 86-23.

Extraction of Bone Marrow Cells and Slide Preparation

All animals were sacrificed by cervical dislocation and their femora were removed. Each bone was opened at the end and the bone marrow was flushed with approximately 2 ml of fetal bovine serum in a centrifuge tube. Bone marrow from both femora of each animal were pooled for slide preparation. The suspension was centrifuged to remove the serum. Portions of the remaining cells were placed on a clean glass microscope slide and a smear was prepared. Two slides were initially prepared for each sample and the remaining cell suspension was refrigerated to prepare additional slides if needed. Following preparation of the smears the slides were allowed to air dry overnight. The slides were stained using a Hema-Tek® 1000 Automated Slide Stainer (Registered trademark of Hema-Tek® 1000 Automated Slide Stainer, Ames Division, Miles Laboratories) and Hema-Tek 1000 Stain Pak which includes Wright-Giemsa stain, buffer and

rinse solutions.

Scoring of Slides

Slides of bone marrow cells were coded prior to distribution and slides were scored without knowledge of the treatment or control groups to which the slides belonged. For each animal, two scorers each evaluated: a) 500 total erythrocytes for polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) and b) 500 PCEs for micronucleated polychromatic erythrocytes (MN PCEs). PCEs and NCEs were distinguished by different staining properties. Micronuclei were identified as uniform, darkly stained, round or oval shaped bodies found in the cytoplasm of PCEs. Bodies in PCEs which were refractile, improperly shaped or stained, or which were not in the focal plane of the cell, were not scored as micronuclei. PCEs containing more than one micronucleus were scored as a single micronucleated PCE. Scoring data were used to calculate, for each animal, the ratio of PCEs to total erythrocytes (PCEs plus NCEs) per 1000 erythrocytes and the number of MN PCEs per 1000 PCEs.

Statistical Analysis

Each individual test animal was the unit used for analysis of micronucleated PCE frequency, PCE/total erythrocyte ratio and body weight change. Micronucleated PCE frequencies observed for each animal were transformed as the square root prior to analysis (Snedecor and Cochran, 1967, MacGregor et al., 1987). PCE/total erythrocyte ratios were not transformed. A Dunnett's test (one sided) was used for comparison of treatment group and positive control values with vehicle control values (Dunnett, 1955). A critical value of p≤0.05 was used for statistical significance.

Data Evaluation

To determine whether a statistically significant response in MN PCE frequency is treatment related the following criteria are considered: (a) whether there are time-dependent effects that are consistent with a treatment-induced response and (b) the degree of the response in relation to both concurrent and historical negative and positive control data.

EXPERIMENTAL DESIGN

Administration of Test Chemical

Solutions or suspensions of the test material were prepared on the day of dosing using corn oil (Sigma Chemical Company, lot: 37F0555) as the vehicle. In the main experiment, animals were treated by a single intraperitoneal injection of corn oil (vehicle control, 10 ml/kg body weight), MON 0818 in corn oil (100 mg/kg body weight), or cyclophosphamide in Hanks Balanced Salt

Solution (60 mg/kg body weight). The positive control used was commercial grade cyclophosphamide monohydrate (Sigma Chemical Company, lot: 67F0155).

Animal Observations

During the study, all animals were observed for visible toxic effects and mortality on the day of dosing, and daily thereafter for up to 72 hours after dosing. Animals were weighed at the time of treatment (all experiments) and at the time of sacrifice for bone marrow extraction (main experiment).

Preliminary Experiments for Dose Selection

An initial rangefinding experiment was conducted using one mouse/sex/dose level. The mice were treated by a single i.p. injection of the test material at doses of 1000 and 5000 mg/kg body weight. Vehicle control animals were dosed with an appropriate volume of corn oil. Based on results of the first experiment a subsequent rangefinding experiment was performed to accurately estimate the maximum tolerated dose. Two mice/sex/dose level were treated at 50, 100 200, 400, 600 and 800 mg/kg body weight.

