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TITLE: Mouse Micronucleus Screening Assay of MON-0818

AUTHORS: [redacted]

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

Mouse Micronucleus Screening Assay of MON 0818

AUTHORS

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STUDY COMPLETED ON

26-Mar-1998

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PROJECT NUMBER

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REPORT SIGNATURE PAGE

This report accurately represents the data developed during the study.

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SUMMARY

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.

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.

TABLE OF CONTENTS

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 Mavournin 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 ± 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

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 $p \leq 0.05$ 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.

Table 1

Design of the Mouse Micronucleus Assay

Treatment Group	Number of Mice Treated		Number of Mice to be Sacrificed at the Specified Time Following Treatment			
			24 hours		48 hours	
	Male	Female	Male	Female	Male	Female
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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APPENDIX I

Data Summary Tables

APPENDIX I - TABLE 1

MICRONUCLEUS ASSAY OF MON 0818

SUMMARY OF TOXICITY RANGE FINDER RESULTS

Dose mg/kg	Number Treated		Number of Deaths										
			3-5 Hours after dosing		24 Hours after dosing		48 Hours after dosing		72 Hours after dosing		Total		Combined [a]
	M	F	M	F	M	F	M	F	M	F	M	F	
0	3	3									0	0	0 / 6
50	2	2									0	0	0 4
100	2	2									0	0	0 / 4
200	2	2			2	1					2	1	3 / 4
400	2	2	2	2							2	2	4 / 4
600	2	2	2	2							2	2	4 / 4
800	2	2	2	2							2	2	4 / 4
1000	1	1	1	1							1	1	2 / 2
5000	1	1	1	1							1	1	2 / 2

[a] Number of deaths per total number of animals treated.

APPENDIX I - TABLE 2

MICRONUCLEUS ASSAY OF MON 0818

SUMMARY OF MEAN BODY WEIGHT CHANGE IN MALE AND FEMALE MICE

Harvest Time (hrs)	Sex	Number	Mean Body Weight Change (g) ± Standard Deviation		
			Vehicle Control [a]	MON 0818 100 mg/kg	Positive Control [a]
24	Male	5	-0.6 ± 4.9	-2.2 ± 4.2	-1.0 ± 0.5
24	Female	5	-3.9 ± 1.7	2.7 ± 1.8	-0.5 ± 0.7
48	Male	5	-1.4 ± 3.1	-2.2 ± 2.4	
48	Female	5	-0.9 ± 1.3	-1.4 ± 1.6	

*$p \leq 0.05$; ** $p \leq 0.01$ by one-sided Dunnett's test.

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

APPENDIX 1 - TABLE 3

MICRONUCLEUS ASSAY OF MON 0818

SUMMARY OF MICRONUCLEUS RESULTS IN MALE AND FEMALE MICE
PCE RATIO DATA

Harvest Time (hrs)	Sex	Number	Mean PCE/Total Erythrocyte Ratio ± Standard Deviation: Vehicle Control[a]	MON 0818 100 mg/kg	Positive Control[a]
24	Male	5	0.39 ± 0.04	0.45 ± 0.03	0.49 ± 0.08
24	Female	5	0.45 ± 0.05	0.52 ± 0.08	0.50 ± 0.06
48	Male	5	0.31 ± 0.06	0.42 ± 0.03	
48	Female	5	0.46 ± 0.11	0.49 ± 0.05	

*$p \leq 0.05$; ** $p \leq 0.01$ by one-sided Dunnett's test.

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

APPENDIX 1 -TABLE 4

MICRONUCLEUS ASSAY OF MON 0818

SUMMARY OF MICRONUCLEUS RESULTS OF MALE AND FEMALE MICE
MICRONUCLEUS DATA

Harvest Time (hrs)	Sex	Number	Mean Micronucleated PCE/1000 PCE ± Standard Deviation: Vehicle Control[a]	MON 0818 100 mg/kg	Positive Control[a]
24	Male	5	2.4 ± 2.2	0.8 ± 1.1	22.0 ± 4.3**
24	Female	5	1.0 ± 1.4	0.4 ± 0.5	27.0 ± 5.6**
48	Male	5	0.6 ± 0.5	0.8 ± 0.4	
48	Female	5	0.8 ± 1.1	0.6 ± 1.3	

* $p \leq 0.05$; ** $p \leq 0.01$ 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

Individual Clinical Signs

Reported only for groups which had clinical signs, groups with no clinical signs are not reported

Table 1 Individual Clinical Signs for Range Finding Experiments

APPENDIX II - TABLE 1

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder I -- Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 1000 mg/kg
Treatment Date: 6-Nov-89

Animal Number	Date of Observation	Observation
L89099-M0002	6-Nov-89	Found Dead
L89099-F0002	6-Nov-89	Found Dead

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder I – Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 5000 mg/kg
Treatment Date: 6-Nov-89

Animal Number	Date of Observation	Observation
L89099-M0003	6-Nov-89	Found Dead
L89099-F0003	6-Nov-89	Found Dead