Mouse Micronucleus Experiment

The highest dose level used in the main micronucleus experiment is the maximum tolerated dose, based on lethality, bone marrow cytotoxicity, or clinical signs of toxicity. In the main experiment, mice were treated once with either test material, vehicle or positive control. All test animals were weighed on the day of treatment and sacrifice, and observed daily for clinical signs of toxicity or death. Design of the mouse micronucleus experiment is summarized in the following table.

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Design of the	he Mouse Microi	nucleus Assay

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This docum.		<u>Male</u>	<u>Female</u>	<u>Malc</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>
	High Dose	10	10	5	5	5	5
	Vehicle Control	10	10	5	5	5	5
	Positive Control	5	5	5	5		

RESULTS

Results of the rangefinding experiments are summarized in Appendix I, Table 1. In the rangefinding experiments, male and female mice were treated with MON 0818 at 50, 100, 200, 400, 600, 800, 1000 and 5000 mg/kg body weight. MON 0818 was found to be toxic to male and female mice at 50 mg/kg body weight and greater as indicated by clinical signs of toxicity. Deaths were observed at 200 mg/kg body weight and higher. The combined male and female LD50 was estimated by nonlinear interpolation to be 165.2 mg/kg body weight.

Based on these results, a target dose of 100 mg/kg body weight (approximately 61% of the estimated LD50 value) was selected as the maximum dose for male and female mice that would insure a reasonable probability of observing signs of toxicity but allow survival of the treated animals through the 48 hour time point.

Results of the micronucleus experiment are summarized in Appendix I, Tables 2-4. Individual clinical signs of toxicity from the rangefinding experiments (reported only for the groups in which clinical signs were observed) are presented in Appendix II, Table 1. Individual animal data (body weights, PCE ratio and micronucleated PCEs) are presented in Appendix III, Tables 1-2.

In the main micronucleus experiment, MON 0818 was not toxic to the male and female mice treated at 100 mg/kg body weight, no clinical signs of toxicity or deaths were observed. No statistically significant decreases in mean body weight change were observed for any of the MON 0818 treated groups or the positive control group compared to the vehicle control group. No statistically significant decreases in mean PCE/total erythrocyte ratio were observed for any of the MON 0818 treated groups or positive control groups.

Analysis of the micronucleated PCE (MN PCE) data indicated no statistically significant increase in mean micronucleated PCE frequency compared to concurrent control values for any of the MON 0818 treated groups

The positive control (cyclophosphamide) group yielded the expected positive responses in micronucleated PCE frequency, indicating the adequacy of the experimental conditions.

CONCLUSIONS

The observations and findings of this study indicate that MON 0818 did not induce increases in micronucleated PCE frequencies in mouse bone marrow cells under the experimental conditions utilized in this study.

REFERENCES

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MacGregor, J.T., Heddle, J.A., Hite, M., Margolin, B. H., Ramel, C., Salamone, M.F., Tice, R.R. and Wild, D. (1987). Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. Mutation Res. 189: 103-112.

Mavournin K.H., Blakey D.H., Cimino M.C., Salamone M.F., and Heddle J.A. (1990). The in One and the property of the pr vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the U.S. Environmental Protection Agency Gene-Tox Program. Mutation Res. 239: 29-80.

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APPENDIX I

Data Summary Tables

Table 1	Summary of Toxicity Rangefinding Results
Table 2	Summary of Mean Body Weight Changes in Male and Female Mice
Table 3	Summary of Micronucleus Assay Results in Male and Female Mice: PCE Ratio Data
Table 4 Table 4 Table 4	Summary of Mean Body Weight Changes in Male and Female Mice: PCE Ratio Data Summary of Micronucleus Assay Results in Male and Female Mice: PCE Ratio Data Summary of Micronucleus Assay Results in Male and Female Mice: Micronucleus Data

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a Number of deaths per total number of animals treated.

APPENDIX I - TABLE 2 DUNE 2000 F. M. CLEUS ASSAY OF M. THT CP. APPENDIX I - TABLE 2 MICRONUCLEUS ASSAY OF MON 0818 SUMMARY OF MEAN BODY WEIGHT CHANGE IN MALE AND FEMALE MICE

Mean Body Weight Change (g)

			and the second second second	and the second s	± Standard Deviation	
	Harvest			Vehicle	MON 0818	Positive
	Time (hrs)	Sex	Number	Control a	100 mg/kg	Control ^a
	24	Male	:000 s	-0.6 ± 4.9	-2.2 ± 4.2	10.10.
	24	Female	2005 His	(**339 ±17.6°	$\frac{-2.2 \pm 4.2}{2.7 \pm 1.8}$	-1.0 ± 0.5 -0.5 ± 0.7
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	48	Male	Ch Sulling		-2.2 ± 2.4	
	48	Female	2 92 40, (-0.9 ± 1.3	-1.4 ± 1.6	
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This document is not the	- X7 ~	0.,,0,	1,00,11		itive control, cycloph	osphamide (60

APPENDIX 1 - TABLE 3 MICRONUCLEUS ASSAY OF MON 0818 RONUCLEUS RESULTS IN MATE PCE RATIO DATE MICRONUCLEUS ASSAY OF MON 0818 SUMMARY OF MICRONUCLEUS RESULTS IN MALE AND FEMALE MICE PCE RATIO DATA Meior Port

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24 Female 5 0.45 \pm 0.05 0.52 \pm 0.08 0.50 \pm 0.06 48 Male 5 0.31 \pm 0.06 0.42 \pm 0.03 48 Female 5 0.46 \pm 0.11 0.49 \pm 0.05	48 Male 5 48 Female 5

APPENDIX 1 - TABLE 4 MICRONUCLEUS ASSAY OF MON 0818 RONUCLEUS RESULTS OF MARKET MICRONUCLEUS MICRONUCLEUS ASSAY OF MON 0818 SUMMARY OF MICRONUCLEUS RESULTS OF MALE AND FEMALE MICE MICRONUCLEUS DATA

Mean Micronucleated PCE/1000 PCE + Standard Deviation

			1100 40	± Standard Deviation	1
Harvest			Vehicle	MON 0818	Positive
Time (hrs)	Sex	Number	Control®	100 mg/kg	Control a
			iges end for the	M. Willibite	
24	Male	5 (0	2.4 ± 2.2	0.8 ± 1.1	$22.0 \pm 4.3**$
24	Female	5.69	(1,0\±1,4	0.4 ± 0.5	27.0 ± 5.6**
48	Male	Noch To	0.6 ± 0.5	0.8 ± 0.4	
48	Female		0.8 ± 1.1	0.6 ± 1.3	

^{*} p≤0.05; ** p≤0.01 by one-sided Dunnett's test. Square root transformed data used for statistical analysis of micronucleated PCE.

^a Vehicle control, corn oil (10 ml/kg body wt.); positive control, cyclophosphamide (60 mg/kg).

APPENDIX II

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coloridate of the signs are not reported

Individual Clinical Signs for Range Finding Experiments

APPENDIX II - TABLE 1 MICRONUCLEUS ASSAY OF MON 0818 INDIVIDUAL CLINICAL SIGNS Range finder 1 — Male and Female CD-1 Mice. Route of Administration: Intraperitoneal Target Dose: 1000 mg/kg Treatment Date: 6-Nov-89 Date of Observation Observation L89099-M0002 6-Nov-89 Found Dead L89099-F0002 6-Nov-89 Found Dead			to documes. I and use
MICRONUCLEUS ASSAY OF MON 0818 INDIVIDUAL CLINICAL SIGNS Range finder 1 Male and Female CD-1 Mice Route of Administration: Intraperitoneal Target Dose: 1000 mg/kg Treatment Date: 6-Nov-89 Date of Animal Number Observation Observation L89099-M0002 6-Nov-89 Found Dead L89099-F0002 6-Nov-89 Found Dead		APPENDIX II - TABLE 1	COS HING WINDING
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APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