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder II -- Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 50 mg/kg
Treatment Date: 7-Nov-89

Animal Number	Date of Observation	Observation
L89099-M0004	9-Nov-89	Listless
L89099-F0011	7-Nov-89	Died at time of dosing, not a treatment-related death

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder II – Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 100 mg/kg
Treatment Date: 7-Nov-89

Animal Number	Date of Observation	Observation
89182M91 001	7-Nov-89	Listless
	8-Nov-89	Listless
	9-Nov-89	Listless
89182M91 002	7-Nov-89	Listless
	8-Nov-89	Listless
	9-Nov-89	Listless
89182F91 001	8-Nov-89	Listless

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder II – Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 200 mg/kg
Treatment Date: 7-Nov-89

Animal Number	Date of Observation	Observation
89182M02 001	7-Nov-89	Listless
	8-Nov-89	Found Dead
89182M02 002	7-Nov-89	Listless
	8-Nov-89	Found Dead
89182F02 001	7-Nov-89	Listless
89182F02 002	7-Nov-89	Listless
	8-Nov-89	Found Dead

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder II – Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 400 mg/kg
Treatment Date: 7-Nov-89

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
89182F03 002	7-Nov-89	Found Dead

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder II -- Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 600 mg/kg
Treatment Date: 7-Nov-89

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	7-Nov-89	Found Dead

APPENDIX II - TABLE 1 (cont.)

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL CLINICAL SIGNS

Range finder II -- Male and Female CD-1 Mice
Route of Administration: Intraperitoneal
Target Dose: 800 mg/kg
Treatment Date: 7-Nov-89

Animal Number	Date of Observation	Observation
89182M05 001	7-Nov-89	Found Dead
89182M05 002	7-Nov-89	Found Dead
89182F05 001	7-Nov-89	Found Dead
89182F05 002	7-Nov-89	Found Dead

APPENDIX III

Individual Test Results

Table 1	Individual Body Weights for Male and Female Mice
Table 2	Individual Slide Scoring Data for Male and Female Mice (PCE/Total Erythrocyte Ratio and Micronucleated PCE's)

APPENDIX III - TABLE 1

MICRONUCLEUS ASSAY OF MON 0818

INDIVIDUAL BODY WEIGHTS FOR MALE MICE

Treatment Group	Animal Number	Time of Sacrifice[a] (hr)	Body Weight (g) Pretest	Final	Difference
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	40.0	2.0
	M92 004	24	38.2	43.6	5.4
	M92 005	24	44.1	39.2	-4.9
MON 0818	M06 001	24	40.1	37.0	-3.1
100 mg/kg	M06 002	24	35.9	38.0	2.1
	M06 003	24	42.0	37.9	-4.1
	M06 004	24	44.2	36.5	-7.7
	M06 005	24	40.0	41.9	1.9
Cyclophosphamide	M07 001	24	34.5	33.3	-1.2
60 mg/kg	M07 002	24	31.3	30.2	-1.1
	M07 003	24	35.0	33.8	-1.2
	M07 004	24	38.3	38.2	-0.1
	M07 005	24	39.6	38.4	-1.2
Vehicle Control	M92 006	48	38.3	37.7	-0.6
Corn oil	M92 007	48	43.8	40.1	-3.7
10 ml/kg	M92 008	48	33.8	31.8	-2.0
	M92 009	48	39.6	35.4	-4.2
	M92 010	48	32.7	36.3	3.6
MON 0818	M06 006	48	39.6	37.3	-2.3
100 mg/kg	M06 007	48	43.1	41.0	-2.1
	M06 008	48	33.3	32.9	-0.4
	M06 009	48	36.7	36.5	-0.2
	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

Treatment Group	Animal Number	Time of Sacrifice[a] (hr)	Body Weight (g) Pretest	Final	Difference
Vehicle Control	F92 001	24	28.0	25.3	-2.7
Corn oil	F92 002	24	30.9	26.0	-4.9
10 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	25.9	-3.3
MON 0818	F06 001	24	26.2	28.5	2.3
100 mg/kg	F06 002	24	26.4	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	26.5	28.4	1.9
Cyclophosphamide	F07 001	24	26.1	25.8	-0.3
60 mg/kg	F07 002	24	26.9	26.0	-0.9
	F07 003	24	27.0	26.1	-0.9
	F07 004	24	26.7	25.8	-0.9
	F07 005	24	25.4	26.0	0.6
Vehicle Control	F92 006	48	26.1	26.0	-0.1
Corn oil	F92 007	48	25.6	23.5	-2.1
10 ml/kg	F92 008	48	26.7	24.3	-2.4
	F92 009	48	22.1	22.0	-0.1
	F92 010	48	28.8	29.1	0.3
MON 0818	F06 006	48	26.2	24.7	-1.5
100 mg/kg	F06 007	48	22.9	24.0	1.1
	F06 008	48	25.8	24.6	-1.2
	F06 009	48	23.0	20.9	-2.1
	F06 010	48	30.8	27.6	-3.2

[a] Hours after treatment.