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	oute of Administration: Intraperitor Target Dose: 50 mg/kg Treatment Date: 7-Nov-89 Date of Observation	olaje i	
Animal Number	Observation	Observation	
L89099-M0004	9-Nov-89	Listless	
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INDIVIDUAL CLINICAL SIGNS

Range finder 11 — Male and Female CD-1 Mice
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Target Dose: 100 mg/kg
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APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

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	Charliegg 40chio	8-Nov-89	Found Dead	
	LES' GUIL GOUTH	rt iner		
	89182102 001	7-Nov-89	Listless	
e ^{jt}	89182F02.002	7.Nov.89	Listless	
ological and the second	L'ALE COLLINE ME	8-Nov-89	Found Dead	
the cont	Enimply of focus			
COLL & BOOK	any line			
is 11.				
iller, Isur				
This document is not the documently.				

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

vder II — Male and Femro

of Administration

Targel Do

Ra	APPENDIX II - TABLE 1 (con MICRONUCLEUS ASSAY OF MO INDIVIDUAL CLINICAL SIG nge finder II — Male and Female (Route of Administration: Intraperi Target Dose: 400 mg/kg Treatment Date: 7-Nov-89	N 0818 A Control of the Control of t
Animal Number	Date of Observation	Observation
89182M03 001	7-Nov-89	Found Dead
89182M03 002	7-Nov-89	Found Dead
89182F03 001	7-Nov-89	Found Dead
89182M03 001 89182F03 001 89182F03 002	Hitting Hills 7-Nov-89	Found Dead

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Inder II - Male and Female CP

of Administration? Inter

Target Dose, 6000

natment

APF MICROI IND Range find	PENDIX II - TABLE 1 (co NUCLEUS ASSAY OF MO IVIDUAL CLINICAL SIC er II – Male and Female (ont.) ON 0818 CD-1 Mice Itoneal Observation	
Route	of Administration: Intraper	toneal	
•	Treatment Date: 7-Nov-89	155 the	
Animal Number	Date of Observation	Observation	
89182M04 001	7-Nov-89	Found Dead	
89182M04 002	7-Nov-89	Found Dead	
89182F04 001	7-Nov-89	Found Dead	
89182F04 002 Etilone 1	7-Nov-89	Found Dead	
89182M04 001 89182M04 002 89182F04 001 89182F04 002		·	9 9 8

Treatment Date: 7-Nov-89	, illo
En City Copylist of Little	.e
Date of	0
Observation	Observat
O COURT TANION	Obscivat

AP MICRO INI Range fine Route	PPENDIX II - TABLE 1 (co DNUCLEUS ASSAY OF MO DIVIDUAL CLINICAL Sto der 11 — Male and Female of Administration: Intraper Target Dose; 800 mg/kg Treatment Date: 7-Nov-89	ont.) ON 0818 CD-1 Mice flooreal Observation	
Animal Number	Observation	Observation	
89182M05 001	7-Nov-89	Found Dead	
89182M05 002	7-Nov-89	Found Dead	
89182F05 001	7-Nov-89	Found Dead	
89182M05 001 89182M05 002 89182F05 001 89182F05 002	7-Nov-89	Found Dead	EHL 89182 Page 24

APPENDIX III

emale Mice of the successful dead of the succ Individual Slide Scoring Data for Male and Female Mice

(PCE/Total Erythrocyte Ratio and Micronucleated PCE)

APPENDIX III - TABLE 1

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL BODY WEIGHTS FOR MALE MICE

		Time of		Body Weight (g)
Treatment Group	Animal Number	Sacrifice a		Jelle	So
		(hr)	Pretest	Final	Difference
				90 Sille	3/1
Vehicle Control	M92 001	24	38.8	39.5	0.7
Corn oil	M92 002	24	39.8	33.6	-6.2
10 ml/kg	M92 003	24	38.0	0 40.0	2.0
	M92 004	24	38.2	43.6	5.4
	M92 005	24	44.1	39.2	-4 .9
			1, 0, 06, cfile	While Sel ships	
MON 0818	M06 001	24	(O) (40.10° C	37.0	-3.1
100 mg/kg	M06 002	24	10 74 0.3270 VA	© 38.0	2.1
	M06 003	24	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	37.9	-4 .1
	M06 004	ر (۱۳۵۵ کا	44\2 C &	36.5	- 7.7
	M06 005	24 8 1	11815 111 40 0 15 11C	41.9	1.9
Cyclambaanhamida	1407 001	Chillegra Co	3 Plizalli Silo	22.2	1.0
Cyclophosphamide 60 mg/kg	M07 001	11/13/16/19	6, 6 360	33,3	-1.2
oo mg/kg	M07 002	0, 224, 90, 910.	77. 031.3	30.2	-1.I
	M07 003	ch My still	35.0	33.8	-1.2
	M07 004	7 65 tol. Mil.	38.3	38.2	-0.1
	M07 005	419,920 Lie 101,	39.6	38.4	-1.2
Vehicle Control	M92 001 M92 002 M92 003 M92 004 M92 005 M06 001 M06 002 M06 003 M06 004 M06 005 M07 001 M07 002 M07 003 M07 004 M07 005 M92 006 M92 007 M92 008 M92 009 M92 010	. C. 18 00 0	38 3	3 7 7	-0.6
Corn oil	M92 007	18 [©]	43.8	40.1	-3.7
10 ml/kg	M92 008	of 348	33.8	31.8	-2.0
	M92 009	**************************************	39.6	35.4	-4 .2
w o'i	M92 010	48	32.7	36.3	3.6
Corn oil 10 ml/kg MON 0818 100 mg/kg	olling of golding				
MON 0818	M06 006	48	39.6	37.3	-2.3
100 mg/kg	M06-007	48	43.1	41.0	-2.1
7. 90° 1	M06 008	48	33.3	32.9	-0.4
is the and	M06 009	48	36,7	36,5	-0.2
is to the doc, and	M92 002 M92 003 M92 004 M92 005 M06 001 M06 002 M06 003 M06 004 M06 005 M07 001 M07 002 M07 003 M07 004 M07 005 M92 006 M92 007 M92 008 M92 009 M92 010 M06 006 M06 007 M06 008 M06 009 M06 010	48	37.8	31.6	-6.2

^a Hours after treatment.

APPENDIX III - TABLE 1 (Cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL BODY WEIGHTS FOR FEMALE MICE

	<u></u>	Time of		Body Weight (g	δ
Treatment Group	Animal Number	Sacrifice a		nerte.	Se.
		(hr)	Pretest	Final &	Difference
				in go sint	Jan.
Vehicle Control	F92 001	24	28.0	25.3 26.0	-2.7
Corn oil	F92 002	24	ى 30.9	26.0	-4 .9
l0 ml/kg	F92 003	24	29.2	22.7	-6.5
	F92 004	24	28.3	26.0	-2.3
	F92 005	24	29.2	25.3 26.0 22.7 26.0 25.9	- 3.3
MON 0818	F06 001	24	26.2	28.5	2.3
100 mg/kg	F06 002	24	© 26.4 d	29.9	3.5
	F06 003	24	23.5	28.8	5.3
	F06 004	24	27.2	27.8	0.6
	F06 005	24 24 24 24 24 24 24 24 24 24 24 24 24 2	28.0 30.9 29.2 28.3 29.2 26.2 26.4 23.5 27.2 26.5 26.1 26.9 27.0 26.7 25.4	28.4	1.9
Cyclophosphamide	F07 001	::(°248) 800 (800)	26.1	25.8	-0.3
60 mg/kg	F07 002	0124 60 ,101	26,9	26.0	-0.9
• •	F07 003	24) (C)	27.0	26.1	-0.9
	F07 004	ith sold of the site of	26.7	25.8	- 0.9
	F07 005	USA BUTTE TOUR	25.4	26.0	0.6
Vehicle Control	F92, 006	66 48 46 6.	26.1	26.0	-0.1
Corn oil	F92 007	18°C	25.6	23.5	-2.1
0 ml/kg	F92 008	o` 48	26.7	24.3	-2.4
, C	F92 009	48	22.1	22.0	-0.1
0 ml/kg MON 0818 00 mg/kg	F06 002 F06 003 F06 004 F06 005 F07 001 F07 002 F07 003 F07 004 F07 005 F92 006 F92 007 F92 008 F92 009 F92 010 F06 006 F06 007 F06 008 F06 009 F06 009 F06 010	48	28.8	29.1	0.3
MON 0818	F92-010 F06-006 F06-007 F06-008 F06-010	48	26.2	24.7	-1.5
00 mg/kg	F06 007	48	22,9	24.0	1.1
7, 902	F06 008	48	25.8	24.6	-1.2
is the sil	F06 009	48	23.0	20.9	-2.1
is not the door and	F06 010	48	30.8	27.6	-3.2