APPENDIX III - TABLE 2

Micronucleus Assay of MON 0818 - Individual Slide Scoring Data

(PCE/Erythrocyte Ratio and Micronucleated PCEs)

Treatment Group	Animal Number	Time (hrs)	PCE / Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld. 1	Sld. 2	Mean	Sld. 1	Sld. 2	Combined
Vehicle Control	M92 001	24	0.410	0.376	0.393	0	3	3
Corn oil	M92 002	24	0.270	0.384	0.327	1	0	1
10 ml/kg	M92 003	24	0.460	0.372	0.416	0	1	1
	M92 004	24	0.356	0.460	0.408	0	1	1
	M92 005	24	0.398	0.390	0.394	1	5	6
MON 0818	M06 001	24	0.530	0.440	0.485	0	0	0
100 mg/kg	M06 002	24	0.436	0.414	0.425	0	0	0
	M06 003	24	0.392	0.470	0.431	0	2	2
	M06 004	24	0.470	0.512	0.491	0	2	2
	M06 005	24	0.410	0.438	0.424	0	0	0
Cyclophosphamide	M07 001	24	0.676	0.456	0.566	11	9	20
60 mg/kg	M07 002	24	0.452	0.354	0.403	12	8	20
	M07 003	24	0.536	0.540	0.538	16	10	26
	M07 004	24	0.426	0.390	0.408	21	6	27
	M07 005	24	0.570	0.466	0.518	9	8	17
Vehicle Control	M92 006	48	0.300	0.174	0.237	0	1	1
Corn oil	M92 007	48	0.342	0.230	0.286	1	0	1
10 ml/kg	M92 008	48	0.320	0.276	0.298	0	1	1
	M92 009	48	0.454	0.348	0.401	0	0	0
	M92 010	48	0.372	0.304	0.338	0	0	0
MON 0818	M06 006	48	0.540	0.410	0.475	0	1	1
100 mg/kg	M06 007	48	0.472	0.348	0.410	0	0	0
	M06 008	48	0.430	0.340	0.385	1	0	1
	M06 009	48	0.416	0.422	0.419	0	1	1
	M06 010	48	0.494	0.334	0.414	0	1	1

[a] Ratio scored per 500 erthyrocytes (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.

APPENDIX III - TABLE 2 (Cont.)

Micronucleus Assay of MON 0818 - Individual Slide Scoring Data

(PCE/Erythrocyte Ratio and Micronucleated PCEs)

Treatment Group	Animal Number	Time (hrs)	PCE / Erythrocyte Ratio [a]			Micronucleated PCE [b]		
			Sld. 1	Sld. 2	Mean	Sld. 1	Sld. 2	Combined
Vehicle Control	F92 001	24	0.390	0.448	0.419	0	0	0
Corn oil	F92 002	24	0.396	0.460	0.428	0	2	2
10 ml/kg	F92 003	24	0.364	0.472	0.418	0	0	0
	F92 004	24	0.614	0.478	0.546	0	0	0
	F92 005	24	0.370	0.516	0.443	0	3	3
MON 0818	F06 001	24	0.602	0.324	0.463	0	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	1	0	1
	F06 005	24	0.648	0.578	0.613	0	0	0
Cyclophosphamide	F07 001	24	0.450	0.586	0.518	23	9	32
60 mg/kg	F07 002	24	0.648	0.522	0.585	11	13	24
	F07 003	24	0.424	0.466	0.445	22	6	28
	F07 004	24	0.480	0.480	0.480	13	19	32
	F07 005	24	0.482	0.432	0.457	11	8	19
Vehicle Control	F92 006	48	0.580	0.576	0.578	2	0	2
Corn oil	F92 007	48	0.526	0.635	0.581	2	0	2
10 ml/kg	F92 008	48	0.386	0.338	0.362	0	0	0
	F92 009	48	0.406	0.418	0.412	0	0	0
	F92 010	48	0.400	0.358	0.379	0	0	0
MON 0818	F06 006	48	0.506	0.506	0.506	3	0	3
100 mg/kg	F06 007	48	0.600	0.464	0.532	0	0	0
	F06 008	48	0.448	0.340	0.394	0	0	0
	F06 009	48	0.574	0.462	0.518	0	0	0
	F06 010	48	0.546	0.446	0.496	0	0	0

[a] Ratio scored per 500 erthyrocytes (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

Scientists and Professional Participating in Study

Study Director: [redacted], B. S.

Technical Support: [redacted]

Supervisory Personnel: [redacted], Dipl., A.C.V.P.

Laboratory Director: [redacted] Ph.D., D.A.B.T.

Location of Study Material:

Type	Location
Specimens	EHL Archives
Raw Data	EHL Archives
Final Report	EHL Archives
Study Protocol	EHL Archives