^a Hours after treatment.

·		APPENI	OIX III -	TABLE 2		inger Enlight.
Mic					lide Scoring Data	on and use
Animal Number	Time (hrs)	PCE / E	rythrocyt	te Ratio ⁸	Micronu	cleated PCE °
		Sld. 1	Sld, 2	Mean	Sld. 1 Si	d. 2 Combined
Moz oot	2.4	0.410	0.274	W 302	192 10:	*******
				0.393	3 Oly Me On	3 3
	- '			0.412	10,00,10,	0 1
				0.410	13:100 01 0	1 1 1 · 1
M92 004 M92 005	24	0.398	0.400	0.394		1 · 1 5 6
1404 001	•	ile.	1 6/ YO	100 5101		
-				$\times 0.485$	g. 0 (0 0
				0.423	U (0 0
						2 2
	24			()		2 2
M06 005	24:0	0.410	0.438	©0.424	0	0 0
M07 001	11024 SUC	0.676	0.456	0.566	11	9 20
M07 002	(0) 24 (1)	0.453	0.354	0.403	12	8 20
M07 003	- / - /2/ - //	_0:000.	0.540	0.538		0 26
M07 004	(*) <u>.</u> 24* (8).	0.426	0.390	0.408		5 27
M07 005	100,59U. "!!	0,570	0.466	0.518		8 17
M92 006	Cip 18:01 W	0.300	0.174	0.237	0	1 1
3 102 802	S . 48 . 2	0.342			•	o i
M92 008	20 ^C 48 ^C	0.320			=	í i
M92 009	48					0
M92 010	JIT 48	0.372	0.304	0.338	· · · · · · · · · · · · · · · · · · ·	o o
Mac noc	40	0.640	0.410	0.475	0	
						1
						-
MOO OOR		0.430		0.385	1 () 1
M06 009	48	0.416	0.422	0.419	0	l 1
	M92 001 M92 002 M92 003 M92 004 M92 005 M06 001 M06 002 M06 003 M06 004 M06 005 M07 001 M07 002 M07 003 M07 004 M07 004 M07 005 M92 006 M92 007 M92 008 M92 009	M92 001 24 M92 002 24 M92 003 24 M92 004 24 M92 005 24 M92 005 24 M06 001 24 M06 002 24 M06 003 24 M06 003 24 M06 005 24 M07 001 24 M07 001 24 M07 002 24 M07 003 24 M07 003 24 M07 004 24 M07 005 24 M92 006 48 M92 007 48 M92 008 48 M92 009 48	Micronucleus Assay of MON (PCE/Erythrocyte Rame	Micronucleus Assay of MON 0818 - I (PCE/Erythrocyte Ratio and II Sld. 1 Sld. 2 Sld. 2 Sld. 1 Sld. 2 Sld.	CPCE/Erythrocyte Ratio and Micronucles CPCE/Erythrocyte Ratio CPCE CPYThrocyte Ratio CPCE CPTThrocyte Ratio CPTThrocyte CP	Micronucleus Assay of MON 0818 - Individual Slide Scoring Data

Ratio scored per 500 enthyrocytes (PCEs and NCEs) for each slide. Mean ratio of both slides (equivalent to ratio for 1000 erythrocytes).

^b Micronucleated PCE scored per 1000 PCEs for each slide and combined micronucleated PCEs for 2000 PCEs scored.

		AF	PENDIX	III - TAI	BLE 2 (Cor	nt.)	57.	SELENION.
	M	licronucleus Ass	ay of MON	0818 - 1	ndividual S	lide Scoring Dat	alisur	15°
		(PCE/Eryti	hrocyte Ra	tio and	Micronucle	eated PCEs)	<u> </u>	and
Treatment Group	Animal Numb	er Time (hrs)	PCE / E	rythrocy	le Ratio a	Mice	onuclea	nted PCE b
			Sld. I	Sld. 2	Mean		1 Sld. 2	Combined
Vehicle Control	F92 001	24	0.390	0.448	0.419		O O	0
Corn oil	F92 002	24	0,396	0.460	0.428	0, 4, 0	2	2
10 ml/kg	F92 003	24	0.364	0.472	0.418	7 60 5 1/2°0	0	0
	F92 004	24	0.614	0.478	0.546	0 0	0	0
	F92 005	24	0.370	0.516	0.443	0,000	3	3
MON 0818	F06 001	24	0.602	0.324	XXV. 1332 X		0	0
100 mg/kg	F06 002	24	0.380	0.488	0.434	0	1	1
- •	F06 003	24	0.584	0.558	0.571	0	0	0
	F06 004	24	0.402	0.564	0.483	i	0	i
	F06 005	24	0.648		0.613	0	0	0
Cyclophosphamide	F07 001	: 324 5UC	0.450	0.586	0.518	23	9	32
60 mg/kg	F07 002	0 24° 0	0.648	0.522	0.585	11	13	24
0 0	F07 003	69 (24 A)	0.424		0.445	22	6	28
	F07 004	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	0.480	0.480	0.480	13	19	32
	F07 005	e ^{Cl} C24()	0.482	0.432	0.457	11	8	19
Vehicle Control	F92 006	is ::048 ct	0.580	0.576	0.578	2	0	2
Corn oil	F92 007	48	0.526	0.635	0.581	$\frac{\overline{2}}{2}$	ő	$\overline{2}$
10 ml/kg	F92 008	48	0.386	0.338	0.362	0	ŏ	0
_0	F92 009	48	0.406	0.418	0.412	Ō	ŏ	ŏ
, pro	F92 010	inis jiri 48	0.400	0.358	0.379	0	0	0
MON 0818 100 mg/kg	F06 006	48	0.506	0.506	0.506	3	0	3
100 mg/kg	F06 007	48	0.600	0.464	0,532	ő	ŏ	ŏ
762	F06 008	48	0.448	0.340	0.394	õ	ŏ	ŏ
sument le Teduch	F06 009	48	0.574	0.462	0.518	Ō	ŏ	ŏ
10,	F06 010	48	0.546	0.446	0,496	ő	ŏ	-

Ratio scored per 500 enthyrocytes (PCEs and NCEs) for each slide. Mean ratio of both slides (equivalent to ratio for 1000 erythrocytes).

^b Micronucleated PCE scored per 1000 PCEs for each slide and combined micronucleated PCEs for 2000 PCEs scored.

SUPPLEMENTAL STUDY INFORMATION

Study Sponsor:	Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167, USA
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Supervisory Personnel:	, Dipl., A.C.V.P.
Laboratory Director:	Ph.D.; D.A.B.T.
Location of Study Material:	or diget such tall the arthorited to
Type Loca	
Specimens EHL Raw Data EHL	Archives
Final Report EHL	Archives
Study Protocol Children EHL	Ph.D., D.A.B.T. Archives Archives Archives Archives